VISION OF THE **ASIAN PIG**
FUTURE OF THE **WORLD PIG**

APVS 2019

2019 아시아양돈수의사대회

ASIAN PIG VETERINARY SOCIETY CONGRESS 2019

ABSTRACT

DATE AUGUST **25-28**, 2019 / **VENUE** GRAND HILTON SEOUL

Contents

Acknowledgement

Dear Delegates,

Since the 1st Asian Pig Veterinary Society(APVS) Congress 2003 in Korea, APVS has contributed to collaboration and update of Asian pig industry. After 16 year from 1st APVS congress, the 9th APV Scongress 2019 is held on August 25-28 in Seoul, South Korea.

Nowadays, Asian countries, including South Korea, stand as the center of the pig industry with more than 50% of the world pig population. However, due to the severe outbreak of African swine fever, Asian pig industry fell in panic state without proper vision and solution.

In this difficult circumstance, Asian veterinarians should be the advisor and leader of pig industry to overcome current difficulties. Additionally, Asian veterinarians need to develop their abilities and network for proper prevention measures against transboundary diseases.

In this point of view, APVS congress 2019 is perfect time to discuss and share your knowledge to solve ongoing swine health issues of high importance in Asia and other parts of the world.

I would like to extend our appreciation to all authors and invited speakers to contribute to this proceeding and scientific program during APVS congress 2019. I wish all attendance can take valuable message and idea from thins congress.

Dr. Hyunkyu Jeong, DVM, Ph.D
Chairman of APVS 2019 Organizing Committee
Dodram Pig Farmers Cooperative

Dear APVS colleague and friends,

It has not been easy journey to hold the 9th APVS in Seoul Korea. My sincere apology to all who felt any inconvenience caused while preparation of the meeting during last one year due to the unexpected ASF outbreaks in Asian continent.

Of good heart and good trust, faithful to ourselves and our profession, we could lift cause of science meeting for pig industry, the cause of the sustainable growth for our next generation, so high no power can tear it down. We are human being first and pig veterinarian second, and the best maximum for our profession is still sacrifice for others, special privileges for none.

True professionalism of APVS friends showed to us was based on the endurance and large measure of humility. I do appreciate for all who support this meeting possible despite of betrayal and groundless attacks from some organization leaders.

I wish you enjoy the APVS 2019 and have a good time in Seoul.

YOUNG S. LYOO, DVM, MSc, PhD
Chairman of APVS 2019 Scientific Committee
Dean/Professor
College of Veterinary Medicine
Konkuk University, Seoul Korea

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History of APVS Congress

2003	Seoul, Korea
2005	Manila, Phillippines
2007	Wuhan, China
2009	Ibaraki, Japan
2011	Pattaya, Thailand
2013	Ho Chi Minh, Vietnam
2015	Manila, Phillippines
2017	Wuhan, China
2019	Seoul, Korea

APVS 2019
Asian Pig Veterinary Society Congress 2019

Country & Regional Reports

Philippines

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The Philippine Swine Industry Country Report

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Ranked 34th largest economy in the world with over a 100 million human population, the Philippines has always been an import dependent country for pork. Notwithstanding its low per capita consumption of pork pegged at 15kg, the additional 2 million mouths to feed each year due to the rapid growth in human population would indeed pose a challenge to the livestock sector of the industry. Adding to the current woes is the fact that the swine industry is growing at a snail pace. In fact, the standing population remained almost flat for over a decade already.

A lot of factors have been attributed to the slow growth of this USD5Billion swine industry. For one, the challenge of diseases such as PRRS, PED, and even Classical Swine Fever cannot be underemphasized. Luckily, the Philippines already succeeded in eradicating Foot and Mouth Disease. Recently, a lot of private stakeholder initiatives have transpired with the end in mind to assist the Philippine Government in its incessant drive to "biosecure" the country against the dreaded African Swine Fever that has already created havoc among neighboring countries in Asia.

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Eradication and control of economically important swine viral diseases in China

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Pig production is always the most important industry in China as pork consumption accounts for roughly 65% among the meat consumption. Thus, prevention and control of the major swine infectious diseases are the priority of the governments, scientific researchers and farmers. Vaccination or medication, together with bio-security, is the mainly measurement for the prevention and controlling the pig infectious diseases, while strict bio-security is currently the approach to prevent African swine fever, a emerging disease in China.

Eradication of swine pseudorabies Basing on the substantial monitoring and surveillance of wild type virus-infected and stamping out policy, more and more herds or pig farms have been pseudorabies free, in particular the national breeding pig farms. As the tradition vaccine such as Bartha strain-based vaccine has a poor protection efficacy against this novel PRV infection, the emerging of the novel pseudorabies virus is still posing a threat to pig industry, however, the viral characterization including pathogenicity, and immunity has been discovered. As a result, the new virus-based inactivated vaccine which can elicit protection against the new PRV has been developed and used while the live attenuated vaccines are still clinically evaluated. A oral vaccine and Elispot assay for evaluation of cellular immunity have been documented. Currently, a regional PR eradication strategy in combination of PR free breeding pig farms and management of pig movement is being carried out.

Eradication of classical swine fever Combination of herd immunization using live attenuated vaccine with stamping-out the CSFV infected pigs, usually together with PR eradication plan, more breeding farms are involving in CSF free campaign. It is worthy to note that the E2-based subunit vaccine is applied, which may pave the way for serological differentiation diagnosis between vaccinated and infected pigs. The serological differentiation test possesses advantage over the traditional RT-PCR that tonsils are collected to prepare template for amplification.

Comprehensive prevention of porcine respiratory and reproductive syndrome. The epidemiological investigation revealed that highly pathogenic and NADC-30 like PRRSVs are prevalent strains. However, the recombination between highly pathogenic PRRSV-derived attenuated vaccine strain and PRRSV field strain is also frequently observed. To prevent the disease, both natural attenuated vaccines and HP-PRRSV derived live vaccines are respectively used in different pig farms while the inactivated vaccine is seldom applied. Due to the concern of potential side-effect of live vaccine, some breeding pig farms focus on the biosecurity measurements and multiple site production, instead of using live vaccine.

PCV-associated disease Among different genotypes, PCV2d detection rate is higher that 2b genotype. Both whole PCV2a- and PCV2 2b virus-based inactivated vaccine and Cap protein-based subunit vaccines are used by different pigs respectively. To control the coinfection of PCV2 with bacteria, PCV2 plus Hemaphillus parasuis or Mycoplasma hyopneumoniae -based bivalent vaccines are being developed. PCV3 is also detected in the samples from pigs with poor growth or reproductive failure in many provinces. PCV3 could be recovery by using infectious clone technology and proved to be pathogenic by pig infection.

Porcine epidemic diarrhea (PED) Higher detection rate of PED variant strains than CV777-like strain has been documented, indicating the immunization failure of CV-777 strain based inactivated or live vaccine. Thus, the development and clinical application of the novel vaccine using variant PEDV becomes necessary and helpful for pig farms to successfully prevention by vaccination of sows before delivery. Meanwhile, the multiplex RT-PCR to simultaneously detect PEDV,TGEV, Rotavirus and IgA-ELISA have also been developed to diagnose rapidly and evaluate the immune response.

African swine fever (ASF) The first ASF case was officially noted on August 2, 2018 and the virus belongs to Genotype II. Transmission of the virus through contaminated vehicles, staffs, porks and infected pigs can be identified. Two batches of rapid diagnostic kits including real-time PCR, LAMP and gold-immunochromatographic strips have been approved by Ministry of Agriculture and Rural Affairs (MARA) of People Republic of China. Laboratory detection of viral DNA and virus isolation must be authorized by governments. Currently, no any vaccines and drugs can be available in China for prevention and therapy purpose. Only ASFV free pigs can be moved from farms to slaughters. The strict bio-security measurements including quarantine and disinfection are recommended and required to fight against this disaster disease.

Acknowledgement The research is granted by China Agriculture Research System (CARS-35).

Swine production and health status in Japan

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Introduction: The swine industry in Japan showed a continuous reduction in number of swine farms, but the number of pigs per farm continued to rise. The swine industry in Japan has become an important agricultural industry. However, swine diseases are a major factor reducing swine productivity in Japan. Common diseases include PRRS, PCVAD, edema disease, and pleuropneumonia etc. Although cattle and pigs had a FMD outbreak in 2010, they are now free of FMD without vaccination. Recently, CSF, a viral disease, was positive in pigs and wild boars.

Swine Production: The number of breeding pigs gradually decreased from the peak in 1989. Therefore, large-scale (over 2,000 fattening pigs) farms extended. After 2004, the number of pigs slightly increased and decreased, and, after 2011, decreased moderately. The number of the breeding pigs was 9,156,000 in 2018. The number of farms, especially small farms, decreased. The number of pig farms in Japan was 43, 000 in 1990 but decreased by 90% to 4, 320 in 2018. The number of breeding pigs per farm increased remarkably. The total number of pigs per farm more than doubled from 275 in 1990 to 2,119 in 2018.

Swine Health Status: Owners are required to report the number of breeding pigs to the prefectural governors. Furthermore, owners (breeding over 5 pigs) are required to report the number of pig shed, following the guideline of the National Standard of the Farm Biosecurity (NSFB).

NSFB: The Act on Domestic Animal Infectious Diseases Control in Japan establishes NSFB, according to which producers should protect the breeding of pigs to a minimum to prevent contagious disease outbreaks and be obligated to observance. The NSFB about the pig is as follows. 1) Isolate the sanitary zone (SZ) and other zones in the farms. 2) Minimize the number of gateways to SZ. Install a "Do Not Enter" signboard near the farm entrance. Do not let unnecessary people enter SZ. 3) People who visit a farm or other livestock-related facility on the same day must not enter SZ carelessly. Moreover, people who visited a foreign country in the past week must not enter SZ carelessly. 4) Heat treat feed, including meat, at over 70℃ for 30 min or at over 80℃ for 3 min before feeding. 5) Do not allow wild animals in the area. 6) Report to the Livestock Hygiene Service Center (LHSC) immediately if a pig shows a specific symptom. 7) Prepare a burying ground (0.9 m^2 / fattening pig). 8) Keep a record of the following items for at least one year: the name, address, organization, date, and purpose of people entering SZ. 9) At large-scale farms, breeding over 3,000 pigs, fix a veterinarian to contact the closed LHSC or medical facility for every farm, and consult a veterinarian regularly for health-care of the breeding pigs.

Swine Diseases: CSF occurred in Japan on September 9, 2018 after an interval of 26 years. The CSF spread to 7 prefectures in 34 cases (July, 2019). Moreover, the CSF spread, in 6 prefectures, to the wild boar, and the total of the CSF-positive wild boars were 927 of 2,758 examined (July, 2019). For a wild boar, oral vaccine administration is carried out. Pigs are not vaccinated. A re-outbreak of PED has been reported locally. AD is still positive in 2 prefectures, but Japan will be free it soon. PRRS still spreads, but satisfactory results of gradual control of PRRS using the regional sanitation strategy were reported. PCVAD and pleuropneumonia are sporadic. Edema disease of pigs is re-sporadic. This may be associated with feed addition of Colistin as antimicrobial growth promoter having become the prohibition (July, 2018). Japan is free, without vaccination of ASF and FMD. However, 45 ASF viral genes were detected in the passenger baggage from ASF-positive countries after August, 2018. The ASF virus was isolated from 2 cases and was closely associated with the SY18China (MH766894) II and Georgia (FR682468) II strains. The Ministry of Agriculture, Forestry and Fisheries Animal Quarantine Service increased quarantine detector dogs to 40 and disclosed 94,000 a year illegal baggage.

Thailand country report. : ASF contingency and preparedness plan.

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African swine fever (ASF) is a severe infectious disease in swine. It causes severe economic impacts in swine industry. Started from 2018 until now, the outbreak of ASF is reported in 21 countries in different regions around the world. In Southeast Asia, the outbreak occurs in six countries. The increasing trend of ASF outbreaks is continuously observed. Although Thailand is free from ASF infection but Thailand has a high risk of ASF introduction into the country. Several risk factors associated with the ASF introduction includes 1) taking pork products illegally from ASF affected country by the tourists at the international airport 2) illegal movement of pork products along the border area 3) the risk of a visit in the ASF affected countries of the farmer or veterinarians which may lead to contaminate of ASF into the country and 4) the risk of ASF contamination onto the vehicles, tools and equipments transported swine and feed after coming back from the neighboring countries. Based on the risk assessment of economic losses in an event of ASF outbreak in Thailand without disease emergency preparedness, effective surveillance as well as disease control measures, it will cause the severe economic losses with total amount not less than 125,000 million baht (4,045 million Dollar) Furthermore, the cost of restoring the occupation and the livelihood of the farmers is high and it takes time for rehabilitation and finally leads to food insecurity. Therefore, Department of Livestock Development in cooperation with all stakeholders and relevant agencies impels the ASF preparedness plan as a national agenda. This plan is comprised of three phases: Pre outbreak, outbreak and post outbreak, which is consisted of 8 important measures such as, administration and driven, integration of disease prevention, increasing efficiency of disease prevention in swine farms, increasing efficiency of disease surveillance, the development of disease diagnosis and establishment of laboratory networks, the development of disease control, capacity building in risk communication and farmer rehabilitation. However the implementation of these measures will not be conducted effectively without the collaboration of all relevant agencies especially swine farmers.

Key words: Thailand, country report, ASF, contingency, preparedness plan

Overview of pig diseases in recent years in Vietnam: from study to clinical

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The world population growth has been boosting supply sources to satisfy demand of food, trade, movement, and even mental that would become the current global challenges. Vietnam is among the high-density countries and is also a agricultural country to serve these needs in which animal husbandry is very important and have a big impact in the national economy. During the decade, the growth rate of livestock has been steadily increasing over the years. Particularly, in 2018, the total number of pigs is 28.151 million, the total number of poultry is 408.97 million, the total number of ruminants is 11.571 million and the huge amount of freshwater and saltwater aquaculture have increased over the years (Department of Livestock Production and General Statistics Office, 2018; Agromonitor, 2018). High density of production and population create the complicated and close interface and relationship among people, livestock (animals) and ecological system more than ever before, then it leads to plenty of challenges of infectiuos and non-infectious disease. Moreover, the challenges of product's price fluctuations, climate change, environmental pollution, poor level of understanding and implement of livestock biosecurity and problems of drug abuse, chemicals, toxic residues in animal product, as well as the emergence of multi-drug resistance microoganisms and pathogens are also very big concerns.

Vietnam pig production comprise a larger proportion of husbandry and has a diversity of scale from backyard to battery models (small, medium, large) and even cooperative farms or mega farms making a complex and diverse ecosystem not only in animal and human populations but also in pathogens, so it becomes very difficult for macro-management. Industrial anmial production is making an imbalance in the normal/healthy state of the animal body, in general, as shown by the organic relationship among the body microorganisms aka "microbiome" (Scotti et al., 2017; Niederwerder, 2018). Therefore, several important non-infectious and infectious diseases have been recorded as a risk for the stability of pig herd and livestock productivity. Common non-infectious diseases such as mastitis metritis and agalactia in sows (MMA), reproductive dysfunction, metabolic disorders, mycotoxins, chemical poisoning,... are prevalent. In addition, infectious diseases always keep the first position, threatening the health of pigs and economy. Not good disease management leads to many infectious diseases categoried into 3 groups: diseases that must be notified epidemic; production diseae, and zoonotic diseases than transmit among pigs and humans

Notified diseases in Vietnam can be listed as follows: Foot and mouth disease, Classical swine fever, Highly pathogenic Porcine respiratory and reproductive syndrome. Especially, African swine fever (ASF) has recently hit the pig production in Vietnam with a hug economic damage. From a report in the first 6 months of 2019, the total number of livestock and poultry of country has a big fluctuation due to the complicated situation of African swine fever (ASF), 3,800,000 pigs were destroyed (MARD, 2019; http://www.fao.org/). The prevalent production diseases (such as syndromes related to PCV2 / PCV3, ulcerative colitis syndrome, ...) were published in several surveys throughout the country (Duy et al., 2015; Quan et al., 2016; Duy et al., 2019). These groups of pathogens (viruses, bacteria and parasites) tend to interact with each other to form many difficult-to-control, and difficult-to-treat syndromes. Important disease syndromes of concern are Procine Respiratory Disease Complex (PRDC), Post-weaning Multisystemic Wasting Syndrome (PMWS), Porcine Dermatitis and Nephropathy Syndrome (PDNS), Post-weaning Wasting Catabolic Syndrome (PWCS), Syndrome of Congenital Tremor, Porcine Acute Diarrhea Syndrome (PEDV, TGEV, PDoCV, PRoA) and Ulcerative colitis syndrome in Pigs. Many national publications mentioned on this issue.

Important novel infectious diseases appear (emerging) and re- emerging every year in this decade and will have been increasing even more in the near future. More notably, dangerous infectious diseases appear to be spread between animals and humans as reported by OIE that 75% of emerging infectious diseases in humans originated from

animals, in which environmental pollution is a bridge link to this interference. The common zoonotic infectious diseases between pigs and humans have been recorded in Vietnam: viral disease (swine flu, hepatitis E, rota group A virus), bacterial diseases (*Streptococcus suis, Bacillus anthracis, Mycobacterium tuberculosis, Campylobacter jejuni, Leptospira* spp …) and parasites (*Taenia solium, Trichinella* spp., Ascaris roundworms) (Juan Carrique-Mas and Juliet Bryant, 2013; Dinh-Toi Chu et al., 2019).

Genetic mutations occur very fast and that lead to genotypic diversity of many important pathogens in pigs are a major challenge in changing pathogenesis, altering immunogenicity and failing diagnostic procedures as molecular and serology have been continueing to occur in pigs in Vietnam. The genetic diversity is noted prominently in the swine flu virus (SIV) (Takemae and c., 2017), the blue ear disease virus (PRRSV) (Duy et al., 2015), the virus associated post-weaning wasting syndrome (PCV2), foot and mouth disease (FMDV) (Department of Animal Health, 2019), acute diarrhea virus (PEDV and PRoA) (Toan et al., 2013; Anh et al., 2014; Kim et al., 2015). Therefore, it is necessary to have continuous supervision of professional agencies and promptly contribute to the prevention and control of molecular epidemiology of pathogens.

The objective of the report is to summarize the important diseases in pigs in recent years in Vietnam with the perpective from research to clinical practice. The focus of the presentation will provide up-to-date information, summaries of epidemiology including molecular epidemiology, and new prospects for better disease control, towards novel strategies about safe, sustainable and cleaner animal production.

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Pig production and pig diseases in Taiwan 2019

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Introduction: The pig industry in Taiwan comprises 6,999 pig farms with 520,000 sows. These figures are from the latest pig survey conducted in May 2019 (Fig. 1). Results from these surveys show that the number of pig farms and the pig inventory has continuously declined year by year, especially for small-scale pig farms and the issue of African Swine Fever (ASF) in the neighbor countries. The rate of pork self-sufficiency is about 90% in Taiwan and pork imports come mainly from the USA and Canada. Recently, ASF is a major pre-border disease which is widespread in the Asian regions. The risk of ASF incursion is increased and we have the strict control measures in place for our border control to minimize the risk of ASF outbreak in Taiwan. In order to stop the travelers carrying meat products we have imposed the heavy fine if they violate the regulation.

Materials and methods:

This report will focus on the issues of our challenges, pig diseases and control measures in Taiwan for the past few years.

The contents are based on the annual reports from annual pig surveillance and disease report in Taiwan.

Results

Challenges in the Pig Industry

Major challenges in the pig industry in Taiwan include international free trade, generational change, a shortage of farm labor and a shortage of young veterinarians to provide services to pig farms. In order to provide some solutions for the pig industry, several projects have been funded by government to train swine veterinary specialists in the veterinary schools in which Master's Degree courses and specialist pig veterinary training courses have been established. Hands-on practical farm management training has been introduced to train the younger generation of staff on farms, about data registration and the batch production system including and adjust pig flow to maximize farm productivity. Some demonstrative farms have been set up to introduce this concept to farmers and to show farmers how to implement these programs. An innovation for training young swine veterinarians is the use of training farms where they can participate in daily farm activities and learn to plan operations such as pig flows on farms that they consult to. A website is provided for swine producers and swine veterinarians to access information aimed at improving their knowledge of pig production and health management. The website can be accessed at: http://pmtw.atri.org.tw/. In the website, are provided videos of our training courses and photos of various common swine diseases.

Pig diseases

Classical swine fever (CSF) and Foot and Mouth Disease (FMD) are targeted diseases for eradication with first control being by compulsory vaccination. Our pig industry stopped FMD vaccination since July 1, 2018 and we do not have FMD cases for over one year now which make us to be able to apply FMD free country without vaccination in May, 2020. With the experiences of FMD control and eradication, our next target disease for eradication is CSF because we do have the disease outbreaks for the past 12 years. In 2014 Porcine Epidemic Diarrhea (PED) was introduced in the farrowing units and caused severe losses in new-born piglets. Currently, the important diseases on pig farms in Taiwan are those caused by Porcine Reproduction and Respiratory Syndrome virus (PRRSV), Porcine circovirus type 2(PCV2), Swine influenza virus (SIV) and co-infection with other pathogens such as Mycoplasma, E. Coli, Salmonella and Streptococcus causing a post-weaning respiratory syndrome which results in severe losses in the nursery piglets.

Control and management

The strategies for controlling the problems are mainly through good husbandry and sound production management aimed at maintaining

pig flow using batch production, all in/all out systems and vaccination programs against Hog cholera/Swine fever (HC) and Pseudorabies. Antibacterial treatment of major diseases, such as those caused by E coli, Salmonella, and Streptococcus is carried out where indicated. In order to provide records on the farms, the white board recording system and PigChamp have been introduced to monitoring the number of pigs per batch. With the "sow board" (Fig.

2), we are able to monitor the pregnant sows in the individual batch to guarantee and prepare enough sows for breeding in the next cycle. The "finishing board" provides a visible record of the losses occurring in each fattening pig batch every week. It is easy to observe the drop of pig numbers in every batch by looking at the white board and then to provide some timely advice on what intervention is needed if there are severe losses in a batch.

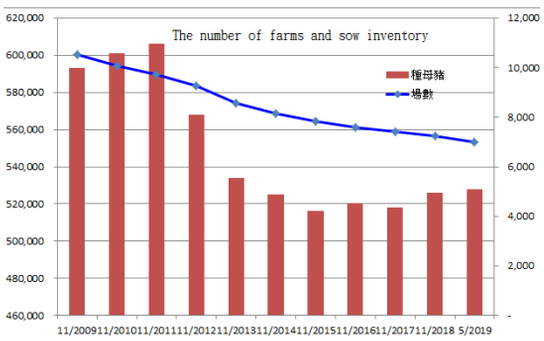

Fig 1. The number of farms and sow inventory for the past 10 years

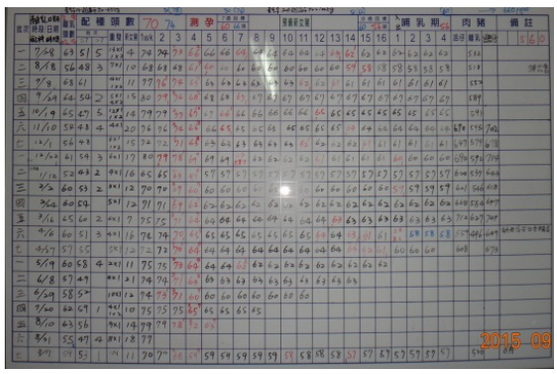

Fig 2. white board recording system has been introduced for monitoring the number of pigs per batch

Conclusions

After working with FMD for more than 20 years, Taiwan finally will become FDM free country without vaccination next year. The model for control and eradication of FMD will be apply to another major swine diseases in Taiwan in the near feature.

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Swine production and disease in Korea

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Introduction

The Korean swine industry showed a continuous reduction in the number of swine farms but the number of pigs per farm continued to rise. As a result, the Korean swine industry became the most important livestock industry in South Korea. Nevertheless, swine diseases are considered one of the most important factors in reducing pig productivity in Korea.

Important diseases include porcine reproductive and respiratory syndrome (PRRS), edema disease, *Clostridium* infection, abortions due to infectious pathogens, and porcine pleuropneumonia.

There was a foot-and-mouth disease (FMD) A type outbreak in swine farms in 2018. In 2019, although new FMD broke out in cattle, it did not infect the swine farms. Recently, another important viral disease in pigs, classical swine fever, was detected in wild boars.

Swine Production

The number of pig farms in South Korea was 6,313 in 2017 but it decreased to 6,188 in 2018, which is a reduction of 2.0%. The total number of pigs per farm more than doubled from 977 in 2007 to 1,831in 2018 (Table 3).

In 2018, 17.37 million pigs were slaughtered and the Marketted-pigs per Sow per Year (MSY) was recorded as 16.3. According to the computer record analysis result by the Korea Pork Producers Association, the top 30% Korean swine farms showed an MSY of 20.9, but the bottom 30% farms showed an MSY of 14.3.

In Korea, meat consumption per capita amounted to 49.9 kg in 2017 (based on MAF statistics). Pork accounted for 24.5 kg, chicken for 13.3 kg, and beef for 11.3 kg respectively. Thus, pork became the most important livestock industry in South Korea.

The Livestock Product Management Act has been enacted since January 2014. It states that animals should not be fed a day before they are slaughtered. However, that act was not enforced properly. From April 2017, this law carried out in its entirety.

Swine Health Status

In 2018, there were several unusual points compared to previous data. First, the PRRS type 1 (European type) virus cases were increased rapidly. On the contrary, PRRS type 2 (North American type) PRRS cases were decreased. Personally, I think PRRS is one of the most important diseases for our country's pig industry. In addition to the visible loss caused by PRRS infection, it is estimated that there will be approximately USD 100 million worth of economic losses in Korea alone in the wake of the loss of feed efficiency.

Accordingly, periodic antigen tests are required to be conducted on breeder farms and artificial insemination centers. In the case of farms, they should require regular PRRS inspection reports when they purchase gilts or semen. Prior to 2014, only type 2 vaccine was used, but since the latter half of the year, type 1 PRRS vaccine has been available. Additionally, other brands of type 1 and type 2 vaccines have been available since the latter half of 2015.

For type 2 PRRS, MLV vaccine strain (Boerhinger Ingellheim) and P129 vaccine strain (Zoetis) have been used. For type 1 PRRS, VP046 vaccine strain from Hipra and DV vaccine strain from MSD have been used.

Although the vaccine manufacturers insisted on cross protect between North American type and European type viruses, there is no evidence to prove it.

There has been a decrease in the cases of E. coli infections and in the number of pigs with edema disease. But in case of *Clostridium perfringenes A*, there were increase of isolated case. There were doubt whether this bacteria is real pathogen or not. Because this bacteria is isolated very often from sick pigs but also from normal pigs. Furthermore, *C. perfringenes* in part of the normal intestinal flora in domestic animals.

Table 1. Statistical analysis of pathogens detected from Korean swine farms.

Pathogen test items	Positive cases			
	2015	2016	2017	2018
PRRSV (Type 2)	339	465	506	600
PRRSV (Type 1)	83	142	212	366
Pathogenic Escherichia coli	211	252	309	342
Clostridium perfringens type A	104	126	185	330
Porcine circovirus type 2	118	142	245	275
PRRSV (Type 1 and Type 2)	76	52	208	260
PCR test associated with abortion	87	143	154	195
Porcine epidemic virus	123	51	92	185
Haemophilus parasuis	59	95	131	133
Lawsonia intracellularis	60	56	105	124

Pathogen test items	Positive cases			
	2015	2016	2017	2018
Mycoplasma hyopneumoniae	39	61	84	118
Streptococcus suis	19	76	122	114
Actinobacillus pleuropneumoniae	54	58	72	92
Salmonella spp.	52	36	84	91
Porcine rotavirus	35	31	49	60
Brachyspira hyodysenteriae	27	15	35	54
Pasteurella multocida	31	51	60	53
Swine Influenza virus	3	7	13	24
Clostridium novyi	5	11	15	20
Mycoplasma hyorhinis	0	11	17	19
Clostridium difficile	1	3	8	18
Staphylococcus hyicus	4	2	3	8
Clostridium perfringens type C	0	1	4	7
Isospora suis (Coccidium)	12	21	11	5
Actinobacillus suis	8	12	4	4
Porcine parvovirus	0	0	1	3
Porcine cytomegalovirus	2	7	2	1
Erysipelothrix rhusiopathiae	0	4	3	0
Transmissible gastroenteritidis virus	7	3	0	0
Leptospira spp.	0	1	0	0
Brachyspira pilosicoli	3	0	0	0
	1562	1935	2734	3501

There were 275 cases of PCV2 in 2018. In 2017, there were 245 cases of antigen detection, indicating that there was an approximately 1.6% increase in the portion of PCV2 compared to 2016. Even if the PCV2 vaccine is inoculated, the PCV2 virus can be detected from the tissue. The detection of antigens does not mean an onset of severe disease.

One finding from the clinical pathology results is the increase in abortion/still birth cases. Abortion and still births may have over 30 different causes, which is why we need to identify the cause of abortion. However, most farms do not have their swine tested in laboratories. Obviously, some of the causes of abortions are nutrition or climate change; however, in cases of abortion by diseases or pathogens, we can improve the situation based on the exact diagnosis.

Porcine epidemic diarrhea is also important pathogen which is affecting the production of Korean swine industry. Lawsonia intracellularis is damaging Korean swine industry. Proliferative ileitis vaccine launched in 2008.

Another disease that damages the Korean swine industry is pleural pneumonia. In 2010, only 3.2% of pleural pneumonia cases were caused by *Actinobacillus pleuropneumoniae* serotype 1, while serotypes 2 and 5 caused 96.8%, thus the main vaccine antigen types were also based on serotypes 2 and 5.

However, cases of serotype 1 pleural pneumonia have continued to increase. In 2014, serotype 1 pleural pneumonia cases accounted for 94.7% out of serotypes 1, 2 and 5 cases (Table 2).

Serotype 1 *A. pleuropneumoniae* infection causes severe damage even to younger piglets, unlike typical pleural pneumonia cases.

Table 2. *Actinobacillus pleuropneumoniae* **isolation rate according to year**

isolation rate of Actinobacillus pleuropneumoniae			
Year	Serotype 1	Serotype 2	Serotype 5
2008	0.0%	53.6%	46.4%
2009	0.0%	43.9%	56.1%
2010	3.2%	19.4%	77.4%
2011	45.9%	2.7%	51.4%
2012	57.9%	12.3%	29.8%
2013	61.0%	18.6%	20.3%
2014	94.7%	0.0%	5.3%
2015	86.1%	2.8%	11.1%
2016	72.2%	8.3%	19.4%
2017	65.7%	2.9%	31.4%
2018	75.0%	0.0%	25.0%

Foot and Mouth Disease

Between March 26, 2018 and April 1, 2018, two cases of A type foot-and-mouth disease (FMD) were reported in pigs.

Due to the outbreak of FMD, 4,435 pigs in two farms were stamped out. In addition, 7,291 pigs in eight farms were stamped out for preventive purposes. Out of ten farms, total 11,726 pigs were destroyed.

Between January 28, and January 31, 2019, three cases of O type FMD were reported in cattle. Korean government succeeded in finishing outbreak of FMD in four days.

Conclusion

In South Korea, the pork industry is considered a very important industry. Nonetheless, MSY is not high compared to other countries. The average MSY of a Korean farm is 16.3. Disease is the main reason for low productivity of farms.

For sustainable development of the swine business, the Korean pig industry will have to overcome various obstacles including high labor cost, lack of reliable employees, alternatives to antibiotics, and environmental factors (manure treatment, odor complaints, soil contamination, etc.). Lastly, animal welfare will also be important considerations in the near future.

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Report of disease status in Korea in 2007, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, and 2018 by Korean Pig Producers Association

Table 3. Changes in swine production in the last eight years (2011~2018, source: Korea Pork Producers Association)

Items	2011	2012	2013	2014	2015	2016	2017	2018
Number of farms	6,300	6,000	5,600	5,177	4,909	4,574	6,313*	6,188*
Pig inventory (k head)	8,171	9,916	9,912	10,090	10,187	10,367	11,273	11,333
Number of sows (k head)	903	962	895	937	958	974	1,058	1,063
Number of pigs/farm	1,287	1,642	1,770	1,949	2,075	2,266	1,786*	1,831*
Number of slaughter pigs	9,851	9,997	16,130	15,688	15,906	16,545	16,729	17,369
Pork imports (k ton)	345.5	236.2	185.0	273.8	357.9	318.4	369.2	463.5
Consumption/capita/y (kg)	18.8	20.3	20.9	22.2	22.8	24.1	24.5	27.0
Feed production (k ton)	3,630	5,639	6,136	5,962	6,092	6,256	6,365	6,554

* Notice: Due to the changes in categorization method of farm from 2017, the farm number looks to be increased. But the real number of pig farms are not increased.

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Keynote Lectures

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Dr. Arunee Thanasarasakulpong

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High quality foot-and-mouth disease vaccines for fmd control and eradication: its importance to ensure success in vaccination programs

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Keywords: foot-and-mouth disease, vaccines, potency, vaccine banks, emergencies

Introduction: Foot-and-mouth disease (FMD) is the most economically important disease of livestock because of its impact on international trade in animals and animal products. One key factor in the success of vaccination programs is the availability of high-quality vaccines. Biogénesis Bagó (BB) is an international company with more than 70 year experience, including scientific and commercial achievements in animal health and animal productivity. Its main objective is to provide effective solutions to major animal diseases that impact animal health and herd productivity around the world.

For decades, it has focused in the research and development of veterinary products with the highest quality standards thus becoming a referent company in FMD vaccine manufacturing and distribution worldwide. BB is globally committed to providing technological tools to the prevention, control and eradication of FMD.

The company has been working with animal health, research and scientific organizations making significant investments in infrastructure and in research and development in order to help eradicate the disease in Latin America and worldwide. During different FMD outbreaks and emergencies, BB provided the necessary vaccines, playing a leading role on the fight against the disease. In 1997, when Taiwan faced a devastating FMD outbreak, BB immediately provided FMD vaccines, becoming the first vaccine approved by the Animal Health Authorities of that country. BB contributed with a large amount of vaccine doses which were administered during emergencies and thereafter in regular vaccination campaigns.

In 2000, BB was selected to create the first South American Bank of Antigens and Vaccines for the prevention of FMD. This bank responded efficiently in emergencies that appeared in Argentina and Uruguay in 2001 and 2002. The international acknowledgement for its technological capability, product quality and production capacity allowed BB to be awarded a supplying contract in 2006, through an international tender of the Antigen and Vaccine Bank of North America, as part of the preparedness plan in case of FMD emergency in United States, Mexico and Canada.

BB also obtained a license for their FMD vaccine in Canada in October 2010 as part of the contingency plan in a country free of the disease.

Furthermore, in July 2011 it became the first company to obtain a "Permit for Sale and Distribution" of their FMD Vaccine "Bioaftogen" in the United States of America.

Today, BB is the only FMD vaccine manufacturer that holds marketing authorizations in every country of Latin America in which FMD vaccination programs are implemented, and with the use of the vaccine approved in case of emergency in Canada, Mexico and USA. Moreover, BB supplies antigens and vaccines to the North American FMD Vaccine Bank and exports vaccines to Asian countries (South Korea and Vietnam).

BB's achievements and contributions to the control of FMD:

1952: First license for FMD Vaccine in Argentina.
1989: First production of FMD Vaccine in the Garín manufacturing site, Buenos Aires, Argentina
1997: Supply for Taiwan emergency
2001-2005: Licensing in Latin America
2006: Selected as a supplier of the NAFMDVB
2010: License in Canada and Brazil
2011: Permit for Sale and Distribution of FMD vaccine Bioaftogen in USA
2013: Start Up of the JV in China – Yangling JINHAI Biotechnology
2016: Supply for South Korea emergency and License of Aftogen Oleo
2017: License of Aftogen Oleo in Vietnam
2018: Supplier for Emergency in Vietnam and licensing of Bioaftogen in South Korea
2019: Vaccine Bank for Taiwan. Bioaftogen obtained the license for regular use in South Korea.

Materials and Methods: The attributes of vaccines produced by BB will be presented. The high potency vaccines were produced in accordance with good manufacturing practice as single water-in-oil emulsion using purified antigen. The quality of the adjuvants was assured by strict quality controls. Final product testing was

carried out on every batch before release for sterility, purity, safety and potency following the OIE Manual guidelines (7) and established national legislation. In addition, antigen content in every batch was monitored using HPLC as previously described (5). The early induction of protective immunity is an essential property of an emergency vaccine to prevent spread of the disease to susceptible animals. Short-term efficacy was evaluated in cattle by direct challenge and by in- contact challenge at 7 days post vaccination.

Regarding vaccine purity, the purification systems used for BB during manufacturing process adequately removes non-structural proteins (NSP) and this has been demonstrated in repeated vaccination experiments (up to three double doses of vaccine applied in a period of six months) and by using several immunodetection systems internationally recognized (6).

Vaccine effectiveness is related also to its capacity to confer cross protection. In this respect, heterologous immunity was studied against a wide range of FMDV isolates belonging to topotypes/lineages currently circulating in Asia (1-2).

Results: The assessment of antigen content in every batch by modern HPLC is a relevant indicator of consistency of production and vaccine potency. The vaccines showed a satisfactory safety profile in vaccinated animals. Regarding vaccine potency in pigs and cattle, antibody titres above protection levels after vaccination revealed compliance with potency requirements of every batch (3, 4). The induction of short-term immunity is an essential feature, particularly for emergency vaccines. Early protection was shown in cattle after virus challenge at 7 days post vaccination.

The use of DIVA vaccines is a central requirement to detect precisely infected animals under vaccination programs. None of the FMD vaccine manufactured with high antigenic payload and after repeated vaccination has shown induction of NSP antibodies post vaccination (6). Regarding heterologous protection, satisfactory vaccine matching results were achieved against relevant field strains belonging to the topotypes/ lineages circulating in Asia. Post vaccination responses in cattle and pigs against a wide spectrum of isolates revealed high neutralizing titres that increased significantly after revaccination.

Conclusions: Vaccination programs properly implemented requires good quality vaccines. BB vaccines showed high potency and proved safety, purity (NSP removal), long-lasting duration of immunity, broad antigenic reactivity and antigenic stability (24 months shelf life). Manufacturing process is sustained on precise methods of quality control that assure the quality of intermediate and final products and production consistency. Furthermore, BB has huge experience in providing emergency vaccines in contingency plans. By 2000-2001 outbreak in Argentina more than 120 million doses of emergency vaccines were provided by BB during the first 6 months after the first case. The vaccine produced by BB is being widely used since more than 3 decades in cattle in South America as part of FMD national control campaigns. Also, Taiwan, South Korea and Vietnam applied BB vaccine in their vaccination programs. Regarding effectiveness of vaccine in generate immunity against emergent field strains, high potency BB vaccines conferred heterologous immunity against a wide spectrum of circulating viruses.

The value of assessing the capacity of vaccine being applied in the field in protecting against the circulating strains is emphasized.

The application of multivalent vaccines of high assured quality among other sanitary measures is a key factor for the success of a FMD control and eradication program. Finally, the support of SENASA OIE FMD Reference laboratory and research entities plays a key role in the innovation and improvement of vaccine quality.

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Advanced Biosecurity: Pathogens and Chemistry

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Introduction: Global swine production now faces several disease challenges including huge economic loss through high-morbidity, high-mortality and medication costs, but also meat sales and market price. Disease prevention and control strategies often include biosecurity as a major point of emphasis for infectious disease prevention. Despite a long history of chemical disinfectant use in animal farming, good level of disinfectant knowledge is limited among its users; the understanding of key issues around disinfectant efficacy is critical to successful biosecurity programs.

Pathogen characteristics: Microorganisms differ in susceptibility to disinfectants: generally bacteria are more sensitive than fungi or viruses, while bacterial spores are the most resistant. Bacteria resistance to several chemistries, however, are increasingly reported. For viruses, based on chemical resistance - authors conclude that the presence of lipids on enveloped viruses and virus size are associated with high susceptibility to disinfectants, while non-enveloped virus are more resistant [1]. Pathogen numbers also influence contact time – the amount of time disinfectants require to inactivate pathogens. The larger the number of microbes, the longer a biocide needs to inactivate them. This reinforces the importance of pre-cleaning before disinfection.

Disinfectant selection: Disinfectant selection in swine farming concerns numerous choices as there is no 'broad-spectrum' disinfectant that inactivates all pathogens in the same dilution and contact time. This is especially true in farm conditions whereby environmental parameters have a great impact upon the activity of chemical solutions; *Temperature*: One of the most important factor affecting disinfection efficacy, studies show that higher temperatures greatly enhance all disinfectant activity, while those below 20℃ deem some ineffective [2]; However some exceptions exist. High temperatures also reduce disinfectant drying time on surfaces, leading to shorter/limited contact times on non-porous surfaces (e.g vehicles and plastic feeding pans).

pH: Common disinfectants such as glutaraldehyde have better efficacy with pH above 7, while QACs have the greatest efficacy at pH of 9-10. pH can also influence the activity of phenolics, hypochlorite and iodine compounds [3].

Water quality: Groundwater, as major water source in farming, tends to be harder than surface water. Studies suggest that water hardness - high levels of calcium and magnesium ions - can inactivate or reduce the effectiveness of certain disinfectants [3].

Organic challenge: Organic matter such as soil, feces, blood, and biofilm can adversely affect biocide activity. Removal of organic materials by cleaning is, therefore, essential before disinfection [4].

Conclusions: Disinfection is an essential part of biosecurity and hygiene in swine farming. The process of careful disinfectant selection should be made with manufacturers, and includes possible factors affecting disinfectant efficacy before making a decision as different application or farm conditions may require different chemistries. Effective disinfection alongside appropriate biosecurity procedures is key to achieving favorable disease prevention outcomes.

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Eradication and control against Foot-and-mouth disease, Classical Swine Fever and African Swine Fever in Japan

- To use antiviral agents in the control against animal transboundary infectious diseases such as FMD, CSF and ASF -

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Introduction: Foot-and-mouth disease (FMD) outbreaks in pigs brought huge economic losses in the FMD free countries without vaccination. In such countries FMD emergency vaccines are usually prepared or concentrated inactivated FMDV antigens are storage in under certain vaccine banks. FMDV-infected pigs usually excrete the viruses within 2 to 4 days after the infection. On the other hand, vaccination will take about 7 days that the vaccinated animals can induce effective antibodies to protect from the FMDV infection. It is considered to be difficult to reduce the expansion of FMD outbreaks in pigs by vaccination in the free countries. To control African Swine Fever (ASF) many scientists have tried to develop the vaccines, however, no effective vaccine against ASF has ever been produced. Regarding to Classical Swine Fever (CSF), if the CSF virus introduces to large scale farm, it will take a long time to slaughter several ten thousand of pigs. They will become another source of infections. For control and eradication against those diseases, new prompt effective tools have to be developed and used in emergency. Antiviral agents can be one of expected tools.

Materials and Methods: (FMD) In vivo efficacy test of an antiviral agent, pyrazinecarboxamide derivatives was performed. 10^6TCID$_{50}$ FMDV O/JPN/2000 were inoculated to one of foot pad of each 6 pigs (4 for treated group and 2 for untreated group: control). 200mg/kg of the antiviral agents were orally administrated for seven days twice a day with food. Initial administration was conducted one hour before the virus challenge. (ASF) In vitro efficacy test of antiviral activity of Acyclovir against ASFV was demonstrated. Antiviral agent, Acyclovir was diluted in a series of 4-time concentration from 1.6mg/ml and added to cultured cells after virus absorption. The virus titers were examined day by day up to 4 days after virus inoculation.

Results: No clinical signs of FMD, no virus excretion in the treated pigs.

Table 1.

Clinical Signs after the virus inoculation

Treatment	No.	Days post inoculation 0	1	2	3	4	5	6	7	8
T-1105	1	−	−	−	−	−	−	−	−	−
	2	−	−	−	−	−	−	−	−	−
	3	−	−	−	−	−	−	−	−	−
	4	−	−	−	−	−	−	−	−	−
Control	5	−	−	+	++	++	++	++	++	++
	6	−	−	−	+	+	++	++	++	++

+Creation of vesicular legion
++ Rupture of vesicle
Lameness and difficulty in standing

Acyclovir inhibits propagation of ASFV at the concentration of 0.4mg/ml or 1.6mg/ml. At the 4 days after ASFV inoculation the virus titers reduce to 1000 times comparing with that of control.

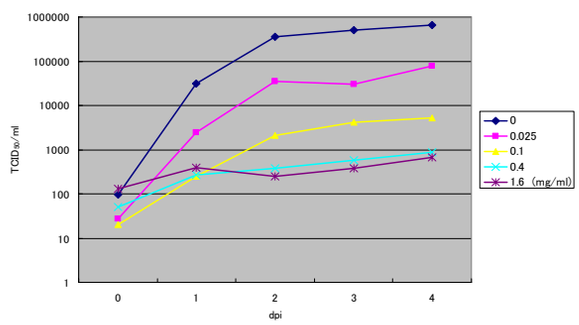

Fig.1

Conclusions: The above animal experiment suggested that antiviral agent, one of pyrazinecarboxamide derivatives can control FMD in pigs and used as a tool to reduce the numbers of FMD outbreaks in many developed countries which belong mostly to FMD free countries without vaccination. Regarding to ASF, the efficacy of antiviral agent of Acyclovir was shown in vitro. Till now no effective vaccine has not been developed against ASF. It is expected to demonstrate efficacy of the antiviral in vivo. Antiviral agent against CSF can be found in the pyrazinecarboxamide derivatives, because the compounds are inhibitor of RNA polymerase and CSFV encodes RNA polymerase gene.

How to manage disease in highly prolific sows

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What is highly prolific sow? Obviously, the definition of a highly prolific sow depends on the type of swine breed we talk about. For an Iberian sow a large litter would be to achieve 10 born alive, while for a modern sow to be highly prolific means something else. There are different genetic lines on the market with differences in prolificacy, but we could define a highly prolific sow, as a sow which its litter is larger than the rearing capacity of its mother. Just because a sow delivers at farrowing more piglets than teats she has, a cascade of events will follow and some of them can have an impact on disease. Large litters put farmers; production experts, veterinarians and nutritionist under a huge pressure to try to wean as much pigs as possible. Often this pressure forces us to forget some basic and important rules when dealing with health. Achieving large numbers of pigs at weaning is a key element in swine production but unless most of them reach market weight, all our effort will be worthless. Let's review what are the key elements when dealing with health:

1. High colostrum intake.

Colostrum provides piglets with passive immunity for protection against pathogens, with the energy necessary for thermoregulation and body growth, and with growth factors that stimulate intestinal growth and maturation (1). Large litters are associated with lower birth weights (2) and piglets with low birth weight have been associated with lower colostrum intake (3). Pigs with low birth-weights, which has low colostrum intake have higher probability to die either pre-weaning or post-weaning and also higher probability of reaching lower weights at the end of finishing (4). Any measure applied directed to assist farrowing will reduce hypoxic pigs during farrowing and will help low birth weights to drink enough colostrum having a positive impact on health (5). Therefore, providing good temperature to the piglets, especially to those with lower birth weight will have an indirect impact on colostrum intake as piglets are stronger to reach the teat and suck (6). On the other hand, any measure directed to increase sow's colostrum yield will be also helpful as individual colostrum intake reduces as larger the litter. Increasing sow's feed intake last days of gestation (from day 108) can increase colostrum yield (7). Also, changing the source of fat in gestation diet can influence the quality of the colostrum (8).

2. Hygiene.

It is common to consider farm's hygiene protocols as correct, without any type of audit. Too often rooms are washed partially, or not let them dry before animals are moved in again. A good washing procedure has to manage to eliminate organic matter, not just from floors, but also from feeders and drinkers. Some studies comparing the efficacy of cleaning and disinfection protocol in different farms found that, too often, drinkers and feeders are not properly cleaned (9). A good all in all out procedure has to include the complete empty of the room and a good cleaning and disinfection. Drying of the room has to be considered a key element of the cleaning and disinfecting procedure to eliminate not just bacteria, present on room but also common viruses in our farms like PRRS(10). Minimizing exposure of suckling piglets to pathogens would be an integral part of controlling pre-weaning mortality, with the keystone being AIAO (11). Moving foster sows from other farrowing room it has to be consider a violation of the all in all out system as contamination coming form another farrowing room will also be moved in, not allowing a real separation or break between batches. Washing sows before farrowing were reported to lower pre-weaning mortality and lower mastitis incidence (12). Minimizing transmission of pathogens between batches requires to apply some basic hygiene rules between them: cleaning the piglet processing trolley among batches, clean and disinfect tools between batches, washing hands and changing boots or shoes between batches (13). Avoiding lesions by not teeth clipping or in case of tail docking cauterising the wound will help to reduce infection in piglets (14). Used needles can potentially spread pathogen form pig to pig (15), changing needles no just between litters but between piglets will help on disease prevention.

3. Batch management.

As we have seen before, large litters have been associated with lower birth-weights and low birth-weights have been associated with lower weights at 42 days of age (16) and

early finishing (17). Days to slaughter are determined by initial weights (18). In practice, to optimize space utilization, it is a common practice to move back slow growers mixing them with younger animals (different batch) in order to give them more time to reach market weights. These movements of pigs are usually done as early as before weaning, or as late as, at the end of finishing. These movements are breaking the integrity of the batch, not respecting the all in-all out practices which have been recognize as one of the most effective tools to control health and to improve performance of pigs during the grow-finishing period (19). Batching systems allow farmers to maintain batch integrity and this have been recognized as being one effective tool when managing disease (20). Working with large litters will put on risk batch integrity when weaning numbers are prioritized, therefore working with batching systems which create a longer interval between batches could have a negative impact on production, but farmers perceive it help to keep a good hygiene on their farms by maintaining batch integrity (21). For some pathogens such as *L. intracellularis*, *M. hyopneumoniae and A. pleuropneumoniae*, an improvement in health status was observed after the change in management system. Moreover, the five-week batch management system showed more consistent improvement over time as compared to the four-week batch management system (22).

4. Keeping litter integrity.

Highly prolific sows are characterized by the fact that they produce more piglets than their actual rearing capacity. Farmers have applied the technique known as cross-fostering to overcome this problem. Cross-fostering is not a technique associated just with large litters, but with them its frequency of use has increase. Foster mothers easily exceed 10% of those present in a farrowing batch, representing at least a double number of piglets transferred as a two-step fostering is the most common one applied. When more than 20% of the piglets are moved around, litter integrity is lost in a majority of them. For certain pathogens, sow's carrying status it is not the same, influencing the health status of their litter at weaning (23,24). The percentage of pigs colonize at weaning can determine the clinical expression for some diseases, as it is the case for *M.hyopneumoniae* (25). For other pathogens mixing pigs will favour their transmission (26). Cross-fostering pigs can influence the immunity status of piglets and therefore the expression of disease, when it is done very soon after farrowing (27,28). Limiting the amount of cross-fostering performed at farms to only moving piglets within the first 24 hours after farrowing and moving the minimum amount needed to fill available teat spaces has been reported to decrease mortality during PRRS outbreaks (29). Little research has been done over the effect of cross fostering on other diseases and their effect in later stages, but a study done at Wageningen University shows that disease spread can be reduced on farm by avoiding mixing from birth to slaughter. Respiratory diseases and treatment costs can be reduced with improvements in pig health and performance (30). With the continuing trend of larger litter sizes, it seems difficult to avoid cross fostering to happen. However, systems such as rescue cups and improved milk replacers, that can supplement sow milk and rearing potential, can be used to maintain litter integrity (31).

Genetic improvement is a challenge for farmers, nutritionists, production advisers and veterinarians. When managing health, it is important to remember that for long time very basic health rules were abandoned as antibiotics could cover the effects of not following them. In such scenario, production was prioritized giving us a wrong impression of what has to be considered a good management. In current conditions, with sows producing larger litters than ever and under the pressure to reduce antibiotic usage, it become essential to start by having the correct sanitation bases. The four points detailed above include the basic rules for managing disease. Obviously, to overcome some of the inconveniences of binging them to practice new technologies will have to be introduced in swine farming and some common practices will have to be changed. We, as swine advisers will have to play an important role helping farmers to understand and implement those changes.

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The efficacy and impact of classical swine fever vaccination

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Classical swine fever (CSF, formerly known as hog cholera) is one of the most devastating and transboundary viral diseases of swine worldwide. CSF is caused by Classical swine fever virus (CSFV), an enveloped single-stranded RNA virus of positive polarity in the family *Flaviviridae*, genus *Pestivirus*. Outbreaks of CSF can immediately lead to huge economic losses in pig industry and encounter enormous tasks for disease control and eradication in epidemic regions. Therefore, World Organization for Animal Health (OIE) is designated CSF on the list of notifiable diseases. At the present time, many countries have successfully eradicated CSF via massive surveillance with intensified long term monitoring and application the most restrict stamping out policy [1]. However, CSF is still sporadic outbreak and/or exhibits endemic in wild boars and domestic pigs in many parts of world according to OIE declaration (www.oie.int). Although non-vaccination and stamping out policy were implemented in most CSF-free status countries, emergency immunization campaigns and prophylactic rim vaccination would be allowed during epidemic outbreaks to prevent disease spreading and decrease substantial costs and social derogations [2,3]. In 2015, Japan was officially announced by OIE and added to the list of CSF-free status countries after conducting a very successful 10 years eradication program; however, several sporadic reemerging incursions transmitted from wild boar in areas previously free of CSF have been reported to OIE in early of this year. This coincidence confirms CSF indeed is a highly contagious and fastidious infectious disease which long term resided in farms and fields.

Due to CSF is a hemorrhagic, multi-systemic and lymphoid damaged viral disease, infected pigs can manifest as acute, subacute, chronic, late onset, or remain subclinical and develop a persistent status based on varied inflammatory progressions and pathological patterns after infection [4,5]. Clinical signs of CSF are also highly variable and strongly determined by the virulence of the strains, age or immune status of infected pigs. It is demonstrated that virulent and moderately virulent strains of CSFV usually express the acute or subacute type of disease, whereas low virulent strains or immunodeficiency due to inadequate vaccination may induce a relatively high proportion of chronic infections. In fact, the subclinical or persistent infection may lead to inapparent or atypical clinical signs that increase difficulties for disease diagnosis and control in endemic regions.

To control the disease in CSF endemic countries, immunization with live attenuated CSFV vaccines produced in cell culture or rabbits developed in the 1960s were widely applied for decades in Asia. These commercially available live attenuated CSFV vaccines can provoke both humoral and cellular immune responses and elicit good protective immunity in vaccinated pigs. The induction of neutralizing antibody (NA) can be detected as early as 5 days post vaccination and the average seroconversion of NA titer greater than 1:32 is considered as a protective standard for individual and an adequate indicator for herd immunity to prevent outbreaks in epidemic areas. Although live attenuated CSFV vaccines are highly efficacious, several major drawbacks including the interference of maternal derived antibody (MDA), variant CSF vaccination protocols in piglets, adverse effects on pregnant sows and feeble piglet, and complications caused by other virus and/or bacterial pathogens, greatly affect the effectiveness of CSF vaccines in pig farms. In addition, another disadvantage of live attenuated live CSFV vaccine is lack characterization for differentiation between infected and vaccinated animals (DIVA) [4,6].

Because there is only one serotype of CSFV and the viral glycoprotein E2, one major envelope protein of *Pestivirus*, has proven to be the most potent immunogen, a strategy employed glycoproteins E2 as antigen and E^{rns} as DIVA marker has been utilized to overcome the major constraint of live attenuated CSF vaccine. Several different types of marker vaccines based on recombinant glycoprotein E2 expressed by baculoviruses in insect cell lines, larva, and silkworms, respectively; or expressed in yeast (*Pichia pastoris*) expression system were successfully developed recently. Many studies illustrated recombinant E2 subunit vaccines in baculoviruses and in yeast expression system showed a good protective immune response against lethal dose of CSFV challenge and prevented horizontal

transmission with cohabitation of unimmunized sentinels after artificial challenge infection [4,7,8]. Although the safety and efficacy of recombinant E2 subunit vaccines was confirmed, some limitations were shown with respect to its expression stability and production cost in comparison with live attenuated vaccines [1,9].

Moreover, several studies have been conducted using transgenic plants including rice, corn, wheat, tomato, or potato to express animal vaccine antigens for the route of oral immunization. The expression of the recombinant E2 protein in transgenic rice has been conducted and the provoked immune responses after oral administration in mice and pigs were confirmed [10]. In addition, a new generation of the recombinant live attenuated marker vaccines has been investigated. This live attenuated marker vaccine is based on chimera pestivirus (CP7_E2alf) that carries the main immunogen of CSFV glycoprotein E2 in a bovine viral diarrhea virus (BVDV) backbone. According to the animal trial results, the chimera live marker virus is a potent live CSF vaccine strain to be applied for both IM and oral routes for young pigs, and showed the limited effect on efficacy by MDA interference [11,12]. However, the humoral immune response induced by these vaccinated animals cannot fully distinguished as DIVA due to the possible serological cross reactions [1]. Nevertheless, it is believed that the limit of efficacy or DIVA may be not a major hampering factor for developing live chimera CSFV vaccines but issues on regulations of genetically modified organisms (GMOs) and vaccine registration processes among different countries before application in fields.

Recently, several studies demonstrated that porcine reproductive and respiratory syndrome virus (PRRSV) infection is one major risk factor to compromise the efficacy of live attenuated CSFV vaccine in pigs. It is also suggested that the PRRSV vaccine may significantly reduce the immune responses of the CSFV vaccination when immunized both vaccines at the same time or within a short interval [13]. Moreover, recent findings illustrated that porcine circovirus type 2 (PCV2) infection exhibits immunocompromised properties in swine and suggested PCV2 infection prior or after CSFV vaccination may interfere the vaccine efficacy [14]. In addition, sequential or concurrent infection of PRRSV and PCV2 would dramatically affect the efficacy of CSFV vaccines [5,15]. However, most studies on concurrent infections associated with the impact of live attenuated CSFV vaccine were conducted and more focusing on PRRSV in SPF animal models, but few studies emphasized in field farms applications.

Although PCV2 vaccines have been widely utilized in field for years and show good efficacy to minimize the clinical symptoms of porcine circovirus associated diseases (PCVADs) in vaccinated pigs, the provoked immunity cannot completely prevent pigs from PCV2 incursion in vaccinated population. These leaky statuses of PCV2 subclinical infected pigs (PCV2-SI) usually showed asymptomatic and were recognized as healthy pigs in farms. Since PCV2-SI is highly endemic in fields, the risks associated with the combination of MDA interference and the potential impact of PCVADs on live attenuated CSF vaccine require intensive evaluation in conventional pig farms where PCV2-SI or PCV2-systemic disease (PCV2-SD) is present. Evaluation of the vaccine efficacy involves several criteria including the dynamic serum antibody titers, viremia level, clinical scores and pathology changes after artificial challenge in animal trials, but variations of individual pigs and several unpredictable factors in pig farms still need to be considered. In our studies, animal trials were carried out using an artificial PCV2 challenge model to mimic PCV2-SD or PCV2-SI while monitoring MDA interference to elucidate whether coexisting factors simultaneously impair live attenuated CSFV vaccine efficacy in pig farms. The trial results revealed PCV2-SI could affect the elicited neutralizing antibody response of live attenuated CSFV vaccine in conventional pig farms. Moreover, results of long-term monitoring of CSFV NA titers in PCV2-SI pigs with minimized interference by MDA suggested that PCV2 infection could compromise the efficacy of live attenuated CSF vaccine in immunized pigs.

In summary, a conventional pig model was established to demonstrate the leaky efficacy of the subunit PCV2 vaccine and its impact on the CSF vaccine in vaccination-challenge trials. Additionally, the impaired efficacy of the PCV2 vaccine resulted in increased PCV2-SI, eventually leading to compromised the live attenuated CSF vaccine induced NA response in field farm applications.

Acknowledgements

We thank Research Center for Animal Medicine, National Chung Hsing University, Taichung, Taiwan for helping with challenge studies and assistances in field trials. We also thank OIE Reference Laboratory for Classical Swine Fever, (Tamsui, Taiwan), Animal Health Research Institute, Council of Agriculture, R.O.C, for providing kind assistance on detecting and comparison of CSFV neutralizing antibodies analysis.

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Understanding African swine fever and major challenges to control the disease

Klaus Depner, Klaas Dietze, Anja Globig, Laura Zani, Thomas C. Mettenleiter

Twelve years ago, African swine fever (ASF) was a so-called exotic animal disease with minor impact on global pig production. Nowadays the picture has changed drastically: ASF is considered as one of the most important threats to the pig-farming sector and no drugs or vaccines are available to cure or prevent the disease.

The ongoing epidemic started in Georgia in 2007 and spread subsequently throughout the Caucasus and the Russian Federation. In 2014, ASF reached the European Union and four years later the first outbreaks were reported in Asia.

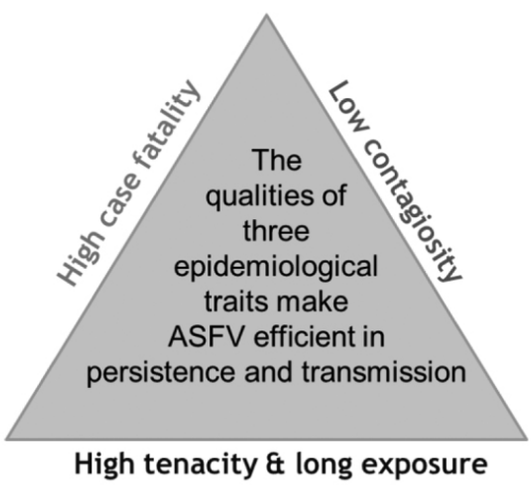

Fig. 1: The persistency triangle Chenais et al.2019

The ASFV strain in the current epidemic belongs to genotype II and is highly virulent. Consequently, in Europe, the disease was expected to either spread rapidly within the wild boar population or fade out due to high case fatality rate and the absence of long term carriers[1]. The current situation, where the disease has become endemic in several countries, shows that none of these predictions held true. In wild boar populations, ASF shows a pattern of habitat bound persistence lacking a tendency of dynamic spatial spread[2]. The infection survived locally in the wild boar population independently from outbreaks in domestic pigs, with steady and low prevalence below 5% and local transmission speed of 2-5 km/month[3]. In addition to local transmission within the wild boar population, long distance jumps into disease-free areas occur. The latter are connected to human activities that also have been identified as main drivers of disease transmission in the domestic pig epidemiological cycle of ASF.[4]

In textbooks, high contagiosity and high mortality are often attributed to the disease. However, field data as well as findings in experimental studies indicate a rather low contagiosity (Fig. 2). The case fatality rate (proportion of infected individuals that succumb to the disease within a certain time period) related to highly virulent ASFV in affected populations of domestic pigs and wild boar is indeed high, often reaching 90-100%. However, the initial mortality within an epidemiological unit is rather low regardless of the generally high case fatality rate. In domestic swine populations this pattern can be considered an advantageous feature reducing the urgency in the implementation of control measures. At the same time, it is complicating early disease detection as the initially low mortality rates can easily get unnoticed in larger farms. For wild boar however, in combination with the environmental stability of the virus and high population densities, the low contagiosity represents a major challenge for effective disease control.

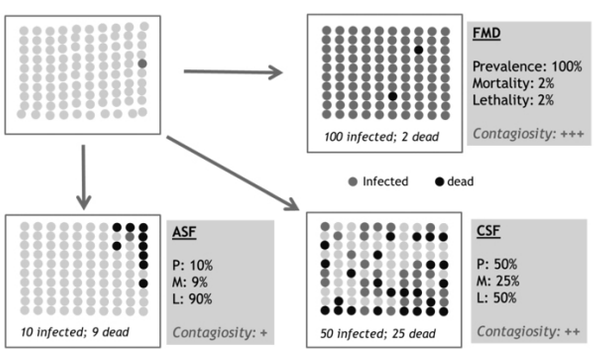

Fig.2: Exemplary disease spread of three major pig diseases highlighting the differences between Foot-and-moth disease (FMD), Classical swine fever (CSF) and African swine fever (ASF)

The qualities of the three epidemiological traits contagiousity, tenacity, and case fatality rate make ASFV efficient in both persistence and transmission4. The combination of high tenacity and a high case fatality rate makes the virus largely available and ensures long-term persistence in the environment; meanwhile the relatively low contagiosity prevents complete depletion of the host population. The interaction of these three parameters

maximize both local persistence and geographical spread of the virus making its eradication a challenge (Fig. 1).

Humans are recognized as the main cause of both long distance transmission and virus introduction into domestic pig farms. Thus, it has become crucial to include social science when planning prevention-, control-, or eradication-measures. By considering only the biological particularities of the disease, contagiousity, tenacity and case fatality rate, but ignoring the human aspects, the epidemic will not be controlled.

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ASFV genes interfering host pathways: Actors for ASF vaccine development

Yolanda Revilla

African swine fever, a devastating disease for domestic pigs and wild boar, is currently spreading in Europe, Russia, China and other regions of Asia, becoming a global threat with huge economic and ecological consequences.

African swine fever virus (ASFV) is a complex, cytoplasmic double stranded DNA (dsDNA) virus that is currently expanding throughout the world. Currently, circulating virulent genotype II Armenia/07-like viruses cause fatal disease in pigs and wild boar, whereas attenuated strains induce infections with various levels of chronic illness. One interesting aspect of ASFV biology is the molecular mechanism leading to high virulence of some strains compared to more attenuated strains, which produce subclinical infections.

Sensing cytosolic dsDNA, mainly by the key DNA sensor cyclic GMP-AMP synthase (cGAS), leads to the synthesis of type I interferon and involves signaling through STING, TBK1, and IRF3.

We demonstrate here that attenuated NH/P68, but not virulent Armenia/07, activates the c GAS/STING- IRF3 cascade very early during infection, inducing STING phosphorylation and trafficking through a mechanism involving cGAMP. Both TBK1 and IRF3 are subsequently activated and, in response to this, a high level of beta interferon (IFN-β) was produced during NH/P68 infection; in contrast, Armenia/07 infection generated IFN-β levels below those of uninfected cells. Our results show that virulent Armenia/07 ASFV controls the cGAS-STING pathway, but these mechanisms are not at play when porcine macrophages are infected with attenuated NH/P68 ASFV. These findings show for the first time the involvement of the cGAS-STINGIRF3 route in ASFV infection, where IFN-β production or inhibition was found after infection by attenuated or virulent ASFV strains, respectively, thus reinforcing the idea that ASFV virulence versus attenuation may be a phenomenon grounded in ASFV-mediated innate immune modulation where the cGAS-STING pathway might play an important role.

Our results show the relationship between the cGAS-STING pathway and ASFV virulence, contributing to uncover the molecular mechanisms of ASFV virulence and to the rational development of ASFV vaccines.

KEYWORDS ASFV, Armenia/07, IFN-β , NH/P68, STING, cGAMP, cGAS, virulence, attenuation

Latest African swine fever status in Asia

Caitlin HOLLEY

Since the first introduction of African swine fever into the Asian region in China in 2018, the virus has continued to spread and cause devastating losses, with a huge impact to the swine population and industry where-ever the disease has occurred.

Asia accounts for over 50% of the world's domestic pig production, largely concentrated in east and south east Asia, where pork is one of the major animal proteins consumed. This region is historically the largest consumer of pork and pig products in the world, seeing importation from other regions to meet the demand.

While African swine fever virus (ASFv) does not affect humans, the most virulent strains are recorded to have 95-97% mortality rate for infected pigs and there is no vaccine or cure. The virus is highly resistant and can survive in the environment and remain infective in meat or cured products for weeks or months, so can be transported over long distances by human activities.

The pig value chains in Asia are extremely complex and interconnected between countries. The range of pig production systems in Asia also varies greatly from free ranging scavenging pigs, small holder systems through to large scale commercial production with vertically integrated companies operating in several countries.

The complexity of pig production and the historical high demand for pork in certain parts of Asia mean when a disease such as African swine fever is introduced to the region, control and prevention is not straightforward.

Early detection, followed by swift action with effective quarantine and biosecurity are necessary in order to contain and control an outbreak, however this requires awareness, understanding and commitment from all sectors involved with swine production to be effective.

Considering the global impact of the disease, currently present in Europe, Asia and Africa, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) have called for a global coordinated control effort from national veterinary services and other public and private institutions, as well as a range of different stakeholders such as pig production industry, universities, research institutes, forestry management bodies, hunter's associations, tourism sector, animal transport organizations, NGOs and other international organisations.

Integration of participatory approaches and stakeholder engagement, participation and ownership in the ASF response are as essential for the development of a regional and global agenda for ASF control as they are for making technical recommendations for use by national Veterinary Services. A strong veterinary service with well-structured and properly implementedcommunication campaigns, targeted for establishing behavioural change and intersectoral collaboration should be an essential component of any ASF control programme and will be needed for long term plans to control ASF.

In Asia, the FAO and OIE are working in partnership with experts and members to ensure current
disease information is shared, and to build expertise and understanding of how the disease is
spreading, what the regional risk factors are and to develop a regional strategy for the short,
medium and long term to control and limit the impact of ASF in Asia.

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Oral Abstracts

- August 26
  - FMD & Transboundary Diseases
  - Biosecurity & Production

- August 27
  - ASF & Viral Diseases I
  - Major Bacterial Diseases (Respiratory & Enteric)
  - Viral Diseases II (PRRS)
  - Major Bacterial Diseases (Enteric) & Swine Enteric Virus

Surveillance of Senecavirus A infection in swine in Thailand, 2016 - 2018

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Introduction: Senecavirus A (SVA) is a virus associated with idiopathic vesicular disease (IVD). The disease is caused economic losses in swine industry with undifferentiated from others vesicular diseases such as foot-and-mouth disease (FMD), swine vesicular disease (SVD), vesicular exanthema (VE) and vesicular stomatitis (VS). SVA was first reported in Thailand in 2016 [1]. In three recent years, the clinical diseases such as lameness and vesicular lesion forming in coronary band and hooves have been increased in pigs in Thailand. In this study, SVA was detected from swine with IVD in high density population of swine farms in Thailand during 2016-2018.

Materials and Methods: The total 236 vesicular fluid, coronary band, or hooves tissue samples were collected from swine farms that affected a disease outbreak associated with IVD in Thailand during 2016-2018 (2016: 30 samples: 6 farms, 2017: 114 samples: 23 farms,and 2018: 92 samples: 15 farms). The viral RNA was extracted from the samples using the Nucleospin® viral RNA isolation kit (Macherey-Nagel Inc., Duren, Germany) according to the manufacturer's instructions. Then, the viral RNA was converted to cDNA using the M-MuLV reverse transcriptase (BioLabs Inc., Ipswich, MA, USA). RT-PCR with specific primer pair (SVA- W-4F and SVA-W-4R) was used SVA amplification as previous reported [2].

Results: The results illustrated that 34 (14.41%) of 236 samples including 3 (10%) of 30 samples 17 (14.91%) of 114 samples and 14 (15.22%) of 92 samples in 2016, 2017, and 2018, respectively, were positive with SVA infection. The number and percentages of positive samples in each year were presented in Table 1

Table 1. Surveillance of SVA in swine in Thailand during 2016-2018

Years	Total samples examined	Number (%) Positive SVA infection	Number (%) Negative SVA infection
2016	30	3(10%)	27(90%)
2017	114	17(14.91%)	97(85.09%)
2018	92	14(15.22%)	78(84.78%)
	236	34(14.41%)	202(85.59%)

Conclusions: This surveillance study presents increasing of trend percentages of positive samples with SVA in each year since 2016 to 2018 in high density population of swine farms in Thailand. The increasing of the virus might due to ineffectiveness of the disease prevention and control. In the future, the knowledges about SVA should be extensively study for usefulness prevention and control SVA infection in swine farms in Thailand.

Acknowledgement: This work was supported by Research and Researchers for industry Program (RRI) (grant number MSD61I0071) and partial funding was provided by the Special Task Force for Activating Research (STAR), Swine Viral Evolution and Vaccine Research (SVEVR), Chulalongkorn University.

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Experimental infection of Foot and Mouth Disease Asia 1 Sindh-08 in conventional pigs

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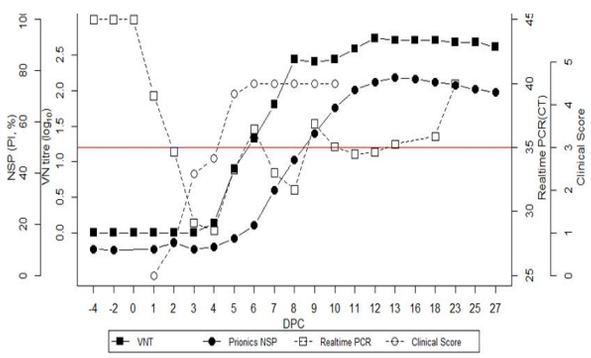
Introduction: Foot and Mouth disease virus (FMDV) is a highly contagious virus that affects cloven-hoofed animal species. FMD virus is divided into 7 serotype (O, A, C, Asia1, SAT1, 2, and 3). It is known that animal immunized with one serotype is unlikely to protect another serotype. Considering previous outbreaks, vaccination against Type O and A is being done in case FMD recur in South Korea. However, it is vulnerable to incursion of FMD Asia 1, even though the possibility of incursion is extremely low. Accordingly. It is important to identify infection dynamics of FMDV in case non-vaccinated serotype outbreak occur. In current study, we investigated the infection dynamic of FMDV Sindi08 in conventional pigs.

Materials and Methods: A total of 12 conventional pigs were used to identify pathogenicity of the FMDV Asia1 Sindh-08 which is kindly given from Pirbrite institute. Two pigs (donor) were sublingually inoculated with the virus ($5.0 \times 10^7 TCID_{50}$) into the tongue. After showing blister in digit, donor pigs were mixed with naïve 10 pigs (contact). Clinical signs (body temp, lameness, blister etc) of contact pigs were checked and pictured on a daily. Samples (sera, oral swabs) were collected for 25 days. To detect antigen, real-time PCR was carried out using the extract from saliva as described. For antibody, virus neutralization test (VN) and NSP ELISA (Priocheck NS) were performed according to recommendation of OIE and manufacturer (Thermo scientific, USA).

Results: Contact-exposed infection dynamic of FMDV Asia1 Sindi08 was shown in Fig.1. FMDV RNA detection in saliva was peak at 3 dpc (days post contact) following contact exposure and steadily detected for 16 days under 35 CT value. Body temperature came to 39-40°C at 2 - 3dpc in most pigs and down to under 39°C at 10 dpc. Clinical signs including vesicle in foot and mouth were firstly detected at 2 dpc in 2 of 10 pigs and all foot-pad of pigs were affected at 6 dpc.

VN increased at 4 dpc and came to more than 1.2 at 6 dpc and plateau in 12 dpc. NSP showed positive 4 out of 10 at 7 dpc and all pigs were seroconverted at 16 dpc. Interestingly, one pig (nos 9) were back to negative at 23 dpc.

Fig.1. Comparison of infection dynamics of contact exposed pigs using FMDAsia1 Sindh08 (n = 10).



Conclusions: Incubation period was supposed to be under 48hr prior to onset of clinical sign following contact exposure. Clinical signs was non-specific within 3 days even though antigen shedding occurred after contact exposure. NSP was seroconverted only 50% at 7 days and one pig were converted to negative.

Acknowledgement: This work was supported by a grant from the Project No. (CAP-16-01-KIST), Korea Institute of Science and Technology (KIST), Ministry of Science and ICT, Republic of Korea.

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Field application of developed 3Diff- Rapid kit for the detection of FMDV O and A in South Korea, from 2018 to 2019.

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Introduction: A total of 11 foot-and-mouth disease (FMD) outbreaks have occurred in Korea so far, Unfortunately, after 2014, FMD has occurred every year. Also, for the first time, two serotypes (O and A) of FMD occurred simultaneously in 2017. In country that carry out vaccination policy as FMD control measure, a rapid and easy tool which can distinguish the serotype of FMDV for the on-site diagnosis of suspected FMD outbreaks is very important. The lateral flow strip kit (3Diff/PAN rapid kit) developed for rapid detection of FMDV serotypes O, A and Asia 1 which mainly found in near countries, South-East Asia (SEA)[1]. The developed 3Diff- Rapid kit were applied to FMDV infected animals during the outbreaks of FMD in 2018 and 2019 in South Korea. The results were compared with results of Ag-ELISA (Pirbright).

Materials and Methods: In this study, the sampling were conducted by local veterinary services as soon as possible after the farm owner reported it. After the immediately sampling at the farms, the sample fluid droped onto the rapid kit as the according to SOP. The serotypes of samples were determined and confirmed by Ag-ELISA and the VP1 coding region sequence.

Results: In March, 2018, according to the case report of suspected FMD, the rapid kit was applied to the nasal epithelium of pigs in Gimpo, Gyeonggi province, and the positive reaction of type A was confirmed within 10 minutes. In addition, as a result of the ELISA and VP1 sequence analysis of the laboratory diagnostic methods, it was confirmed that the FMDVA type and the genotype was ASIA/SEA97. Also, on January, 2019, a local veterinary officials were dispatched to the farm where the suspected case of FMD occurred in cattle in Anseong, Gyeonggi Province. The rapid kit was applied to the tongue-epithelial tissue of cattle which was showing clinical symptoms. Within 5 minutes, the tissue was diagnosed with FMDVO. Antigen-ELISA and VP1 sequencing analysis of the laboratory tests revealed O / ME-SA / Ind-2001e. Also, on January 31, suspected FMD was reported at Hanwoo farm in Chungju, Chungcheong province. As a result, it was diagnosed as FMDVO type by the rapid kit, and finally FMDVO/ME-SA/Ind-2001e was found in laboratory diagnosis(Fig 1).

Fig1. The results are based on application of the epithelial tissue from animals infected with FMDV in 2018 and 2019, South Korea. A(on the left) was determined to be FMDVA using the nose-epithelium of FMDV infected pig, 2018.B(on the right) was determined to be FMDVO using the tongue-epithelium of FMDV infected cattle, 2019.

Conclusions: In order to control FMD outbreaks, an early and accurate identification of the causative FMDV is very important. In countries using a “Vaccination” policy, serotype identification is crucial to allow selection of the most appropriate vaccine. In this study, the developed rapid kit could simultaneously detect not only common of FMDV but also distinguish serotype O and A using the epithelial tissue. The rapid kit will be very useful where Lab diagnosis is not available and for the on-site diagnosis of suspected cases of FMD.

Acknowledgement: This research was supported by the Research of Animal and Plant Quarantine Agency (Project No. M-1543082-2018-19-02), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Evaluating the Classical Swine Fever (CSF) Serum-Neutralization titers in Vietnam from 2015-2018

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Introduction: Classical Swine Fever (CSF) is a devastating viral infectious disease causing severe economic losses in swine production [1]. CSF vaccination in both of breeder and piglet is an effective tool to control this disease. It is important to know the serological status in the farm, to design a good vaccination scheme. The antibodies measured at 5 weeks of age are typically maternal derived antibodies (MDA). Their level (SN < 32, or $5\log_2$) allows the vaccination without significant interference [2]. Piglets are passively protected if their serum has a certain minimum level of MDA ≥ 8 ($3\log_2$) [3]. Active immunization of piglets protection against challenge following vaccination with Thiverval strain with SN ≥ 4 ($2\log_2$) are protected[3]. The objective of this study was to describe the level of SN antibodies in various animal categories in commercial farms in Vietnam.

Materials and Methods: Blood samples were collected from 87 farms with 5419 samples from all parts of Vietnam during 2015-2018. None of the farm had clinical symptoms of CSF at time of sampling. The vaccination schedule applied was: 5 weeks before farrowing sows, before mating in gilts, twice per year in boars, and 6 to 7 weeks of age in piglet. Samples were divided in 5 groups which were Boar, Gilt, Sow, Piglet and Finisher. Serum neutralizing (SN) antibody titer against CSF virus were determined by a qualified local lab in Vietnam. The average SN titer was calculated from $\log_2$ of each group. Results are summarized by group of pigs.

Table 1. Number of serum sample divided from each group of pig

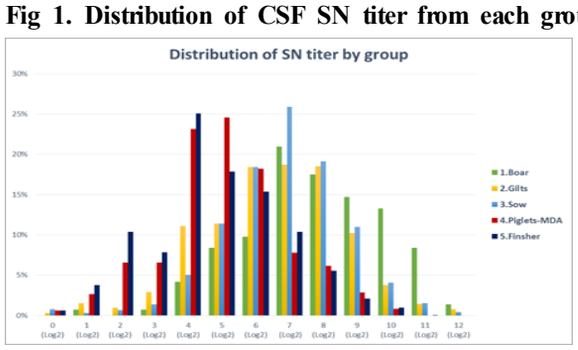
Group	Number	Remark
Boar	143	
Gilt	1037	24-28 week of age
Sow	2403	P1-7, 3 weeks after farrowing
Piglet	488	5-6 weeks of age
Finisher	1348	10-24 weeks of age

Results: Most samples (72-80%) had a titer higher than 32 ($5\log_2$) in the Gilt and Sow groups, and 86%in Boar. In the Finisher group 85% were considered protected (SN ≥ $2\log_2$) and CV 41%. Mean of titer in Piglet group (MDA level at 5-6 weeks of age) was 4.98 $\log_2$ with CV 37%.

Table 2. log2 CSF SN titers by category of pigs

Group	Mean	SD	%CV	< 8	8-32	>32
Boar	7.81	2.05	26%	1%	13%	86%
Gilt	6.58	2.05	31%	3%	25%	72%
Sow	6.89	1.82	26%	2%	18%	80%
Piglet	4.98	1.84	37%	10%	54%	36%
Finisher	4.76	1.97	41%	15%	51%	34%
Total	6.15	2.15	35%	6%	31%	63%

Fig 1. Distribution of CSF SN titer from each groups



Conclusions: The results of this survey show the distribution of CSF SN titer is more uniform in breeder (Boar, Sow and Gilt group); most of the pig in these groups have a titer above the minimum protective level ($2\log_2$ for active immunity). Meanwhile, SN titers in piglets are less uniform; from problem sow or colostrum management. The highest CV is found in finisher group, the reason might be interaction by other diseases or MDA level at vaccination time. The high MDA level in the piglet group (36% with SN>32) might interfere with vaccination. The first vaccination needs to delay after this age (5-6 weeks). To design a good vaccination scheme the CSF serological status monitored in farms in Vietnam, good colostrum management to have high homogeneity of MDA and optimal timing vaccination for piglet are recommended.

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Modified zearalenone as potential cause for hyperestrogenism in suckling piglets-from a field case to current challenges in the diagnosis of mycotoxicosis

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Introduction: The mycotoxin zearalenone (ZEN) interacts with estrogen receptors, thus impairing pig health. Many countries have established maximum levels for ZEN in feed. However, these regulations do not consider the occurrence of modified mycotoxins, which are formed by plants, fungi or mammals through modification of the mycotoxins' structures. For example, fungi and plants are capable to produce zearalenone-14-sulfate (ZEN-14-S), which is effectively hydrolyzed during digestion in pigs [1]. Since a substantial proportion of ZEN is liberated, ZEN-14-S can pose a hidden risk for pig productivity.

Materials and Methods: In a conventional farm in Germany (200 sows), severe reproductive disorders were observed, such as swollen genitals of piglets and higher rates of weak live-born piglets. Within weeks, piglet losses increased to 30% during lactation. Sows showed no signs of infection. As ZEN toxicosis was suspected, feed components were analyzed for ZEN by ELISA. Positive results were only obtained for hay pellets, which served as fibre source in the sows' diets. Notably, hay was the only feed component being exchanged prior to the onset of clinical signs. To confirm ELISA results, hay samples were subjected to multi-mycotoxin analysis by LC-MS/MS.

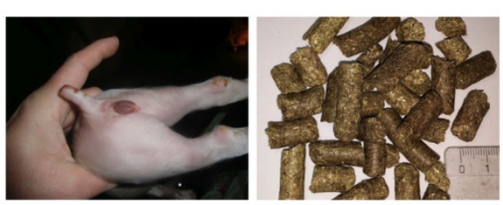

Figure 1. Left: Swollen vulva of 2-days old piglet. Right: contaminated hay pellets. Both pictures were originally published in [2].

Results: Botanic inspection of hay pellets did not reveal the presence of herbs or potential phytoestrogen producers. Via LC-MS/MS, 52 fungal metabolites were detected in the hay sample. While major mycotoxins (e.g. aflatoxin B_1, deoxynivalenol) were absent, ZEN was found at 479 µg/kg. In addition, ZEN-14-S was present at a concentration of 530 µg/kg, thus even exceeding the levels of its parent toxin. Considering the inclusion rate of hay pellets in the diet, estimated levels of ZEN and its metabolites were between 52 and 73µg/kg. After replacement of contaminated hay pallets, no further clinical signs were observed.

Discussion and conclusions: Based on performed analysis, ZEN and ZEN-14-S in hay samples remained as the only plausible cause for the observed clinical signs. Levels of ZEN and its metabolites in the total diets were relatively low, although chronical ingestion might have caused toxin accumulation. Due to the absence of suitable biomarkers for ZEN, we could not confirm our suspected diagnosis at animal level. Currently, neither the measurement of ZEN residues in biological specimens, nor the analysis of hematological or biochemical parameters allow solid prediction of ZEN intake of pigs under field conditions [3]. Thus, the present case shows the importance of appropriate feed analysis, not only to detect modified mycotoxins, but also to consider dietary fiber as potential mycotoxin source.

Acknowledgement: We thank the farmer for cooperation in this field case.

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A survey of Asian respiratory health in finishing pigs at slaughter age

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Introduction: Lung scoring at the slaughterhouse is a valuable tool for assessment of the respiratory health status of a large number of animals at a single visit, at relatively limited cost. Different from post-mortem investigation, it assesses lung health across the whole batch of animals. Moreover, a clear relation between lung lesions present at slaughterhouse and economic impact of respiratory disease has been reported [1], making lung scoring an attractive tool for decision making and effect monitoring of veterinary interventions.

To facilitate efficient and hygienic lung lesion scoring at slaughterhouses, Ceva provides a scoring methodology Ceva Lung program (CLP). A tablet- based software tool allowing for rapid recording of the results and their processing is a part of CLP.

Materials and Methods: In between January 2017 and April 2019, a total of 521 batches of pigs (23,032 animals) were scored at time of slaughter, using CLP. Lung scorings were performed in Cambodia (12), Malaysia (18), Philippines (177), South Korea (32), Taiwan (11), Thailand (82) and Vietnam (189).

Lungs were scored following the CLP method [2], with presence, type and extension of lung lesions described by:

- Enzootic pneumonia (EP)-like lesions following a modified Madec methodology.
- Cranio-ventral pleurisy, to describe EP-associated secondary pleurisy.
- Scarring, describing prevalence of fissures associated with older EP-like lesions.
- Dorsocaudal pleurisy, to describe *Actinobacillus pleuropneumoniae* (APP)-like lesions
- Actinobacillus pleuropneumoniae Index (APPI), using prevalence and grade of dorsocaudal pleurisy (scale 0-4).

Results: Results for the Asian region are presented in Table 1 and 2 using percentiles (P_{25}-median-P_{75}).

Table 1. EP-like lesions

	P_{25}	Median	P_{75}
Prevalence bronchopneumonia	51.8%	72.5%	86.7%
% lung surface with bronchopneumonia	2.5%	5.3%	9.7%
% Cranio-ventral pleurisy	3.3%	12.9%	31.1%
Scars	0.0%	4.0%	16.7%

Table 2. APP-like lesions

	P_{25}	Median	P_{75}
% Dorsocaudal pleurisy	6.4%	14.0%	29.9%
APPI index	0.13	0.36	0.77

Conclusions: Results clearly indicate there is room for improving the respiratory health of finishing pigs in the sampled countries. Both lesions associated with *M.hyopneumoniae* and *A.pleuropneumoniae* have a high prevalence. While the data by country (not shown) suggests some differences between countries exist, these have to be interpreted with caution as farm selection was not randomized. Nevertheless, the distribution reported above could be a useful tool for interpretation of lung lesion scoring results, as well as for setting targets for farms aiming for improvement of their respiratory health.

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Effective mycotoxin detoxification strategies to ensure safer feed, a review

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Mycotoxins are secondary metabolites of various fungi commonly found in feed and foodstuffs, often co-occurring at low levels [1]. Based on their occurrence and observed effects on human and animal health, aflatoxins, fumonisins, deoxynivalenol, ochratoxin A and zearalenone are recognized as the five most important mycotoxins in animal husbandry. The most cost-effective strategy to counteract the effects of these low levels of mycotoxins in the feed industry is to use mycotoxin detoxifying agents. This paper aims at reviewing the different strategies of detoxification available in the market and their efficacy, considering the evaluation method.

We can distinguish two main categories within the wide group of mycotoxin detoxifying agents: adsorbing agents, which the aim is to decrease mycotoxin bioavailability by including them in the compound feed, leading to a reduction of mycotoxin uptake; and biotransforming agents, which the aim is to degrade mycotoxins into less toxic metabolites by using microorganisms or enzymes. Within the adsorbing agents the most commonly used are activated carbons, aluminosilicates (HSCAS), modified aluminosilicates and yeast cell walls. Within the biotransforming agents, there are a wide array of bacteria, fungi and different enzymes, although few of them are used in commercial products.

The efficacy of mycotoxin detoxifying agents is very often assessed in static in vitro models measuring the percentage of adsorption or the degree of detoxification achieved by the agent. However, Vekiru et al.[2] and Versantvoort et al. [3] showed that detoxifying agents generally become less efficient when gastro-intestinal conditions are simulated. Their efficacy may be overestimated in static models making more reliable the use of dynamic gastro intestinal models.

The efficacy of adsorbing agents depends on the intermolecular interactions between the adsorbing agent and the mycotoxin, such as electrostatic, hydrophobic or shape effects and the characteristics of the adsorbing agents, including atom composition, total charge and charge distribution, size of the pores or accessible surface. The efficacy of biotransforming agents depends on the activity and survival of the microorganism in the digestive tract, the specificity of the enzyme and its substrate, the time for the enzymatic reaction to occur, the toxicity of the produced metabolites and their intestinal absorption compared to the parent mycotoxin.

Many parameters influence the efficacy of a mycotoxin detoxifying agent, from their own characteristics to the evaluation method used to study them. In vitro results should be analyzed and interpreted carefully and complementary in vivo trials on performance should be available to conclude on an agent efficacy.

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Coping with drawback period by using 42 Degree® in nursery pigs: its effect on growth performance

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Introduction: In nursery house, one of the most important problem is a drawback period (setback) which occurs during the first 2 weeks of entering the nursery house, which result in growth retardation and respiratory and gastrointestinal diseases [1]. There are many factors that cause a drawback period, for example, stress from weaning, from transportation, from nutritional changes, environmental changes and also social stress from fighting [2]. During nursery period, it has been shown that pig faced with many stressors in the nursery house. In practice, if one can find a product in which can manipulate this drawback period, this will guarantee that there is no negative effect on growth performance during the nursery period. The 42 Degree® is a natural product, compose of willow bark, horse tail, string nettle, lysine, methionine, sodium chloride, magnesium chloride and propandiol which claims to provide quick recovery from elevated body temperature, alleviates pain and mobility problem, keeping animal active, maintain feed intake and therefore prevent body weight losses. Therefore, this study aims to test the product, namely 42 Degree®, forminimizing the effect of drawback (i.e. setback) period on growth performances in nursery pig.

Materials and Methods: Altogether 60 nursery pigs were allocated into 2 different groups as follows: Treatment group 1, the 30 nursery pigs, were fed with 42 Degree® by water dripping at the recommended dose for nursey pig for a period of 6 weeks (age between 4 and 10 weeks old); control group 2: The 30 nursery pigs, were fed with normal feed and normal water dripping for a period of 6 weeks (age between 4 and 10 weeks). They were vaccinated against PCV-2, CSF, FMD at 32, 41 and 61 days old, respectively. Data recording and sample collection: Body weight recording (n=30 in each group): weight in (kg; at 21 days=4wk), weight out (kg; at 71 days=10wk) and body weight at 43 and 56 days old were also recorded (n=20 in each group).

Results: Clinical signs of respiratory infection (i.e. nasal discharge, ocular discharge, abdominal breathing) and of diarrhea in control and treatment groups are absent. The body temperature in control and treatment groups during experiment are varied between 37.38-38.73 °C and 37.82-38.78 °C, respectively. There is a significantly higher ADG in treatment group at day weight at day 43 (381.03 vs 304.13 g; P = 0.013) than in the control group, however at 56 the difference between groups was not found (410.35 vs 397.08 g; P = 0.7) and a tendency of higher ADG (993.33 vs 859.40 g; P = 0.08) at day 71 was found in treatment group than in the control group (Figure 1).

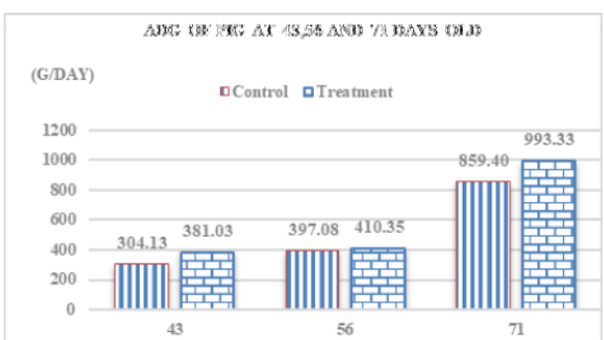

Figure 1 The average daily gain (ADG, gram) in control (n=20) and treatment (n=20) groups at 43, 56 and 71 days old.

Discussion and Conclusion: The above results implied that 42 Degree, containing active ingredient such as salicin, flavonoid and tannin, with its anti-inflammatory and anti-fever action, are able to minimize the negative effect during a drawback period and then promote growth performance in treatment group.

Acknowledgement: This work was supported by a grant from Innovet Corporation Co., Ltd., Samutprakarn, Thailand.

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Fusarium mycotoxin contamination in feed effects performance, organ health and immune status - Review on field trials

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Introduction: In general, pigs are considered highly susceptible to mycotoxins, with young animals and female breeders being the most sensitive groups. Feed quality control and feed safety regulations may lead to the conclusion that mycotoxins are under control and not of concern for pig health, but the interactive and subclinical effects, even at low mycotoxin contaminations, are underestimated. Pigs show a high sensitivity to deoxynivalenol (DON). Growth reduction, impaired immune function and decreased reproductive performance are the most frequently observed effects. Due to its cytotoxicity, DON alone and in combination with other mycotoxins can negatively affect the tight junction proteins. Therefore, DON can play an important role in the leaky gut syndrome, a serious condition, where the gut barrier is impaired. Fumonisins (FUM) can cause immune-modulation and organ specific alterations depending on dose and duration of exposure. FUM disrupt the sphingolipid metabolism by blocking ceramide synthase leading to accumulation of free sphinganine. Consequently, the production of complex sphingolipids, necessary components of nerves, muscles and membranes, is interrupted. Pigs are also very sensitive to zearalenone (ZEN). ZEN contaminated feed induces swelling and reddening of the vulva, false heats and false pregnancy. This abstract provides a summary of four field trials with weaning piglets to show the negative impact of low to moderate mycotoxin contaminations.

Materials and Methods: Trial 1 was performed with 24 female grower pigs and a mycotoxin contamination of 200 ppb ZEN. Performance and reproductive organs were evaluated in a three-week trial period. Blood and immunoglobulin status of piglets receiving 2 ppm DON over a period of 56 days were evaluated in trial 2. Trial 3 included 720 mixed sexed weaning piglets with a DON contamination below 900 ppb for a trial period of 42 days. Performance and impact on ear necrosis and diarrhea incidences were evaluated. In trial 4 the effect of 3.8 ppm DON, 200 ppb ZEN and 2.5 ppm FUM on performance and intestinal histology were evaluated. In all four trials one group was supplied with a mycotoxin counteracting feed additive.

Results: All parameters investigated revealed a significant negative impact of mycotoxins on the animals. Villus length and crypt depth were impaired by mycotoxins, ear necrosis incidences as well as the number of diarrhea days were increased. Besides reduced performance, weight and size of the reproductive organs (uterus, vulva) were significantly increased in the ZEN groups ($p<0.05$). Some blood parameters, such as hematocrit, hemoglobin as well as IgA were negatively influenced ($p<0.05$) by DON contamination of the feed. The negative impacts were reduced by the addition of the feed additive.

Conclusions: Mycotoxin contamination even at low levels is a risk factor in swine production. A proper mycotoxin risk management is crucial to reduce subclinical mycotoxicosis in piglets and a resulting loss in performance.

Efficacy of Acetaminophen (Pracetam®) on immune levels In sow colostrum and lactating unit performances

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Introduction: Colostrum is considered to be an important factor that indicates farrowing unit performance in swine farm. Colostrum mainly contains energy and maternal passive immunity which are necessary for newborn piglets [1]. Inadequate colostrum consumption of piglets causes piglets pre-weaning mortality that may continue to increase the mortality rate in nursery and fattening pigs. Sows have fever during late pregnancy, the period of mammary tissue development with immune cells accumulations, which can have a negative influence on colostrum yield [2]. The objective of this study was to evaluate the effect of Acetaminophen (Pracetam®) administration on immune response and performance of lactating sows.

Materials and Methods: The present study was conducted on commercial swine farm in Thailand. Forty-three sows were randomly selected and divided into two groups, control (n=21) and treatment (n=22). The treatment group, Pracetam® was administrated to sow (30mg/kg BW) in feed for 10 consecutive days (start at five days before farrowing). Rectal temperature were measured for 5 consecutive days (start at 2 days before farrowing). Sow colostrum samples were collected within an hour after the first piglet was born. The colostrum samples were tested for the levels of total immunoglobulin G (IgG) and specific immunoglobulin A (IgA) against porcine epidemic diarrhea virus (PEDV ELISA). Performance in the farrowing unit was recorded. The levels of immune levels and performance parameters were compared between groups using linear regression model of R program version 3.5.2. Body temperature of sows were compared using repeated measure. A value of $p < 0.05$ was considered statistically significant difference.

Results: The results indicated that sows treated with Pracetam® (treatment group) had significantly lower body temperature than the control group from 2 days before farrowing until 3 days post farrowing ($p = 0.009$). Interestingly, the sows treated with Pracetam® had higher levels of total IgG than control sows (48.73±32.17 and 41.55±20.83mg/ml) and the S/P ration of PEDV-specific IgA also higher in the treatment group than the control (1.66±0.25 and 1.56±0.25). More over, the present study demonstrated that performance parameters in farrowing unit of sows treated with Pracetam® were better than those of the control group. However, all of them were not significantly different(Table1).

Table 1. Sow performance parameters (mean ± SD).

	Control	Treatment	p-value
Total born/litter (heads)	14.33±4.25	15.59±4.19	0.40
Born alive/litter (heads)	12.67±3.32	14.50±4.03	0.13
Stillborn (%)	3.89±6.84	3.15±4.71	0.44
Mummies (%)	5.58±9.73	3.67±4.99	0.48
ADLWG (kg/d)	2.50±0.42	2.65±0.45	0.29

Conclusion: The Pracetam® administration in sow feed during late pregnancy and lactating periods can significantly reduce rectal temperature of sows during the farrowing period. Pracetam® supplementation in feed might be expected to increase total IgG and IgA levels in colostrum and could improve the farrowing unit performance. Thus, the effect of Pracetam® on immune response and performance should be further studied for more information.

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Gain more control and confidence in detecting African Swine Fever Virus in difficult sample material

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Introduction: African Swine Fever (ASF) is a threat to animal welfare and one of the most severe diseases affecting wild boars and domestic pigs worldwide. Due to higher risks of the ASF-Virus (ASFV) spreading through contaminated meat, animals or vehicles, accurate detection of the virus is key. The new virotype ASFV 2 Plus PCR Kit from INDICAL BIOSCIENCE (formerly QIAGEN Animal Health) enables rapid and reliable identification of ASFV-DNA. The newly developed test kit comes with a double control strategy that offers a higher level of confidence in the interpretation of qPCR results, especially when dealing with difficult sample material.

Materials and Methods: ASFV-DNA was extracted using the silica column-based IndiSpin Pathogen Kit (formerly: QIAamp cador Pathogen Mini Kit) and the magnetic bead-based IndiMag Pathogen Kit (formerly: MagAttract 96 cador Pathogen Kit) from INDICAL. The extracted nucleic acids were then analyzed using INDICAL's new virotype ASFV 2 Plus PCR Kit. This triplex qPCR assay contains all the reagents necessary to identify ASFV-DNA, including a positive and a negative control. To exclude the possibility of false negative results, the virotype ASFV 2 Plus PCR Kit comes with two internal controls:

- An endogenous internal control (porcine β -actin) ensuring sample presence and quality
- An exogenous internal control added to the lysis buffer, monitoring whether the extraction procedure was successful
- Both controls monitor PCR inhibition as well as the correct PCR setup and cycler program.

The performance of the virotype ASFV 2 Plus PCR Kit was tested using in-vitro ASFV-DNA. The validation was conducted on ASFV-positive samples from pigs and wild boars experimentally infected with three different genotypes as well as field samples covering different genotypes. ASFV-negative samples and pig samples positive for other porcine pathogens were also tested.

Results: Testing a titration series of in-vitro ASFV-DNA showed the detection limit of the test kit to be five copies of ASFV-DNA. When testing 245 ASFV-positive samples, the assay demonstrated a high sensitivity. A specificity of 100% was determined by testing 226 ASFV-negative samples. In addition, no cross-reactivity was detected with other porcine viral pathogens such as Classical Swine Fever Virus (CSFV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Swine Influenza Virus (SIV) or Porcine Circovirus 2 (PCV2). Testing the intra- and inter-assay variance showed excellent reproducibility and repeatability. Inhibition of qPCR shown by failed or weak control signals and failed endogenous control signals due to sample quality proves the double internal control strategy.

Conclusions: The virotype ASFV 2 Plus PCR Kit is a highly sensitive and specific solution for detection of ASFV-DNA in samples from pigs and wild boars. The double internal control system provides more confidence in test results, especially when dealing with difficult sample material. In combination with the IndiSpin Pathogen or IndiMag Pathogen extraction kits, INDICAL provides a complete workflow solution for accurate ASFV detection saving precious lab time.

Disinfecting power of sodium dichloro-iso-cyanurate/ potassium mono-persulphate disinfectant on african swine fever virus under real farm conditions

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Introduction: African Swine Fever (ASF) was introduced in Russia in 2007. No specific preventative measures against ASF virus (ASFv) exist and epidemiological studies have shown that the transport of infected pigs is the main cause of ASF outbreaks [1]. Disinfection is one of the most important actions needed to reduce the spread of the virus. The Pirbright Institute, UK tested a disinfectant with active ingredients Sodium Dichloro-Iso-Cyanurate (SDIC) and Potassium Mono-Persulphate (PMP) and results show it produces complete inactivation of ASFv (log reduction >3.3 Log_{10}) in 30 seconds, at less than half of the label dose and without soiling [2].

The objective of the present study was to determine the disinfecting power of the SDIC/ PMP disinfectant against a virulent strain of ASFv on contaminated surfaces that mimic the surfaces of swine farms. Two parameters were evaluated: (1) the infectivity of a specific ASFv strain in primary Cell Cultures of Swine bone Marrow (CCSM), (2) the disinfecting power of SDIC/ PMP disinfectant on concrete surfaces sprayed by a mix of ASFv and pig feces.

Materials and Methods: The study was performed in accordance with official Russian guidelines [3, 4]. To determine the infectivity of the Stavropol strain ASFv isolated from pig blood, its tenfold dilutions and negative control were prepared, infused into petri-dishes of CCSM and incubated at 37℃ for 7 days. To evaluate the disinfecting power of the SDIC/ PMP disinfectant *in vivo*, a mix of 1.5 ml liquid containing 7 log HAU_{50} of virus per ml (HAU: Hemadsorbing unit) and 0.3 g of sterile pig feces (SPF) was prepared, evenly distributed onto 100 cm^2 of concrete wall and dried for 2 hours. SPF was used to provide mechanical protection to the virus. Then, the SDIC/ PMP disinfectant dilutions (1%, 2%, 3% and 4%) and negative control (distilled water) were evenly sprayed (0.3 l/m^2) to the concrete walls and dried for 1 or 3 hours. Organic material from the concrete walls was scrapped with 4.5 mL of Hanks medium, extracted at ambient temperature for 30 min and centrifuged for 15 min at 3000 rpm. The resulting supernatant was used to infect pigs weighing 18-25 kg. A bioassay was performed on 14 animals: 13 tests and 1 control. Inoculated pigs were observed for 21 days.

Results: Table 1 summarizes the virucidal power of the SDIC/ PMP disinfectant under real farm conditions. A single treatment of the SDIC/ PMP disinfectant, applied at a rate of 0.3 L/m^2, successfully disinfected concrete walls contaminated with ASFv protected by pig feces with a 3% dilution / 3-hours contact time and 4% dilution / 1-hour contact time.

Table 1. Determination of the virucidal power of a SDIC/ PMP disinfectant on ASFv protected by pig feces on concrete walls (solution applied at 0.3 L/m^2)

Dilution	Contact time (min)	Incubation (days)	Mortality (days)	Mortality (dead pigs/ inoculated pigs)
1%	60	7	2-4	1/1
2%	60	8	2-4	1/1
3%	60	16	2-4	2/2
3%	**180**	/	/	**0/3**
4%	**60**	/	/	**0/3**
4%	**240**	/	/	**0/3**
Control	/	4	3	1/1

Conclusions: This study showed that a single disinfection with a SDIC/ PMP disinfectant treatment could prevent pig mortality due to ASF.

Acknowledgements: Independent study performed at the National Institute of Veterinary Virology and Microbiology, Russian Academy of Agricultural Sciences in 2012. Full report available on demand.

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Development of DNA-protein vaccine strategy against African swine fever and evaluation of protection from challenge with African Swine Fever virus Arm 07 strain

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Introduction: African swine fever virus (ASFV) causes high morbidity and mortality in swine (Sus scrofa), for which there is no commercially available vaccine due to the complexity of the virus. Recent outbreaks of the virus in Trans-Caucasus countries, Eastern Europe, Belgium and Asian countries including China, Vietnam and Cambodia highlight the urgent need to develop effective vaccines against ASFV. Activation of both humoral and cellular immunity have been suggested to be necessary to obtain a protective vaccine against African Swine Fever Virus (ASFV). Therefore, the objective of this study was to develop protection using a new approach based on heterologous prime-boost vaccination, combining ASFV antigens encoded by DNA plasmids and recombinant ASFV proteins.

Materials and Methods: seven selected proteins of ASFV were expressed in either Baculovirus (Sf9 cells) or *E.coli* and six ASFV genes were cloned into pcDNA 3.1 expression plasmids. The immunogenicity of the constructs was tested in two independent experiments. In total, 75 piglets were immunized with different combinations of proteins and DNA plasmids. Serum and blood samples from vaccinated animals were evaluated by ELISA and virus neutralization assays. Pigs vaccinated with a combination of p15, p35 and p54 recombinant proteins with pcDNAs CD2v, p72 and p32 were challenged with virulent ASFV Armenia 2007 and then evaluate the protective effect with observation of clinical sign and viremia using RT-PCR.

Results: The results identified some promising combinations of plasmid DNAs and antigens. ELISA antibody titers were high with a number of ASFV proteins including p32, p15, CD2v-E, p54, p72 and p35. Interestingly, the combination of these proteins with plasmid DNA, showed increased levels of virus neutralization capacity. Specifically, neutralizing antibodies were more efficiently induced in pigs immunized with combinations of ASFV proteins and heterologous plasmid DNAs (i.e. p35 protein and CD2v pcDNA). Some of these combinations reached up to 80% of ASFV infection inhibition levels in vitro. After challenging with virulent Arm 07, none of them survived. Interestingly, viremia and clinical signs were observed earlier in the vaccinated pigs compared to the non-vaccinated controls. ASFV induced pathology was also enhanced in the vaccinated pigs. Furthermore, while the vaccinated pigs developed antigen-specific antibodies, immunized pig sera at the time of challenge lacked the capacity to neutralize virus, and instead was observed to enhance ASFV infection in vitro.

Conclusions: The results from this work opens a new opportunity for future studies on the most appropriate combination of ASFV-specific pcDNAs and proteins to develop a rationally designed, safe, efficacious and DIVA-compatible vaccine for ASF.

Acknowledgements: This work was funded by grants of the U.S.Department of Homeland Security under Grant Award Number DHS-2010-ST-061 for the Center of Excellence for Emerging and Zoonotic Animal Disease (CEEZAD) and the state of Kansas National Bio and Agro-Defense Facility(NBAF) transition fund.

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Comparison of a porcine circovirus type 2a (PCV2a)- and PCV2d-based vaccine against a PCV2d challenge

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Introduction: PCV2d is now the predominant genotype in Korea [1] and has been previously isolated in PCVAD cases in vaccinated herds [2]. As PCV2 vaccines were initially produced from PCV2a genotype, this study was performed to compare efficacy of a recently registered vaccine from PCV2d genotype with a vaccine from PCV2a genotype according to a challenge by a PCV2d strain isolated from an outbreak of PCVAD in Korea.

Materials and Methods: Eighty colostrum-fed conventional piglets were randomly allocated to 4 groups of 20 each. They were negative against PCV2, PRRS and *M. hyopneumoniae* (Elisa and PCR tests). Groups T and C were vaccinated with PCV2 vaccines (respectively Suigen® PCV2, Virbac and Ingelvac CircoFLEX®, Boehringer Ingelheim) at 3 weeks of age (1 ml/pig by IM route). UnVac/Ch and UnVac/Un Ch groups were injected with 1 ml/pig of PBS by IM route. Groups T, C and UnVac/Ch were inoculated intranasally by 2 ml (1 ml/nostril/pig) inoculum containing 10^5TCID$_{50}$/ml of a PCV2d strain at 7 weeks of age. UnVac/UnCh group was inoculated by 2 ml (1 ml/nostril/pig) of uninfected cell culture supernatant. Blood samples were taken on all pigs every 2 weeks between vaccination and challenge, then weekly between challenge and necropsy on 28 days after challenge. Serum PCV2d genomic copies (by qPCR), serum PCV2d neutralizing antibodies (NA) titer, number of PCV2d interferon-γ secreting cells (IFN- γ -SC) in peripheral blood mononuclear cells (PBMC) and number of PCV2 infected cells in superficial inguinal lymph node (by immunohistochemistry: IHC) were quantified by previously published methods. Data were analyzed by a general linear mixed statistical model.

Results: No side effects were reported in vaccinated groups. NA titers and IFN-γ -SC were detected in vaccinated groups from 14 days after vaccination and peaked respectively 21 and 14 days after challenge. The mean NA titers and IFN-γ -SC were significantly higher in vaccinated groups than in UnVac/Ch group between challenge and 28 days after challenge. Viremia peaked on 21 days after challenge. The mean serum genomic copies of PCV2d were significantly lower in vaccinated groups than in UnVac/Ch group between 2 and 4 weeks after challenge. The mean lymph node IHC scores were significantly lower in vaccinated than in UnVac/Ch group. The above criteria remained below limits of quantification in UnVac/UnCh group during the whole study.

Table 1. Mean ± SD of peak immunity criteria, viremia and lymph node viral burdens

Group	T	C	UnVac/Ch
Log$_2$NAtiters	7.0 ± 1.5^a	7.3 ± 1.2^a	2.3 ± 1.0^b
IFN-ɣ-SC/10^{6}PBMC	76 ± 27^a	87 ± 30^a	36 ± 28^b
Log$_{10}$copies/ml	3.4 ± 1.8^a	3.1 ± 1.9^a	6.2 ± 0.8^b
IHC scores	3.7 ± 1.4^a	2.8 ± 1.1^a	18.3 ± 4.0^b

$^{a,\ b:}$Different superscripts indicate significant differences

Conclusions: Both vaccines were effective in inducing specific immunity against PCV2d and reducing blood and tissue viral burdens, thus confirming cross protection between genotypes. No significant difference was found between vaccines according to the challenge model tested. In a more severe model combining PCV2 and PRRS infections, viremia was better controlled to some extent with homologous genotype between vaccine and challenge [3]. Thus PCV2a and d vaccines could be further investigated in a model combining PCV2d and PRSS infection, as combination of both viruses induces more severe disease [4].

Acknowledgement: The author's research was supported by contract research funds from Virbac (Grant no. 550-20180009).

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Control of PCVD via sows and piglets vaccination with Circovac® in case of early PRRSv infections

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Introduction: The efficacy of vaccines may be compromised when piglets are vaccinated at the same time when a PRRS infection occurs. CIRCOVAC® is a PCV2 vaccine registered for the use in sows and piglets. Vaccination of piglets and if needed also of sows has been shown to be an effective strategy to control clinical and subclinical PCVD in pigs. The objective of this study was to improve the production results in the fattening phase by an optimization of the vaccination program against PCV2.

Material and Medthods: The study was carried out on a commercial farrow to wean pig farm of 1200 sows. Piglets were vaccinated against PCV2 at 4-6 weeks of age (woa) but at the same age they were infected with the PRRSv. An alternative vaccination protocol was used against PCV2 to avoid piglets vaccination at the same time as PRRSv infection occurred. Two groups were compared in the same period during two consecutive years:

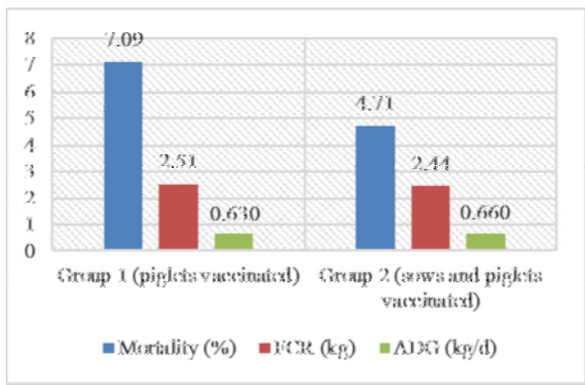
Group 1: piglets vaccinated against PCV2 with Circovac® at 4 woa

Group 2: sows and piglets vaccinated against PCV2 with Circovac® at day 90 of gestation (D90) and at 10 woa, respectively.

Results were analyzed by a parametric test Anova.

Results: In total, 30795 pigs were included in the study (group 1: 14335 pigs, group 2: 16460 pigs). In group 1 the mortality was 7,09%, Feed Conversion Ratio (FCR) was 2,51 and Average Daily Gain (ADG) 630 gr. In group 2 the mortality was 4.71% ($p<0.001$), FCR was 2,44 kg ($p=0.013$) and ADG was 660 gr ($p=0.047$).

Graph 1: Productive results comparison between groups



Conclusions: Sows and late piglets vaccination with Circovac® in early PRRSv infection cases in post-weaning stage improves the productive parameters compared to piglets vaccinated against PCV2 at 4 woa (at the same time PRRSv infection) and could contribute to reduce economic impact of PCV2 due to a better control of it.

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Identification of Novel Conformational Epitopes of the Porcine Epidemic Diarrhoea Virus Spike Protein

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Introduction: Since 2010, newly identified variants of porcine epidemic diarrhoea virus (PEDV) have caused high mortality in neonatal piglets which has devastated the swine industry all over the world. The spike (S) glycoprotein of PEDV contains multiple neutralizing epitopes and is a major target for PEDV neutralization and vaccine development. Recently, five core cell attachment domains of the PEDV S1 protein, $S1^0$, $S1^A$, $S1^B$, $S1^C$, and $S1^D$, were proposed based on the 3D structures cryo-EM of Alphacoronaviruses. To understand the antigenicity of the new PEDV variant, we characterized the neutralizing epitopes of a new genotype 2b PEDV isolate from Taiwan, PEDV Pintung 52 (PEDV-PT), by the generation of neutralizing monoclonal antibodies (NmAb).

Materials and Methods: A panel of purified monoclonal antibodies (mAbs) was generated by immunization of BALB/c mice with inactivate PEDV-PT viral particles. The neutralizing ability of each mAbs was examined by the neutralizing assay against PEDV-PT and only the mAbs which had an excellent neutralizing ability were selected to conduct further epitope mapping. Moreover, different truncated S proteins covering different structural domains were constructed and expressed by HEK cells for applying on the indirect ELISA and immunocytochemical staining (ICC) to evaluate the binding affinity of these mAbs. The dot blotting of different truncated S proteins were also conducted to confirm the conformational significance of the neutralizing epitopes.

Results: Two NmAbs, P4B-1, and E10E-1-10 that recognized the ectodomain of the full-length recombinant PEDV S protein and exhibited neutralizing ability against the PEDV-PT virus were selected. Recombinant truncated S proteins were used to identify the target sequences for the NmAbs and P4B-1 was shown to recognize the C-terminus of the COE epitope at amino acids (a.a.) 575-639 of the PEDV S. Interestingly, E10E- 1-10 could recognize a novel neutralizing epitope at a.a. 435-485 within the $S1^A$ domain of the PEDV S protein, whose importance and function are yet to be determined. Moreover, both NmAbs could not bind to linearized S proteins, indicating that only conformational epitopes are recognized.

Conclusions: Novel conformational neutralizing epitopes of PEDV S protein identified in the present study could improve our understanding of the antigenic structures of the PEDV S protein and facilitate future development of novel epitope-based vaccines.

Acknowledgement: This work was supported by the Ministry of Science and Technology, Taiwan, R.O.C. for grants MOST106-2311-B-002-028-MY3, MOST107-2321-B-033-002-6, 108L7842 and 107L7842 from National Taiwan University, Taiwan, ROC.

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Emerging, epidemiology and evolutionary analysis of porcine deltacoronavirus in South East Asia

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Introduction: Porcine deltacoronavirus (PDCoV) was first reported in Hong Kong in 2009 and subsequently reported in many countries [1]. The clinical signs of PDCoV affected pigs are similar to that of porcine epidemic diarrhea virus (PEDV) [2]. PDCoV is an enveloped, single-stranded, positive-sense RNA virus in the genus Deltacoronavirus, family Coronaviridae. The genome arrangements are in the order of 5' UTR, ORF 1a/1b, spike, envelope, membrane, Nsp6, nucleocapsid, Nsp7 and 3' UTR. This agent has been decreasing productivity in the swine industry, especially in Vietnam which the largest scale of pork production in SEA. From the previous study [1], the isolates of SEA were forming their own cluster and there phylogeographic study describing the introduction of the virus into these countries. In this study, we did the analysis of all available complete genomes up to date, using BEAST [3] to describe its global phylodynamic distribution into SEA.

Materials and Methods: All available complete genomes of PDCoV deposit in GENBANK® up to 2018 in a total of 107 sequences were used in this study. The alignment was performed using MAFFT and phylodynamic analysis of PDCoV was estimated using BEAST 1.10.4 [3]. TN93 with gamma and invariant model was applied using coalescent Bayesian skyline tree with the lognormal clock. The result will be acceptable at 200 effective sample size (ESS) or more and will be used for further analysis. The result will be interpreted using Tracer 1.6.1. Trees were annotated using TreeAnnotator 1.10.4 and convert to be displayed using FigTree 1.4.3. The result will be then converted to phylogeography using SPREAD 1.0.7 and displays in Google Earth [4].

Results: The result demonstrates that the virus was reaching from China close to the border of Vietnam during 1990-1991 prior to the introduction to Vietnam in 1994. The virus was suspected to be circulating in Thailand and SEA since 2003 prior to the introduction to Lao PDR in 2016. The evolutionary rate of the virus in this study was estimated at 1.3286 x 10^{-4} substitution/site/year.

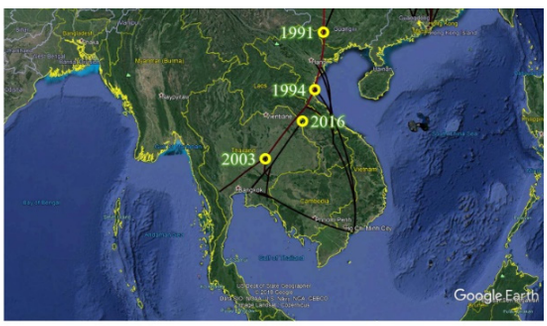

Figure 1. Phylogeography demonstrating the distribution of PDCoV in SEA

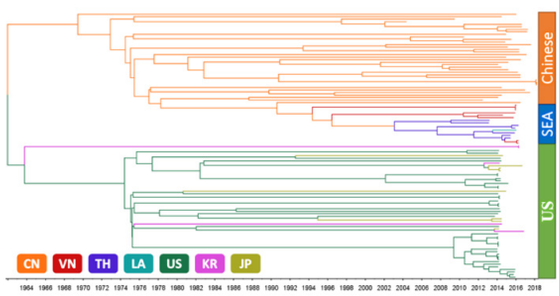

Figure 2. The phylogeographic tree is shown the estimated divergence of nodes through the years from 1961-2018. The abbreviations are shown the color of each country displaying in the tree

Conclusions: PDCoV in SEA was originated from China and suspected to be introduced via border area of Vietnam since 1991 prior to the introduction in 2003 and completely circulating in SEA.

Acknowledgment: This research was financially supported by the National Research Council of Thailand and the Agricultural Research Development Agency (Public Organization). Partial funding was provided by the Special Task Force for Activating Research (STAR), Chulalongkorn University.

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Correlation between detection of *Mycoplasma hyopneumoniae* from the larynx of suckling piglets and their lung lesion scores at slaughter, and comparison of the efficacy of two commercial vaccines

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Introduction: *Mycoplasma hyopneumoniae* (Mhp) is the causative agent of enzootic pneumonia and one of the main pathogens causing the porcine respiratory disease complex. Gilts may shed Mhp after introduction to the breeding herd resulting in colonization of their piglets [1]. The presence of Mhp in nasal swabs at weaning has been related to percentage of lung lesions seen at slaughter [2]. In the present study, we evaluated the correlation between the presence of Mhp in laryngeal swabs taken at pre-weaning and lung lesion scores at slaughter, as well as the efficacy of two commercial Mhp vaccines which were administered when the sows were young piglets in a commercial pig farm in Japan.

Materials and Methods: In April 2017, the Mhp vaccine was changed to RespiSure One® from a local vaccine in both piglets and a breeding herd and the vaccinating timing to piglets was also changed from at weaning to 4 days of age. Laryngeal swab samples were taken from 20 pre-weaning piglets, which were born from only primiparous sows. These piglets were individually tagged and kept in the same farm until market. The lung lesions at slaughter were checked based on the technique by Straw et al. [3] and the scores were calculated. These activities were conducted 7 times in total, being done every few months from January 2018. Mhp specific DNA was detected in laryngeal swabs by real-time PCR. The primer was set by targeting the mhp165 and mhp183 genes based on Strait et al. [4].

Results: Mhp specific DNA in laryngeal swabs was detected from 0 to 35% of piglets depending on the sampling batches. Average batch lung lesion scores were from 0.1 to 20.5, with a correlation rate of 0.89 (Figure 1).Gradual improvements in the positive rate of Mhp specific DNA and average lung lesion scores were observed after the change of Mhp vaccine ($P<0.05$) (Table 1).

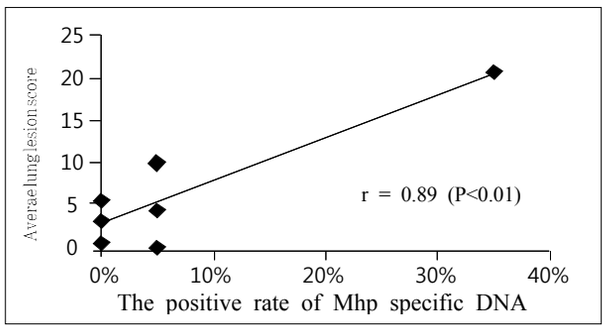

Figure 1: Correlation between Mhp specific DNA positive rate and average lung lesion score.

Table 1: Change in the positive rate of Mhp specific DNA and average lung lesion scores in the offspring with the change of vaccine administered to the sows as young piglets. (ab, cd: $P<0.05$)

# times	Date of laryngeal swabbing	Positive rate of Mhp specific DNA	Average lung lesion score	Vaccination when the sows were young piglets
1	Jan-18	35^{a}	20.5^{c}	Japanese local vaccine
2	Feb-18	5^{b}	10.1^{c}	
3	Jun-19	5^{b}	0.1^{d}	RespiSure One®
4	Aug-19	0^{b}	0.7^{d}	
5	Sep-19	5^{b}	4.4^{d}	
6	Oct-19	0^{b}	5.5^{d}	
7	Nov-19	0^{b}	3.4^{d}	

Conclusions and Discussion: A strong correlation was detected between the positive rate of Mhp specific DNA in laryngeal swabs and average lung lesion scores. RespiSure One® improved both parameters, probably due to reduction of shedding of Mhp from vaccinated sows. It is suggested that sows vaccinated with RespiSure One® as young piglets were shedding less Mhp than sows vaccinated with a local product. The data also support the view that Mhp vaccination of young piglets is important, and that reduction of shedding of Mhp from sows to offspring is an important aspect of Mhp control.

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Comparison of two different combinations of vaccines against Mycoplasma hyopneumoniae and Actinobacillus pleuropneumoniae in the protection lung health and production parameters

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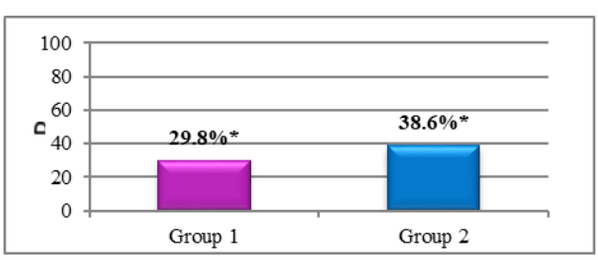
Introduction: Mycoplasma hyopneumoniae (Mhyo) and Actinobacillus pleuropneumoniae (A.p) are very common pathogens in swine farms worldwide and cause severe respiratory diseases with great economic impact [1]. In this study, the effect of two different combinations of vaccines against Mhyo and A.p in pigs' lung health, production parameters and mortality was investigated.

Materials and Methods: 500 animals in a commercial farm were vaccinated against Mhyo and A.p with Hyogen® \ and Coglapix® (group 1), respectively, and 500 with vaccines A and B (group 2). Per farmer's request, both Mhyo vaccines were administered in 2 doses, in 7th and 28th day of life, with 1 ml per dose for Hyogen® and 2 ml per dose for Vaccine A. Blind lung evaluation was applied at slaughter by using the Ceva Lung Program. Also, animals were weighted after weaning, when entering the pre-fattening and fattening units and before slaughter. Average daily gain (ADG) for the different stages of pigs' growth was calculated and mortality was recorded.

Results: In total 664 lungs were examined, 312 in group 1 and 352 in group 2. The percentage of lungs with Ep-like lesions differed significantly (P<0.05; Pearson's chi-square test) between groups 1 and 2 (Figure 1). The frequency of dorsocaudal pleurisy was 7.05% (22 out of 312 lungs) and 9.94% (35 out of 352 lungs) in groups 1 and 2 (P>0.05; Pearson's chi-square test), respectively.

Figure 1. The frequency (%) of EP-like lesions



*P<0.05; Pearson's chi-square test.

The production parameters were measured for 248 pigs in group 1 and 342 in group 2. ADG from weaning to slaughter differed significantly between the two groups (P<0.05; Two sample t-test) (Table 1). Mortality from pre-fattening to slaughter did not differ (P>0.05; Pearson's chi-square test) between groups 1 and 2, with 2.7% and 1.4% dead animals, respectively.

Table 1. Mean (± SD) daily gain (gr) in the different growth stages

Growth stage	Group 1	Group 2	P-value*
Weaning to pre-fattening	612 (±37)	639 (±14)	<0.05
Pre-fattening to fattening	925 (±65)	869 (±74)	<0.05
Fattening to slaughter	998 (±150)	992 (±40)	>0.05
Weaning to slaughter	829 (±45)	796 (±56)	<0.05

The odds of having Enzootic pneumonia-like lesions were 2.1 times lower (P<0.05; Logistic Regression Model adjusted for "slaughter age and room effect") for animals vaccinated with Hyogen® and Coglapix® compared to Vaccines A and B. Also, animals vaccinated with the first combination of vaccines for Mhyo and A.p were on average 2.81kg heavier at slaughter (P<0.05; Linear Regression Model adjusted for "slaughter age and room effect") compared to the second one.

Conclusions: Under the conditions of this study, the use of Hyogen® and Coglapix® together had a beneficial effect in pigs' lung health and production parameters compared to the other combination of vaccines. Those results indicate that vaccination with Hyogen® and Coglapix® could be an effective measure for better control of Mhyo and A.p infections in swine farms.

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Survey on Mycoplasma hyopneumoniae gilt acclimation practices in Korea

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Boehringer Ingelheim Animal Health Korea[1]

Introduction

Vertical transmission of M. hyopneumoniae (M.hyo) from an infected sow to her piglets is generally recognized as the initial pathway of enzootic pneumonia. Infected sows are considered a risk factor for M.hyo infection in the pre-weaning period (1). M. hyo shedding through direct contact with vertically infected piglets is the initial infection source in susceptible pig populations during the nursery and growth-finishing periods. The objective of this study was to understand the current gilt acclimation practice against M.hyo in Korea.

Material & Method

A survey with 22 questions was designed to understand current gilt acclimation practices for M. hyo in Korea. The survey was completed by 11 swine veterinarians for 32 swine farms, representing 23.295 sows from different regions in Korea. The average herd size was 725 sows.

Results

The most important findings were:

- 50% of farms receive gilts from M.hyo negative multipliers
- 78% have late (age) gilt acclimation, beyond 20 weeks of age
- 56.3% use vaccines against M.hyo during the acclimation
- 15.6% use young piglets, 0% use cull sows and 9.3% use culled piglet as source of exposure during the acclimation process
- 97% do not perform diagnostics to verify an adequate acclimation
- 12.5% of acclimation sites are continuous flow
- 84.4% use antibiotics during the acclimation process (Tiamulin, Lyncomycin, Amoxaciline & Macrolide treatment for 2 weeks)
- In 46.9% of the farms the assessment of the stability in the sow herd is based on clinical signs (lung lesions at slaughter: 25% of farms / ELISA test: 18.8%)
- 40.6% of the responders feel that their acclimation protocol keeps their sow herd stable to M. hyo

Table 1. Comparisons of major risks on gilt acclimation among three geographical regions.

Risk Factor	US[2]	Spain[3]	Korea
Receive Naïve Gilts into Positive Herds	55%	29%	21.8%
Have>50% replacement rate	41%	n.a.	6.2%
Do not acclimatize to herd specific strain	60%	62%	72%
Late gilt acclimation, beyond 20 w.o.a	53%	34%	78%
Validate exposure & recovery methods	20%	7%	3%

Discussion and conclusions

All swine veterinarians agree that an appropriate gilt acclimation program is important to control M.hyo. However, many of them do not have a clear definition of appropriate acclimation methods. Compared with US and Spian, Korea shows low replacement rate and late timing of gilt acclimation. The results of this survey are a good foundation to develop an effective strategy for the acclimatization of gilts in Korean.

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Monitoring of Atrophic Rhinitis and Lung Lesion Scores in Slaughtered Pigs from 15 Farms

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INTRODUCTION

Atrophic rhinitis (AR) is a respiratory disease in pigs caused by toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* type D toxin (PMT). Also, AR can cause economic loss, like retarded growth performance. Besides, Bb coinfection with different pathogens, including *Haemophilus parasuis, Streptococcus suis and Mycoplasma hyorhinis*, may enhance AR.

To monitor the status of AR in Korea, nasal scores were evaluated in slaughtered pigs from 15 farms. Also, lung lesion scores were evaluated, as well as their correlation with AR.

MATERIAL & METHODS

Fifteen farms (four of them genetic) were evaluated, and 20 slaughtered pigs were randomly selected from each farm.

Regarding nasal scoring, atrophy of each nasal scroll was scored a maximum of 4 points, and each deviated septum bone was scored a maximum of 2 points (4*4 scrolls + 2 septum scores = total maximum of 18 points) [1].

To classify the status of AR, all pigs were divided in three different groups based on nasal scores (Mild: 0-4, Moderate: 5-8, Severe: 9-18).

On the other hand, maximum lung lesion score was 28 points (4 points per lobe) [2].

RESULTS

Based on AR monitoring data, 40% of pigs were scored less than 4 points, which is considered a mild lesion. However, 35.3% of pigs showed severe AR lesions, even though all monitored farms conducted vaccination against AR. Besides, the groups of pigs with moderate or severe AR showed significantly higher lung lesion scores compared with mild AR group.

Figure 1. Nasal score distribution of 300 heads of pigs (maximum score: 18 points).

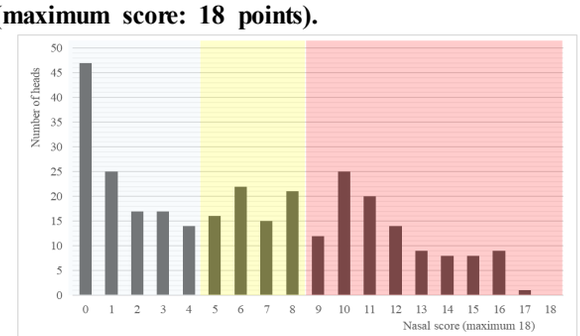

Figure 2. Relationship between nasal score (maximum score: 18) and lung lesion score (maximum score: 28).

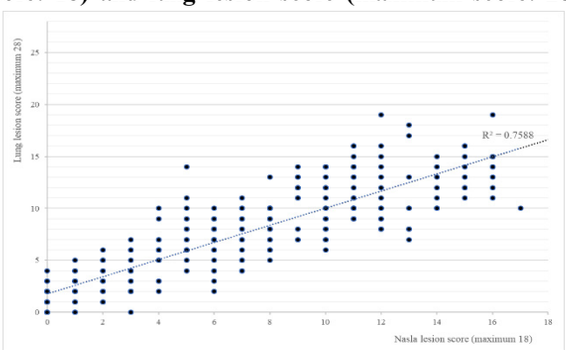

Table 1. Mean lung lesion score by group based on atrophic rhinitis status.

Group based on AR status*	Mild group	Moderate group	Severe group
Number of samples	120	74	106
Nasal lesion score (Mean ± SD)	$1.38_{\pm 1.42}$	$6.55_{\pm 1.12}$	$11.85_{\pm 2.20}$
Lung lesion score (Mean ± SD)	$2.59_{\pm 2.01}$	$7.65_{\pm 2.24}$**	$11.57_{\pm 2.89}$**

*AR status was divided into three groups based on nasal score: Mild (0-4), Moderate (5-8), and Severe (9-18).

***p*-value <0.001, Student's t-test

DISCUSSION & CONCLUSION

Although all monitored farms were using vaccines against AR, 60% of slaughtered pigs showed mild-to-severe AR lesions. Considering these results, Korean pig farms' protective measures are still inefficient to control AR.

Besides, pigs with AR had higher lung lesion scores. These correlation data show that an effective control of AR is important to prevent coinfection and improve lung lesion scores.

Thus, efficient prevention against AR is recommended in Korean pig farms to improve productivity and respiratory conditions.

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Utilizing Lung Lesion Scoring to Monitor Lung Health Improvement in a Commercial Swine Farm

Ernesto C. Balolong Jr[1], *Roselle Cudal*[1], *Mary Grace Alba*[1],

[1]*Elanco Philippines*

Introduction:

Swine Enzootic Pneumonia (SEP) remains a very important respiratory disease condition in swine causing economic losses due to growth retardation and reduced feed efficiency (1). *M. hyopneumoniae* is the primary pathogen of SEP and plays a central role in the Porcine Respiratory Disease Complex (PRDC) as it interacts with other bacterial and viral respiratory pathogens causing a more severe pneumonia with serious consequences (2,3).

In the 3rd quarter of 2017, a >2,500 sow level farrow to finish commercial farm in the Visayas, Philippines reported respiratory symptoms leading to high mortality (>3%) and poor performance at the grower-finisher stage. Lung lesion scoring (LLS) done at the abattoir to observe *Mycoplasma*-like lesions and to determine incidence and severity of pneumonia following the implementation of a control program using antibiotics.

Materials and Methods:

A baseline lung lesion scoring for *Mycoplasma*-like pneumonia lesions which occur as cranioventral consolidations and using the 55 pts method (4) was done in October 2017 prior to adding Pulmotil® (tilmicosin phosphate) in the grower feeds at 2kg/ton for 21 days. Also, noted were the presence of adhesions and pleurisy, suggestive of *Actinobacillus pleuropneumoniae, Haemophilus parasuis and Pasteurella multocida* infection (4). In February 2018, Pulmotil® was included in the lactation diet 1 week before farrowing and 2 weeks after farrowing. By Sep 2018, use of Pulmotil® in the grower feeds was discontinued but remain in the lactating feed until February 2019. LLS was done, when possible, in succeeding months after the baseline.

Results:

An improvement of LLS score from an average score of 25.3 pts to an average of 12.22 pts was observed 4 months after the change in grower feed medication. The percentage of lungs with a score of > 10pts, considered as having a moderate to severe pneumonia and to affect pig performance, improved from 92.5% to an average of 29.9% of the total lungs starting at 4 months after the implementation of the medication program involving Pulmotil until February 2019. Improvements in the incidence of adhesions and pleurisy were also be observed in months where Pulmotil was used.

Table 1.

Month	Medication program		% Lungs Classification (*Mhyo* Scores)			Average LLS	% Lungs with	
			Normal (0-10)	Moderate Pneumonia (11-45)	Severe Pneumonia (46-55)		Adhesions	Pleurisy
Oct-17	Other	Grower	7.50	68.50	24.00	25.85	78.30	59.70
Nov-17	Pulmotil	Grower	35.71	53.57	10.71	20.70	60.71	46.42
Jan-18	Pulmotil	Grower	28.89	60.01	11.11	20.64	82.22	35.55
Feb-18	Pulmotil	Grower + Clean Sow	58.82	39.21	1.96	12.22	49.01	17.64
Mar-18	Other	Grower	24.44	64.44	11.11	22.58	80.00	62.22
Apr-18	Pulmotil	Grower + Clean Sow	66.66	28.57	4.76	9.31	25.00	10.00
May-18	Pulmotil	Grower + Clean Sow	66.00	32.00	2.00	10.30	38.00	6.00
Jun-18	Pulmotil	Grower + Clean Sow	84.85	15.15	0	6.00	28.53	10.00
Aug-18	Pulmotil	Grower + Clean Sow	78.26	19.57	2.17	7.19	32.11	10.67
Feb-19	Pulmotil	Clean Sow	66.00	34.00	0	10.26	60.00	24.00

Conclusions:

Baseline LLS results provided valuable information on the incidence and severity of SEP in the farm and when monitoring improvements of lung health. LLS results provide evidence of improvement in the succeeding months based on reduction of gross lesions in the lungs.

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Field efficacy trial of a novel inactivated injectable vaccine against *Lawsonia intracellularis* (Porcilis Ileitis®) in Cebu, Philippines

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Introduction: *Lawsonia intracellularis* is an obligate intracellular bacterium that is the etiologic agent of ileitis in pigs[1,2]. It causes thickened mucosal lining primarily of the small intestine due to infected crypt cell proliferation. Clinical symptoms of ileitis include stunted growth and diarrhea in young, growing pigs whereas hemorrhagic diarrhea and sometimes sudden death in adult pigs. Because of this, the disease continues to be a problem in pig production and is responsible for substantial economic losses worldwide[3,4]. Vaccination and use of antibiotics have been the top control options for ileitis[4]. In this study, the efficacy and safety of a novel inactivated injectable vaccine against *L. intracellularis* (Porcilis Ileitis®) were evaluated. Materials and Methods: A total of three thousand two-hundred ten (3210) 24-day-old piglets were used in this study. These animals were assigned randomly to one of the two groups: TREATMENT (n=1605), which were vaccinated with Porcilis Ileitis® vaccine 2ml intramuscularly (IM) and CONTROL (n=1605), unvaccinated.

For the trial, serial serum samples were collected as the pigs age. Samples were tested for *L. intracellularis* antibodies with blocking ELISA using Ileitis-ELISA kit in accordance to the manufacturer's instructions (Svanova Biotech AB, Sweden). For the safety trial, production indices were acquired throughout the production cycle.

Results: In Figure 1, both control and treatment groups were negative for *L. intracellularis* at 4 weeks of age. Only in their 10th week when positives started to develop in the control group, whereas the treatment group has been maintaining a positive reading for *L. intracellularis* antibodies as early as 6 weeks up until 18 weeks of age, which highly suggests prolonged protective titers. Furthermore, table 1 illustrates the observed statistical difference between treatment and control groups based on their production indices. Within assumptions under local conditions, the treatment group calculates a positive economic impact with regards to prime pigs sold, carcass sales, and FCR benefit with a net benefit gain of PhP 499.8 (USD 9.61) per pig compared to the control group.

Figure 1. Percent positivity of pigs (%) to *Lawsonia intracellularis* antibodies as they age.

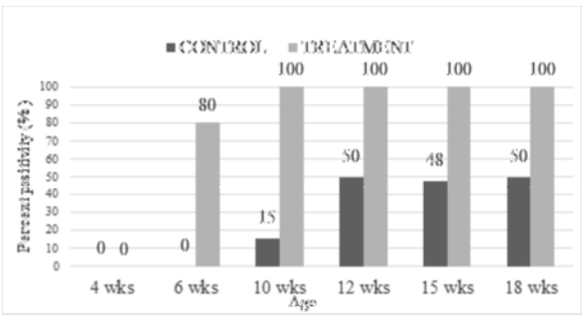

Table 1. Production indices of fattener pigs (T=treatment, C=control groups). a, b: value with different superscripts in each column represent statistically significant differences ($p<0.05$).

	ADG	Birth weight(kg)				
T	0.75^a	1.44^a	7.47^a	33.37^a	117.37^a	139.14^a
C	0.74^a	1.43^a	7.45^a	34.05^b	116.15^b	137.98^b

Conclusions: Porcilis Ileitis® demonstrated efficacy in terms of maintaining persistent protective titers, and safety in commercial herds with significant differences over the control pigs for some parameters (transfer, carcass, and market weights) with positive economic effect. As a new, innovative product, Porcilis Ileitis® is a viable option for producers to combat ileitis-related diseases.

Acknowledgement: MSD Animal Health wishes to thank the staff of the farm involved for participating in the study.

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Phytogenic Feed Additive In Controlling Presence Of Lawsonia *Intracellularis* In Young Fattening Pigs

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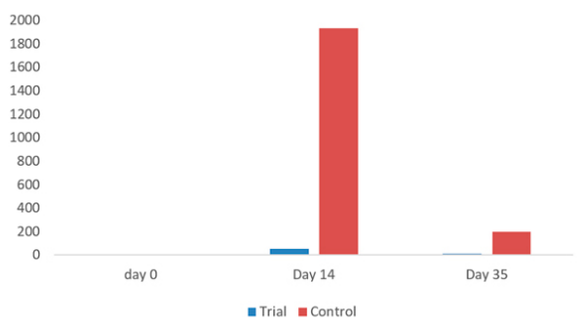
Introduction: *Lawsonia intracellularis*, causative agent of pig ileitis (PI), is one of the most economically important pathogens in pig production all over the globe. The biggest challenge in controlling PI, is the most common form, subclinical PI, where the only symptom is a financial loss. Poor pig production is a consequence of ineffective digestion and reduced nutrient absorption, influenced by *L. intracellularis*. The increase incidence of PI is considered to be a direct consequence of withdrawal of antibiotic growth promotors (AGPs) from pigs' diets (1). Therefore, there is a strong scientific and commercial urge to find safe, reliable and cost-effective replacement to AGPs. Phytogenic feed additives (PFA) have the potential to act as an alternative to AGPs and there are many evidences for beneficial effects of PFA on pig gut health. Our recent publication has also provided evidence of effective application of plant-based feed additives in controlling *L. intracellularis* associated diseases (2) in post weaned piglets. The aim of this trial was to asses efficacy of the same PFA on young fattening pigs transferred to the farm where *L. intracellularis* was previously determined.

Materials and Methods: The first day of the trial was also the first day of fattening period for a total of 20 pigs which were physically divided into two groups. Trial group was fed with diet where 2 kg/ton of commercial PFA (PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia) was added, while in control group neither antibiotics nor other PFA were added. On day 0, 14 and 35, blood and fecal samples from each pig were collected alongside the fecal samples from the floor of each group. Number of DNA copies of *L. intracellularis* was determined with real-time PCR in every fecal sample. *L. intracellularis* antibodies (Ab) was determined by ELISA. Presence of *Brachyspira pilosicoli, E. coli* F4 and *E. coli* F8 was excluded with real-time PCR. The data was analyzed using ANNOVA.

Results: On the 0th day of the trial presence of *L. intracellularis* was not detected in all fecal samples from Control and Trial group by real-time PCR, while serology test showed Ab only in sample of one pig from each group. The presence of Ab was not observed in every pig on day 14th too. On contrary, *L. intracellularis* presence was confirmed in every fecal sample. On 14th day, the number of bacterial DNA copies were statistically significantly ($p<0.05$) higher in control group in comparison to the trial group. On same day, the number of *L. intracellularis* DNA copies was 35, higher in Control group that did not previously consumed PFA (Figure 1.). Statistically significant ($p<0.05$) difference between groups in number of bacterial DNA copies in individual fecal was detected on day 35th too, and control group had 13 times more DNA copies than trial group in floor fecal samples.
However statistical difference between groups in number of Ab was not found at the end of the trial.

Figure 1. The number of ***Lawsonia intracellularis*** DNA copies in floor faecal samples measured by real-time PCR in both groups on day 0, 14 and 35.



Conclusions: The results of this trial suggest that PFA could be used in controlling PI without interfering development of natural immunity.

Acknowledgement: This work was supported by company PATENT CO. DOO. The trial was conducted by PORCUS (Denmark). All samples were analyzed at DTU, Technical University of Denmark.

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Porcine reproductive and respiratory syndrome virus nsp1-beta protein interacts with nucleoporin 62 (Nup62) to promote viral replication and evasion from antiviral immunity

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Introduction: PRRSV replication takes place in the cytoplasm but blocks host cell mRNA to the cytoplasm, suggesting a novel mechanism to enhance viral replication. PRRSV nonstructural protein (nsp) 1β was identified as the viral protein playing the role for host mRNA nuclear retention and subversion of host protein synthesis. A consensus sequence was identified in nsp1β. *In situ* hybridization unveiled that mutations in this region were unable to cause nuclear retention of host cell mRNAs and did not suppress host protein synthesis. In addition, nsp1β mutants did not suppress innate immunity. Using the reverse genetics, a series of mutant PRRS viruses were constructed. These mutant PRRSV did not suppress IFN production and were clinically attenuated in experimentally-infected pigs. In the present study, we delineate the basis for nsp1β -mediated innate immune evasion of PRRSV.

Materials and Methods: MARC-145, HeLa, Vero, and 3S4/21 cells were cultivated in DMEM or RPMI-1640 containing 10% FBS. PRRSV NVSL 97-7895 is a PRRSV-2 strain, and FL12 is the infectious cDNA clone of NVSL 97-7895. FL13 is a modified version of FL12 to allow transcription in mammalian cells. Reporter assays were conducted using Dual Luciferase Reporter Assay system (Promega). Co-immunoprecipitation, GST-pull down assay, and immunofluorescent co-staining were performed to determine protein-protein binding.

Results: Nucleoporin 62 (Nup62) was found to bind to PRRSV nsp1β, and the C-terminal 328-522 residues of Nup62 was the binding domain to nsp1β. The nsp1β L126A mutant in the SAP domain did not bind to Nup62, and in L126A-expressing cells, the host mRNA nuclear export occurred normally. The vL126A mutant PRRSV generated by reverse genetics replicated at a slower rate, and the titer was lower than wild-type virus. In nsp1-beta overexpressing cells or siRNA-mediated Nup62 knock-down cells, the viral protein synthesis increased. Notably, the production of type Iinterferons (IFNs-α /β), IFN-stimulated genes (PKR, OAS, Mx1, ISG15), IFN-induced proteins with tetratricopeptide repeats (IFITs) 1 and 2, and IFN regulatory factor 3 decreased in these cells. As consequence, the growth of vL126A mutant PRRSV was rescued to the level of wild-type PRRSV. These findings are attributed to the nuclear pore complex disintegration by nsp1β , resulting in increased viral protein production and decreased host protein production including antiviral proteins in the cytoplasm. Our study reveals a new strategy of PRRSV for immune evasion and enhanced replication during infection which is a key determinant for PRRSV pathogenesis.

Table 1. Properties of nsp1β for member viruses in the family Arteriviridae

virus	Viral factor	Host mRNA nuclear retension	IFN suppression	Nup62 binding
PRRSV-1	nsp1β	+	+	+
PRRSV-2	nsp1β	+	+	+
LDV	nsp1β	+	+	+
EAV	nsp1	-	-	-
SHFV	nsp1β	+	+	+

Conclusions:

1. The nsp1β protein binds to nucleoporin 62 (Nup62).
2. The nuclear pore complex becomes disintegrated and the nucleocytoplasmic trafficking of host mRNAs and host proteins are blocked.
3. PRRSV gains dual benefits; 1) inhibition of host antiviral protein expression and 2) exclusive usage of host machinery for viral protein production. As a result, PRRSV replication is enhanced.

Acknowledgement: This work was supported by AFRI Competitive Grants no. 2013-67015-21243 and 2018-67015-28287 from USDA NIFA.

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Immune response of pigs intramuscularly or intradermally vaccinated with type 1 porcine reproductive and respiratory syndrome virus modified-live vaccine and protective efficacy against single type 2or co-challenge with type 1

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Introduction: Co-existence of type 1 and 2 porcine reproductive and respiratory syndrome virus (PRRSV) had been increasingly reported in several Asian countries, including China, Korea, Vietnam and Thailand [1]. Recently, modified-live vaccines (MLV) had been used to control PRRSV infection and the protective efficacy was investigated. However, improved version of known MLV are being introduced and updated information on the efficacy is highly demanded. Therefore, the objective of this study was to test the efficacy of UNISTRAIN® PRRS when administrated intramuscularly or intradermally in pigs against either single challenge with type 2 PRRSV (HP-PRRSV) or in co-challenge with type 1 PRRSV (Pan European) and HP-PRRSV.

Materials and Methods: Forty-two, PRRSV-free pigs at 3-week-old were randomly allocated into 7 groups with 6 pigs each, including VacIM/PRRS2, VacID/PRRS2, NV/PRRS2, VacIM/PRRS1+2, VacID/PRRS1+2, NV/PRRS1+2, and NV/Unch, respectively. Groups VacIM/PRRS2 and VacIM/PRRS1+2 were intramuscularly vaccinated with UNISTRAIN® PRRS. Meanwhile, groups VacID/PRRS2 and VacID/PRRS1+2 were intradermally vaccinated with UNISTRAIN® PRRS. Groups NV/PRRS2, NV/PRRS1+2 and NV/Unch were left non-vaccination. Blood sample and peripheral blood mononuclear cells (PBMC) were collected and assayed for immune response using ELISA and ELIPOT assays. At 35 days post-vaccination (DPV), groups 1, 2 and 3 were challenged with HP-PRRSV and groups 4, 5 and 6 were co-challenged with type 1 and HP-PRRSV. Pigs in group NV/Unch were left as no challenge. Pigs were necropsied at 7- and 35 days post-challenge (DPC). PRRSV RNA, lung lesion and pathological examination were evaluated.

Results: Following vaccination, there was no difference in antibody response among vaccinated groups. ID vaccinated pigs had shorter viremia phase and lower RNA level compared to IM vaccinated pigs. ID vaccinated pigs had significant lower IL-10 levels than IM vaccinated pigs (Figure 1), but IFN-γ-producing cells were significantly higher (Figure 2). Following challenge, ID vaccinated pigs had significantly lower PRRSV RNA and lung lesion compared to IM vaccinated pigs at 7 DPC but showed no difference at 35 DPC.

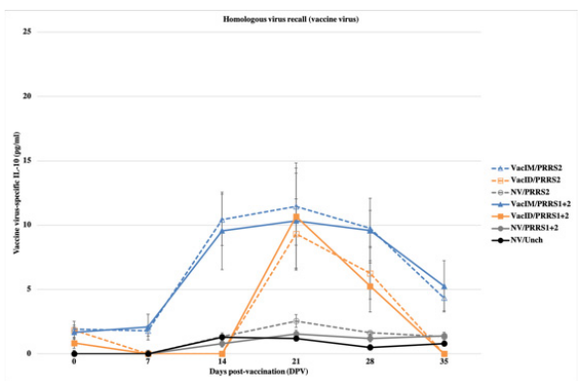

Figure 1. IL-10 production

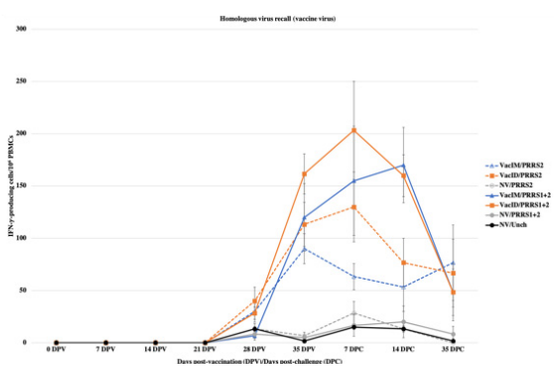

Figure 2. IFN-g-producing cells

Conclusions: The results suggested that UNISTRAIN® PRRS administrated, either ID or IM, can provide protection against challenge with HP-PRRSV, either alone or in co-challenge with type 1 PRRSV, as demonstrated by reducing lung lesion and viremia.

Acknowledgement: This work was supported by Research and Researchers for Industries (Grant No. PHD59I0040).

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Cross-protection of a modified-live porcine reproductive and respiratory syndrome virus (PRRSV)-2 vaccine against a heterologous PRRSV-1 challenge in late-term pregnancy gilts

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is an enveloped, single stranded, positive sense RNA virus that belongs to the new genus *Porartevirus*, in the *Arteriviridae* family and order *Nidovirales*. PRRSV has been recently reclassified into two major distinct species bases on genetic and antigenic differences, PRRSV-1 (former Type 1 genotype from European origin) and PRRSV-2 (former Type 2 genotype from North American origin) [1]. The objective of this study was to evaluate and compare the efficacy of the PRRS subunit vaccine with a PRRS MLV vaccine in endemic PRRS farms. A PRRSV-2 MLV vaccine (Fostera™ PRRS, Zoetis, Parsippany, NJ, USA) was licensed in 2017 in most Asian countries for use in gilts and sows to protect against reproductive failure caused by PRRSV-2. The objective of this study was to determine whether this PRRSV-2 MLV vaccine can cross-protect against a heterologous PRRSV-1 challenge in gilts in terms of reproductive failure.

Materials and Methods: At -135 days post challenge (dpc, 6 weeks prior to breeding), gilts in the Vac2/Ch1 and Vac2/UnCh groups were intramuscularly injected with a 2.0 mL dose of PRRSV-2 MLV vaccine (Fostera™ PRRS, Zoetis, Lot No. 169588, Serial No. 163540/159469, Expiration date 28 NOV 17, PRRS Potency 4.5 Log_{10} $TCID_{50}$/dose). At 0 dpc (93 days of gestation), pregnant gilts in the Vac2/Ch1 and UnVac/Ch1 groups were inoculated intranasally with 6 mL of tissue culture supernatant containing 10^4 $TCID_{50}$/mL of PRRSV-1 (SNUVR090485, 2nd passage in alveolar macrophages). Pregnant gilts in the Vac2/UnCh group were similarly inoculated with 6 mL of uninfected cell culture supernatant. Pregnant gilts were monitored daily by the same personnel. Farrowing data, including litter size [total number, live-born, stillborn, mummified, and light (<1 Kg body weight) per litter] at birth and when weaned at 21 days of age, were also recorded.

Results: Pregnant gilts from the UnVac/Ch1 group had premature farrowing with significantly shorter ($P < 0.05$) gestation periods compared to the Vac2/UnCh group which had normal farrowing. Gilts from the Vac2/Ch1 group had a significantly ($P < 0.05$) longer gestation period compared to the UnVac/Ch1 group however, they still had some premature farrowing with a significantly shorter ($P < 0.05$) gestation period compared to Vac2/UnCh group. Pregnant gilts from the Vac2/UnCh group had a significantly ($P < 0.05$) higher number of live-born and weaned piglets compared to pregnant gilts from the Vac2/Ch1 and UnVac/Ch1 groups. Pregnant gilts from the Vac2/Ch1 group had a significantly ($P < 0.05$) higher number of live-born and weaned piglets compared to pregnant gilts from the UnVac/Ch1 group. Pregnant gilts from the Vac2/UnCh group had a significantly ($P < 0.05$) lower number of stillborn piglets compared to pregnant gilts from the Vac2/Ch1 and UnVac/Ch1 groups. Pregnant gilts from the Vac2/Ch1 group had a significantly ($P < 0.05$) lower number of stillborn piglets compared to pregnant gilts from the UnVac/Ch1 group. No local or systemic adverse effects relative to vaccination were observed throughout the entire pregnancy period.

Conclusions: The results presented here demonstrate that a PRRSV-2 MLV vaccine can confer cross-protection against a heterologous PRRSV-1 challenge in late-term pregnancy gilts. Vaccinated gilts after challenge had a higher number of live-born and weaned piglets and at the same time a significantly lower number of stillborn piglets, compared to unvaccinated gilts. Good cross-protection of the PRRSV-2 MLV vaccine against PRRSV-1 is clinically significant.

Acknowledgement: The author's research was supported by contract research funds (Grant no. 550-20160053).

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Fostera® PRRS vaccinated pigs showed improved growth performance under field conditions in Japan

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases for the swine industry globally. In Japan almost all isolated PRRS viruses are Type 2 (North American genotype), which are classified into 5 clusters by ORF 5 sequence analysis [1]. Effective control of PRRS via vaccination strategy has been a main focus in the Japanese swine industry. Fostera® PRRS is a modified live vaccine containing a Type 2, Cluster I virus, which is classified in lineage 8 using the common international system. It was approved in Japan in 2018 for administration to piglets of 1 day of age or older. The product has already been reported to contribute to successful control of PRRS in North American and Asian countries [3]. The objective of the present study was to evaluate the efficacy of Fostera® PRRS vaccination to suckling piglets under field conditions in Japan.

Materials and Methods: A commercial pig farm of 320 sows, comprising a breeding-weaning site and a finishing site, was utilized for the study. Pigs were weaned weekly and each group was separated. The farm had suffered from PRRS at around 40 to 50 days of age and a commercial PRRS vaccine (Ingelvac® PRRS MLV) was being administered to sows four times a year, but the level of control was not considered sufficient. In August 2018, while continuing administration of the PRRS vaccine to sows, Fostera® PRRS vaccination to 3 day of age piglets was started. Blood samples were collected monthly from piglets of 3 to 7 weeks of age (5 piglets per week of age). All samples were tested for ELISA titers against PRRS virus, *Mycoplasma hyopneumoniae* (Mhp) and Porcine circo virus type 2 (PCV2), and for PRRS virus, Mhp and PCV2 by real-time PCR. When PRRS real-time PCR was positive, the sample was submitted for virus sequence analysis. Average daily gain (ADG) and mortality rate in the weaning stage were calculated and were compared to performance before starting the study.

Results: Clinical symptoms in weaned piglets improved after the start of vaccination of Fostera® PRRS to suckling piglets, and ADG was significantly improved ($p<0.05$) compared to the past when PRRS vaccination in suckling piglets was not implemented (Table 1). However, the mortality rate was not significantly different. During Fostera® PRRS vaccination, two wild types of PRRS virus were detected. Both wild type viruses were classified as Cluster IV by sequence analysis. ORF 5 homology between these wild type viruses and Fostera® PRRS were 87.2-89.5%. There were no significant changes in Mhp ELISA except once and PCV2 DNA was not detected during the study.

Table 1: Comparison of ADG and mortality rate between the periods when Fostera® PRRS was administered, or not administered, to suckling piglets.

	Jan - Aug 2018	Sep - Dec 2018
ADG	505.1g/day[a]	553.1g/day[b]
Mortality	5.4%	5.6%

(ab: $P<0.05$)

Conclusions: Fostera® PRRS was effective against heterologous PRRS wild type strains in Japan, as has been reported in other countries [3]. Vaccination of Fostera® PRRS to suckling piglets prevented loss of ADG even with PCR detection of PRRS wild type strains after starting vaccination. It was suggested that productivity and performance should be prioritized to evaluate vaccine efficacy, rather than detection of RNA of wild type strains.

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Tracking after outbreak of Porcine reproductive and respiratory syndrome in a negative farrow-to-finish farm without vaccination strategy

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is regarded as one of the most important health issues affecting pig production (1). Abortion storm is often seen when first infected to sow in a PRRS negative farm(2). This case report is a one of tracking record after PRRS outbreak in a negative farrow-to-finish farm in Korea.

Materials and Methods: 1. History - A. Information: 700-sow, farrow-to-finish, maintained PRRS negative farm before outbreak, B. PRRS confirmed: A farm manager reported to clinic seen abortion storm in gestation stage. PRRS confirmed via lab analysis by RT-PCR and ELISA on serum and aborted fetus. 2. Strategy - A. Farm owner wanted to do without PRRS vaccination protocol, so, herd closure, nursery depopulation, serum monitoring was selected. B. Herd closure: new gilts for using during 4-5 months were isolated before herd closure started. C. Nursery depopulation: it was performed when farrowing site was confirmed to stable via serum analysis. D. Monitoring: serum analysis was performed once a month. 3. Action plan - A. Gilts: herd closure. Health acclimation. B. Farrowing sows: elevating replacement rate, rebuilding parity structure. C. Suckling piglets: intensive care. D. Disinfectant: Virocid®. E. Biosecurity: installation of shower booth, parking zone (visitor only) outside farm. 3. Medication / Feeding additives - A. Sow: feeding immune boosters (PMCplus®), antibiotics to minimize co-infection (tylvalosin, bacitracin), B. Suckling: feeding premium meal (TheEU®), C. Weaning piglets: feeding electrolytes, vitamins, antibiotics (amoxycillin).

Results: Serum analysis results showed in table 1. Compared with at PRRS outbreak time, it was going well clinical sign, shown better productivity (total born, average piglets weaned per sow, PSY, etc) within first 6 months. General health status was recovered after 6 months abortion storm in gestating sow (data not shown).

Table 1. serum analysis for the detection of PRRSV antigen by the RT-PCR and antibody by the ELISA

	Outbreak		D+1 month		D+9 months		D+14 months	
	Ag	Ab	Ag	Ab	Ag	Ab	Ag	Ab
Gestating	EU	P	N	P	N/T	N/T	N	P
Lactating	EU	P	N	P	N	P	N	P
Suckling	EU	P	N	P	N	N	N	N
Weaning	EU	P	EU, NA	P	N/T	N/T	EU	P
Growing	EU	P	EU, NA	P	N/T	N/T	N	P

*Outbreak (PRRS outbreak), D+1 month (herd closure started), D+9 months (nursery depopulation started), Ag (antigen), Ab (antibody), EU (PRRS type Europe), NA (PRRS type North America), P (positive), N (negative), N/T (not tested)

Conclusions: Farm performance (productivity, health condition, PSY, etc.) is going well after 6 months from PRRS outbreak. But, despite herd closure, nursery depopulation, intensive care was performed, it only remains scientific PRRS positive farm.

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Reduction of PRRSV Vertical Transmission and Stabilization of Herd Immunity towards PRRS after Replacing Type I (EU) PRRS Vaccine with whole herd Ingelvac® PRRS MLV.

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure in sows and respiratory problems in growing pigs. The virus can be transmitted horizontally between pigs and vertically from sows to fetuses. In PRRS positive farms in Malaysia, the prevalence of type 2 (US) PRRSV is 81-100%, while it is only 25% for type 1 (EU) PRRSV. Furthermore, immunization by EU PRRSV only provide protection against secondary homologous EU strain PRRSV challenge but not heterologous US strain PRRSV; while US PRRSV immunization provides cross-protection against both strains. This study reports a case of PRRS outbreak, followed by improvement of herd immunity after replacing EU PRRS vaccine with whole herd Ingelvac® PRRS MLV (US strain).

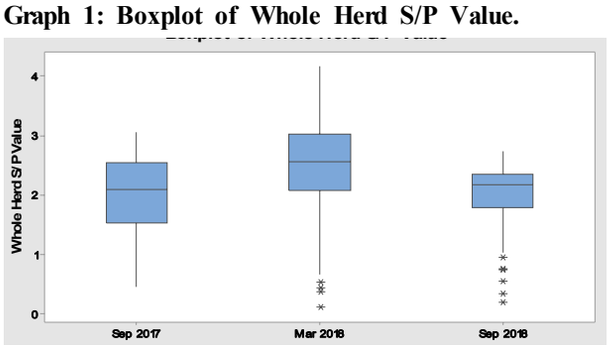
Materials and Methods: The farm is a single site, farrow to finish farm in Malaysia. In September- October 2017, the farm experienced spike in pre-weaning piglet mortality at 47.5% in one unit; increased nursery mortality at 20%; high abortion rate at 15%; and increased mummified fetus percentage to 5%. Necropsy was performed with only findings of lymphadenopathy and starvation. Lymph nodes and serum samples were sent for laboratory diagnosis. PCR results showed that the samples were negative for classical swine fever virus; serum samples of 1 and 4 weeks old (to be weaned) piglets were tested positive for US and EU strain PRRSV with 54% positive for US strain PRRSV (Table 1). While ELISA results showed that the farm is negative for Aujeszky's disease. With the diagnosis, the farm decided to stop EU PRRS MLV in breeders and EU PRRS inactivated vaccine in porkers and started to use Ingelvac® PRRS MLV whole herd (breeders and piglets) vaccination in November 2017, followed by sow booster vaccination a month later and revaccinate every 3 months. Serum samples were taken every quarter after the new vaccination program for PCR and ELISA test against PRRSV to monitor the herd health.

Results: After change of vaccination program; PCR results showed that the percentage of US and EU strain PRRSV positive piglets has been reduced from 54% to 0% and 14.3% to 0% respectively (Table1). For ELISA results, the median value and variability of whole herd sample to positive (S/P) ratio has been reduced (Graph 1). While farm performances have improved as pre-wean and nursery mortality was reduced to 9.9% from 47.5%. Results are summarized in Table 1 & Graph 1.

Table 1: Percent positive of PRRSV in 1 week old piglets.

PRRSV	Oct 2017	Mar 2018	June 2018
US Strain	54%	8.3%	0%
EU Strain	14.3%	0%	0%

Graph 1: Boxplot of Whole Herd S/P Value.



Conclusions: The reduction in percentage of PRRSV positive piglets to 0% in June 2018 after application of Ingelvac® PRRS MLV shows that the breeder herd immunity status has been stabilized, there is reduction of vertical transmission of PRRSV to the piglets and also elimination of field PRRSV. Besides that, the reduction of both median value and variability of whole herd S/P ratio can be interpreted as reduction of circulation/exposure of field virus and reflects a successful stabilization process. These results shows that whole herd breeder mass vaccination and piglet vaccination with Ingelvac® PRRS MLV is safe, besides being efficacious to provide protection and to eradicate field virus.

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Heat inactivation of multidrug resistant Salmonellae and potential of DNA fragments for antimicrobial resistance gene transformation to *E. coli* ATCC 25922

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Introduction: Antimicrobial resistance is a global issue. *Salmonella* contamination in fermented pork sausage (Nham-moo) in Northeastern Thailand has been demonstrated with high prevalence [1]. Nham-moo made from *Salmonella* contaminated pork could result in food poisoning if eaten raw. Should one be concern when eating dead bacteria. How much temperature and time are required for killing bacteria? *E. coli* is found elsewhere particularly in digestive tract. This study tried to demonstrate whether *E. coli* could pick up DNA fragments of *Salmonella* after heat inactivation.

Materials and Methods: Three multi-drug resistance *Salmonellae* (MDR) isolated from Nham-moo were chosen and used as antimicrobial (AMR) gene donor (1-Sal. RISSEN, 2- Sal. TYPHIMURIUM, 3-Sal. TYPHIMURIUM). The recipient for AMR genes was *E. coli* ATCC 25922 (antimicrobial sensitive-AMR gene free). The AMR of donors (1-3) and recipient were identified by disk diffusion (Cefotaxime-CF, Enrofloxacin-ENR, Nalidixic acid-NA, Streptomycin-S, Sulfamethoxazole-trimethoprim-SXT, Tetracycline-TET, (Oxoid^{TM}, USA), minimal inhibition concentration (Colistin-CT), and PCR (AMR genes-tetA, tetB, aadA, sul1, sul2, sul3, dfrA12, qnrS, parC, gyrB). To assure the donors were completely inactivated, they were pre-tested with heating at (55, 60, 70, 80, 90, 100 ℃ for 1, 2, 5, 10, 20, 30, 60 min.), re-cultured and enumerated. Two ml of *Salmonella* in sterile saline (0.5 McFarland) was heated at 100℃ for 30 min, and used as DNA donor. The resulting solution was checked for no viable bacteria after adding 1 ml into 9 ml Buffered peptone water (Difco^{TM}, USA) and incubated overnight at 37℃. Then 100 µl of *E. coli* ATCC 25922 (0.5 McFarland) was mixed with the inactivated donor suspension and set at 37℃ for 24-48 hr. The recipient *E. coli* was then cultured and biochemical identified, and tested for resistant patterns according to donor's resistance (TET, SXT, NA, S) by method following guideline of the CLSI 2017 standard. The transformation assay of each donor was repeated 50 times.

Results: The AMR characteristics were demonstrated in the Table. The resulting *E. coli* recipient was susceptible to all tested drugs.

Salmonella	Drug Sensitive	Drug resistance	tet A	tet B	aad A	sul1	sul2	sul3	dfr A12	qnr S	par C	gyr B
1	CF, ENR, NA, CT	TET, SXT, S	+	+	+	+	+	-	-	-	+	+
2	CF, ENR, NA, SXT, CT	TET, S	+	+	+	-	-	-	-	-	-	+
3	CF, ENR, SXT, CT	TET, NA, S	+	-	+	-	+	+	+	+	+	+

Tested by Disk diffusion method (Oxoid^{TM}, USA); Cefotaxime-CF, Enrofloxacin-ENR, Nalidixic acid-NA, Tetracycline-TET, Streptomycin-S, and by Minimum inhibition concentration-MIC (Colistin-CT)

Conclusions: We concluded that, AMR gene transformation from heat inactivated *Salmonella* isolates to *E. coli* was unlikely to occur under our experimental set. Since transformation is a rare event many replications or increased duration of exposure are needed. Although another researcher demonstrated horizontal transmission of plasmids between natural populations (live) of *E. coli* and *Salmonella* enterica [2]. Our experiment attempted to mimic natural way when eating cooked *Salmonella* contaminated meat.

Acknowledgement: to Miss Nawarat Rattanadilokna Phuket and the Regional Medical Sciences Center 7th, KhonKaen, Ministry of Public Health, KhonKaen, Thailand for *Salmonella* typing.

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Application of synbiotics-based feeding program to protect ileitis in pigs

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Introduction: Porcine proliferative ileitis is a major economic burden for the swine industry, affecting growing pigs and young adult pigs [1] for nutritional interventions to improve the development of intestinal structure and the maturation of the immune system, with lifelong effects. Synbiotics have both probiotic and prebiotic properties and were created in order to overcome some possible difficulties in the survival of probiotics in the gastrointestinal tract [2]. Therefore, an appropriate combination of both components in a single product should ensure a superior effect, compared to the activity of the probiotic or prebiotic alone. In this study, the protective efficacy of probiotics and prebiotics administration against LI infection was evaluated under LI challege experiments.

Materials and Methods: Twenty-five, four-week-old pigs on a commercial farrow-to-finish farm were used in this experiment. The pigs were randomly divided into 5 groups, and 4 groups were treated with different concentration of probiotics and/or prebiotics. All the group of pigs were necropsied at 28 days post-LI challenge. Incidence and severity of ileitis were evaluated by gross and microscopic observation of ileal tissues. Colonization of the gut after challenge was examined by LI-specific immunohistochemistry, and qPCR of ileal scrapings. Following the challenge, all pigs were observed daily for clinical signs of ileitis, including body condition, alertness, appetite, and consistency of the feces. Fecal consistency was scored as normal, soft or watery (mild diarrhea), bloody or tarry (severe diarrhea). Gross lesions were scored on the basis of the severity of mucosal thickening (0- normal, 1-slight edema/hyperemia, 2-moderate hyperemia/mucosal thickening, 3- severe mucosal thickening, 4-severe hemorrhaging/ necrosis/fibrinous exudate). Scores 2–4 were considered indicative of clinical ileitis. Histopathological examination following H&E staining to evaluate microscopic lesions, mainly proliferation, consistent with *L. intracellularis* (0- no lesions; 1- mild proliferation; 2- marked proliferative enterocolitis).

Results: Signs of clinical disease were monitored during the challenge phase of the experiment. Severe diarrhea was observed in 4 of 5 animals in the control group (80%), but not in any of the symbiotics-treated animals. Ileum gross lesion scoring is summarized in Fig. 1A. In the control group, 5 of 5 pigs (100%) were scored (score >3), whereas only 2 of the 5 (LC Pro + HC Pro group) (40%) were scored as positive. The occurrence of clinical ileitis was also evaluated by histopathological examination, on an ordinal scale 0–2 (Fig. 1B). Of the 5 controls, 5 (100%) were positive (score >0), with 2 pigs presenting with score 1 and 3 pigs with score 2. Of the LC Pro + HC Pro group, 2 of 5 (40%) were positive for microscopic lesions.

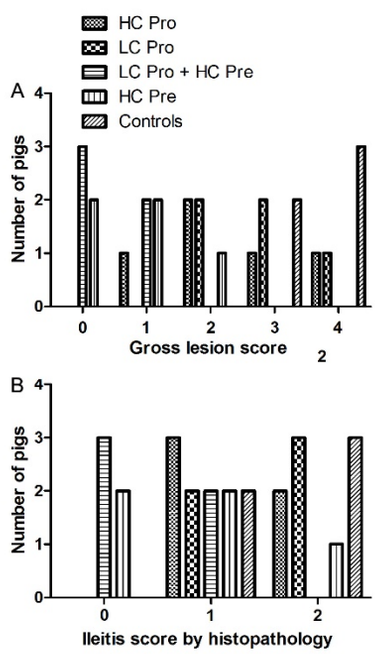

Fig. 1. Gross lesion and histopathology scoring of the protective efficacy of probiotics and prebiotics administration against LI infection.

Conclusions: The data demonstrated that this synbiotics-based feeding program was very effective to protect ileitis in pigs.

Acknowledgement: This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ013 22301)" Rural Development Administration, Republic of Korea.

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Species-level bacterial community profiling of the sow fecal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes

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Introduction: The sow barn accommodates sows in different parities from 1st parity to 8th more parity. It is already well known that piglets born to 2nd or 3rd parity-sows have a more stable health condition than piglets from 1st parity-sows. The importance of animal gut microbiome is widely acknowledged as its pivotal roles in the overall health and well-being of animals. The animal gastrointestinal tract is colonized by a complex ecosystem of microorganisms. Intestinal bacteria are not only commensal, but they also undergo a synbiotic co-evolution along with their host. Beneficial intestinal bacteria have numerous and important functions, e.g., they produce various nutrients for their host, prevent infections, and modulate the immune function. Newborn piglets in every farm have presented variable health conditions and response to vaccination, infection or clinical intervention. Those difference might be solved through exploring their mothers' gut microbiome. This study was performed to solve that question. The health of newborn piglets would vary according to the gut microbiome of sows in different parities, which were analyzed in this research on the assumption. It is necessary for the improved health condition of animals to achieve, restore, and maintain a favorable balance and its activity of intestinal microbiome in the gut ecosystem. In this study, we characterized the gut bacterial communities of 25 sows in different parities from feces via high-throughput sequencing technology on PacBio platform.

Materials and Methods: Twenty five fecal samples from 25 sows with different parity (parity 1, 2, 3, 4 and 8) were used to analyse in this study. The DNA was amplified with Illumina adapter and indexed PCR primers using a dual-index sequencing strategy to target the bacterial V1V2 16S rRNA gene. Each PCR was done in triplicate with 20 cycles with the same cycling conditions. PCR product was purified with AMPure XP beads (Agencourt Bioscience). The library size was confirmed on a Tape stations (Agilent Technologies) before submitting for MiSeq sequencing using the 600 cycle MiSeq reagent kit V3.

Results: As a result, the Parity 1 sow group consisted of 12 phyla, the Parity 2 and 3 sow groups consisted of 11 phyla, the Parity 4 and 8 sow groups consisted of 14 phyla. The result of relative abundance analysis suggested that the proportion of bacteria in *Tenericutes* disappeared as the parity increased (P<0.01). The principal coordinate analysis (PCoA) resulted that those sow groups had their own distinct clusters (Fig. 1).

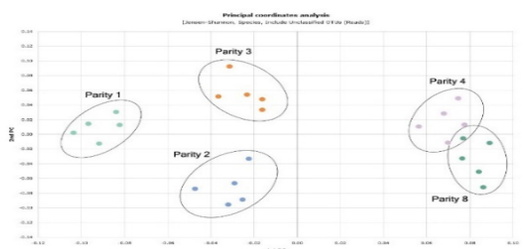

Fig. 1. Principal coordinate analysis (PCoA) was showed that the sows harbor distinct bacterial taxa. Based on membership, bacterial communities from sows with parity clustered together and separated from those from different parity sows which explained the largest amount of variation.

Conclusions: Pan-bacterial 16S rRNA microbiome surveys performed with massively parallel DNA sequencing technologies have transformed community microbiological studies. Herein, we present a microbiome analysis pipeline that takes advantage of PacBio circular consensus sequencing (CCS) technology to sequence and error correct full-length bacterial 16S rRNA genes, which provides high-fidelity species-level microbiome data. By parity distinct clusters of gut microbiome were observed and it is expected that those results and further related research will help to grasp more precise relationships between gut microbiome of sows in different parities and their piglets health status.

Acknowledgement: This research was supported by Technology Development Program (Project No. 1116043-1) for Bio-industry, Ministry for Agriculture, Food and Rural Affairs, Republic of Korea.

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A Comparative Study of Avirulent Strain of F4 and F18 *E.coli* Vaccines on Piglets Diarrhea

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Introduction: Post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) diarrhea are the most common enteric diseases in piglets. In general, supplementation of antibiotics is commonly used in swine industry in Thailand. However, reducing use of antibiotics has been implementing in the industry. The objective of this study was to study the efficacy of avirulent strain of F4 *E.coli* vaccines (Enterovac®) and F18 *E.coli* vaccines (Edemavac®) on performance in controlling diarrhea in piglets being challenged with enterotoxic $F4^+$ and $F18^+$ *E.coli*, respectively.

Materials and Methods: A total of 60 free-PRRS weaned pigs (18 day-old) were included in this study. Pig was individually raised in an open housed system. Exp. 1, pigs were divided into 3 groups, control 1 (C1) group (n=10), T1 group (n=10) challenged at 7 days post-vaccination with F4 *E.coli* vaccines (Enterovac®, Arko laboratories Ltd, USA.) (PV) and T2 group (n=10) challenged at 21 days PC. Exp. 2, pigs were divided into 3 groups, control 2 (C2) group (n=10), T3 group (n=10) challenged at 7 days post-vaccination with F18 *E.coli* vaccines (Edemavac®, Arko laboratories Ltd, USA.) and T4 group (n=10) challenged at 21 days PV. Pigs in Exp.1 were challenged with wild type of F4+/F18- *E.coli*, Exp.2 were challenged with wild type of F4-/F18+ *E.coli* isolated from necropsied pigs submitted to Veterinary Diagnostic Center, Kasetsart University at $2x10^8$ CFU/pig, orally at 7 and 21 days, accordingly. Clinical signs, fecal scoring, fecal *E.coli* count and production performances were recorded and analyzed.

Results: The number of fecal *E.coli* in vaccinated groups was lower than those of control group, from D1 until D10 post-challenged (PC), especially in T1 and T3 groups that challenged at 7 days PC ($p<0.05$). Pigs challenged at 21 days PC showed no differences in the number of fecal *E.coli*. Production parameters were shown in Table 1 and 2, for Exp. 1 and Exp. 2, respectively.

Table 1. production parameters in Exp. 1 pigs vaccinated with F4 *E.coli* vaccine (Enterovac®).

	Group					
	challenged 7 days PC			challenged 21 days PC		
	C1	T1	p	C1	T2	p
Wt gain (kg)	2.16 (0.61)	3.57 (1.02)	0.02	6.21 (1.86)	6.27 (1.63)	0.99
ADG g/day)	108 (30.28)	178 (50.9)	0.02	188 (56.38)	190 (49.2)	0.99
FI (kg)	4.41 (0.52)	5.38 (1.38)	0.07	10.35 (2.77)	11.61 (1.96)	0.40
FCR	2.18 (0.69)	1.53 (0.20)	0.10	1.73 (0.47)	1.93 (0.43)	0.46

Table 2. production parameters in Exp. 1 pigs vaccinated with F18 *E.coli* vaccine (Edemavac®).

	Group					
	challenged 7 days PC			challenged 21 days PC		
	C2	T3	p	C2	T4	p
Wt gain (kg)	1.85 (1.37)	3.25 (0.96)	0.05	8.01 (2.58)	6.79 (1.18)	0.24
ADG g/day)	92.50 (68.42)	162.5 (48.2)	0.05	242.73 (78.04)	205.6 (35.8)	0.24
FI (kg)	4.21 (0.88)	5.19 (1.08)	0.09	12.82 (3.39)	12.26 (1.44)	0.74
FCR	5.89 (7.42)	1.66 (0.31)	0.27	1.63 (0.26)	1.84 (0.28)	0.18

Conclusions: This study provides more information on vaccination-to-infection time of Enterovac® and Edemavac® vaccine against the wild type enterotoxic F4+ and F18+ *E.coli,* respectively. The results demonstrated that when *E.coli* infection occurred as early as 7 days after vaccination, the vaccine could provide higher protection against the infection. However, the vaccine could provide protection against the wild type F4+ and F18+ *E.coli* in both short and long vaccination-to-infection models.

Sensitivity of two serological Erysipelothrix rhusiopathiae tests on vaccine induced immune stimulation and protective immunity

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Introduction: No commercial test is available for indicating Erysipelothrix rhusiopathiae (Ery) protective immunity. The tests available are designed to reveal Ery field infection. This lack of readily available tools to evaluate vaccine induced protective immunity and vaccine take with a sufficient reliable sensitivity following Ery vaccination has already been addressed in experimental setup [1,4]. Anti-SpaA antibodies are identified as the central protective components in swine-Ery immunity[2,3]. A rSpaA415-microbead based serological fluorescent immunoassay (*SpaA-FMIA*) available for project-based testing has proven to have 100% sensitivity and specificity; far higher than commercial tests when compared[1], and following Parvoruvax®(Ceva) Ery-immunisation[4], under experimental settings. The aim of this study was to compare sensitivity in a commercial Ery-ELISA to the sensitivity of the *SpaA-FMIA*[1], in serum sampled in farms vaccinating with Parvoruvax® for Ery-protection.

Materials and Methods: In 2018, two Spanish and two German swine farms, with no apparent Ery circulation and already using Parvoruvax® for over one year, were sampled. On each farm, sera were collected cross-sectional in female breeding stock in 5 groups of 10 animals each. In all farms 10 non-vaccinated gilts of approximately 6 months of age and 10 gilts 4 weeks following the second of the two primo-vaccinations were sampled. As well as 3 different groups of sow parity one to five, >8 weeks following booster vaccination. The Ery protective immunity was assessed in a Luminex® based *SpaA-FMIA* [1], and the commercial test for Ery field infection, INgezim Mal Rojo (*INgezim MR*), Ingenasa, were both performed at the Roslin Institute, University of Edinburgh, Scotland, UK. Logistic regression was used to model the probability of positive *INgezim MR* test as a function of log10 *SpaA-FMIA* titre. The predicted positivity rate at 3.5 log10 of *SpaA-FMIA* with its 95% CI was obtained by the delta method.

Results: At low, protective *SpaA-FMIA* titres the *INgezim MR* provided a very high rate of negative results; At the lower positive titre (3000/3.5 log10) of *SpaA-FMIA, INgezim MR* has a predicted positive rate of only 8% (95% CI: 1% to 15%); hence 92% negatives (Fig.1). Increasing titres increases agreement of *INgezim MR* to *SpaA-FMIA*; close to the model predicted full agreement at 50,000/4.7log10 (Fig.1).

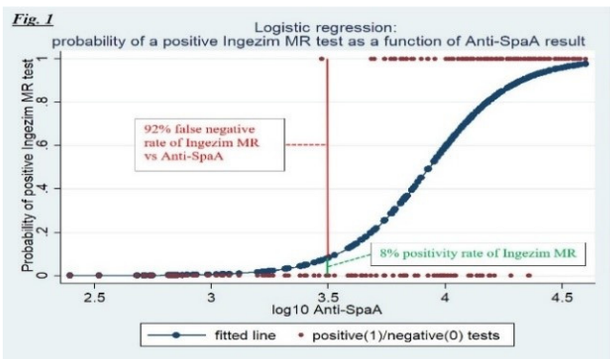

Fig 1. *Regression probability of a positive Ingezim MR test as a function of anti=spa result*

Conclusions: It takes a SpaA-based ELISA or FMIA to evaluate Ery vaccine efficacy and protective immunity [1]. Commercial Ery-ELISAs are not reliable tools for evaluating vaccine-take and protective efficacy, but designed for detecting previous Ery infection. Compared to the *SpaA-FMIA* test, the *INgezim MR*, is far less sensitive. At the low level of positive anti-SpaA antibodies the probability of a false negative is found to be 92% (Fig.1). The observation of low sensitivity on vaccine induced immune response in commercial Ery-ELISAs is in accordance with previous studies [1,4], showing the sensitivity of Ery-ELISAs, following vaccination, tested against the proven the 100% sensitive *SpaA-FMIA* revealing: only *22%* sensitivity of the *INgezim MR*, and only *44 %* sensitivity of the *Civtest Suis SE/MR*, Hipra [1].

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The correlation of porcine epidemic diarrhea virus virulent between serial passage in *in vitro* and *in vivo*

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Introduction: Porcine epidemic diarrhea virus (PEDV) is main causative agent of enteric disease in all ages of pigs especially in naïve piglets (1). Crucial clinical signs of PEDV are acute watery diarrhea, vomiting, dehydration, and death in eventually. Main virulent gene of the virus is spike (S) gene, consist of S1 and S2 domains, which play an important role in cell entry. Moreover, S gene have higher diversity rate (2). The aims of this study were evaluated genetic variation of S gene compared to prototype strain and viral distribution in small intestine.

Materials and Methods: The study is divided into two ways: 1) *In vitro*; PEDV P3-3/18 JPF strain was passaged in Vero cells (ATCC®CRL-1586™) for 5 passages, called P0-P5. Ten ml of virus P0, P2, and P5 were separately orally challenged in 3 days-old paiglets (F1, n=3), called P0F1, P2F1, and P5F1, respectively.2) *In vivo*; the intestinal samples of P0F1, P2F1, and P5F1 were chopped and 10 ml (1 mg/ml) of each sample were inoculated to new 3 days-old piglets (F2, n=3), called P0F2, P2F2, and P5F2, respectively. Then, the intestinal samples of P0F2, P2F2, and P5F2 were chopped and 10 ml (1 mg/ml) of each sample were inoculated to new 3 days-old piglets (F3, n=3), called P0F3, P2F3, and P5F3, respectively. Intestinal samples were collected from all piglets for whole S gene characterization. Moreover, five parts of small intestine were fixed in 10% formalin and immunohistochemistry (IHC) was performed IHC score was determined by semi-quantitatively scored with criteria: 0 = no staining; 1= 1-10%; 2 = 10-25%; 3 = 25-50%; and 4 = 50-100% positive signal in enterocyte.

Results: The result of sequence analysis showed that sequence identities were 99.9% in P.1, 99.6% in P0F1, 99.8% in P0F2, 99.9% in P0F3, 98.1% in P2F2, 99.7% in P2F3 and 95.9% in P5F3 compared to the original strain. Nevertheless, P2F1, P5F1, and P5F2 were negative by RT-PCR. Amino acid (aa) insertions at position 312-316 (^{312}EDLKS^{316}) and 1162-1163(^{1162}LI^{1163}) were found in P5F3.IHC score (mean±SD) was showed in Table 1

Table 1. Immunohistochemistry score in mean±SD of five sections small intestine samples

Passage in cell	Parts of intestine	Piglets (mean±SD)		
		F1	F2	F3
P.0	Duodenum	2.67±0.57	2±0.00	2.67±0.57
	Prox. jejunum	1.33±0.57	1.33±0.57	3.67±0.57
	Mid. jejunum	3±0.00	1.33±0.57	3±0.00
	Dis. jejunum	3.83±0.28	2±0.00	3.33±1.15
	Ileum	3.67±0.57	2.67±0.57	3.67±0.57
P.2	Duodenum	0.33±0.57	1.33±0.57	3±0.00
	Prox. jejunum	0.33±0.57	1.67±0.57	2.33±0.57
	Mid. jejunum	1±0.00	3±0.00	0.67±0.57
	Dis. jejunum	0.67±0.57	3.67±0.57	0.67±0.57
	Ileum	1±0.00	0.67±0.57	1±0.00
P.5	Duodenum	0.67±0.57	0.67±0.57	1.67±0.57
	Prox. jejunum	0	0.67±0.57	2.67±0.57
	Mid. jejunum	0	2.67±0.57	1.33±0.57
	Dis. jejunum	0	1.33±0.57	1±0.00
	Ileum	0	1.67±0.57	0

Discussions: The result showed that P2F1, P5F1 and P5F2 were negative for PEDV by RT-PCR but positive by IHC. After in vivo passaged, the virus was returned to positive by RT-PCR and IHC. The results confirm existence of the virus, but the virus was attenuated by passaged in Vero cells. The reasons of insertion in P5F3 is unknown but might be the attenuated virus have tried to turn virulent in natural host. In the future, many questions from this study should be clarified.

Acknowledgement: Partial funding was provided by the Special Task Force for Activating Research (STAR), Swine Viral Evolution and Vaccine Research (SVEVR)

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Double-inactivation of nsp16 methyl transferase activity and S protein endocytosis signal increases innate immune response and confers complete protection from PEDV infection

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Introduction: Porcine epidemic diarrhea virus (PEDV) causes high mortality in neonatal piglets. Viral-vectored subunit vaccine and inactivated vaccine are available, but their efficacies remain less satisfactory. From the knowledge of other swine enteric diseases, oral vaccination of sows with a live attenuated virus (LAV) is a promising approach to protecting neonates from virulent infection. A reversion-resistant vaccine candidate may be rationally designed and generated by reverse genetics using an infectious clone. The PEDV S protein contains a specific endocytosis signal in the cytoplasmic tail, and nsp16 is one of three viral methyltransferases and catalyzes 2'-O methylation in the 5'cap of the viral genome. In the present study, these two functions have been removed from PEDV. A mutant PEDV has been generated and their virulence and protective efficacy have been examined in gnotobiotic pigs.

Materials and Methods: Vero and IPEQ-DQ cells [1] were cultivated in DMEM containing 5% serum and RPMI 1640 containing 10% serum, respectively. To remove the endocytosis signal of the S protein, Y1378A mutation was introduced using an infectious clone [2]. For nsp16, KDKE^{4A} mutations were introduced. Subsequently, a double-mutant PEDV for S and nsp16 was generated. For determination of type I and type III IFNs, Dual-luciferase reporter assays were conducted using the IFN-λ 1-Luc or IFN-β -Luc plasmid (0.3 μ g /well) and pRL-TK (0.03 μ g /well) for 24 h. Cell lysates were prepared, and luciferase activities were determined (Promega). Gnotobiotic (Gn) piglets were obtained from two PEDV-naïve sows. Total 29 piglets were randomly divided into four groups, four piglets per group. At 4 days of age, Gn piglets were orally inoculated with recombinant PEDV (100 PFU/pig). At 21 dpi, each pig was challenged orally with 6 $\log_{10}$ PFU of PEDV and maintained for 9 more days to evaluate protection.

Results: A main hypothesis is that inactivation of the 2'-O methyltransferase activity of nsp16 and the endocytosis signal of the S protein attenuates PEDV but retains the immunogenicity in pigs. Using the virulent PEDV infectious clone, KDKE to AAAA substitution (KDKE^{4A}) was introduced in the catalytic tetrad of nsp16. Then, KDKE^{4A}-SYA was constructed by mutating the endocytosis signal of the Spike protein. KDKE^{4A}-SYA virus replicated in cells and induced stronger type I and type III interferon responses. In gnotobiotic piglets, the virulence of KDKE4A-SYA was significantly reduced, and mortality rate was 0%. At 21 days post-inoculation, the animals were challenged orally with a high dose of virulent PEDV. The KDKE^{4A}-SYA-inoculated pigs were protected from the challenge and no pigs in this group developed diarrhea, whereas all pigs in the control group developed severe diarrhea accompanied with 33% mortality. No reversion occurred in pigs for the KDKE^{4A}-SYA virus after three passages.

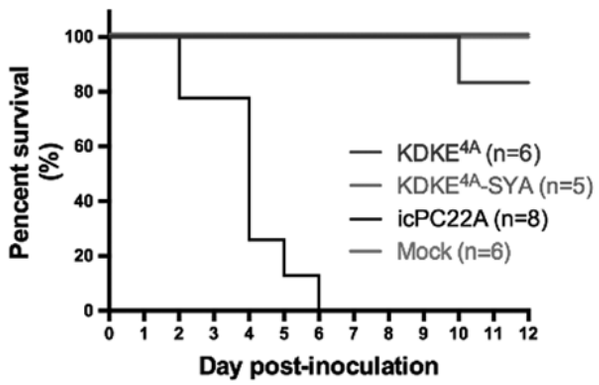

Figure 1. Protection of pigs vaccinated with the S and nsp16 double-mutant PEDV.

Conclusions: The data demonstrates that KDKE^{4A}-SYA may be an effective PEDV vaccine candidate.

Acknowledgements: This project was supported by USDA NIFA Competitive Grants no. 2015-67015-23067 to QW and no. 2018-67015-28287 to DY.

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Effect of supplemental nutrition through an isotonic protein solution provided days 2 to 8 of life on pre-weaning mortality of piglets and their lifetime performance

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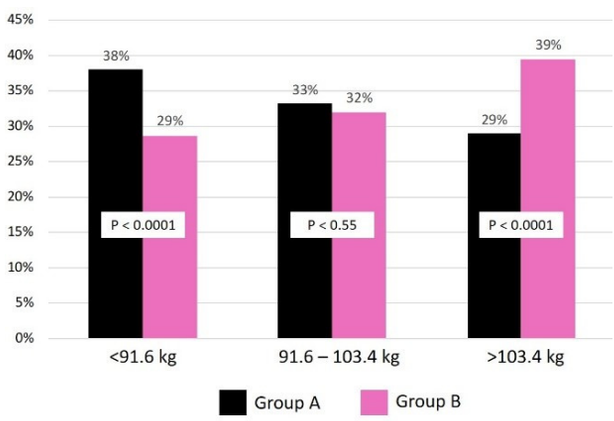
Introduction: Right after birth, the small intestine of the piglet goes through tremendous development. This period is described as a "window of opportunity"[1] for nutritional interventions to improve the development of intestinal structure and the maturation of the immune system, with lifelong effects. The aim of this study was to test if an isotonic protein solution provided in early life would increase farrowing survival and weaning weight, and if those improvements in weight would persist through harvest.

Materials and Methods: The study involved 3862 piglets farrowed from 353 gilts. Litters were allocated to one of 2 groups, control (Group A-1969 piglets) or supplementation with an isotonic protein drink (Group B-1893 piglets). All piglets were identified at day 2 with individual ear tags. Group A litters received only pelleted creep feed from birth to weaning. Group B litters were given 250 ml of a 3% isotonic protein solution on day 2 of age, and 500 ml from days 3-8 of age, once daily in an open pan. Group B also had access to pelleted creep feed before weaning. Group B litters also received the 3% solution three, two, and one days before weaning. Wean age was from 17 to 25 days. Pigs were moved to two separate nursery sites, where Group A pigs received the standard nursery diet in their feeder and free choice water. Group B pigs received the isotonic protein solutions in open feeding pans as follows: day of arrival - 7.5 litres per pen of 3% liquid (no feed added), day 1 - 7.5 litres per pen of 1.5% liquid mixed with feed, day 2 - 7.5 litres per pen of 0.75% liquid mixed with feed. Individual pig weights for all pigs were recorded at birth, weaning, end of nursery and 1 week pre-harvest. Mortality was recorded for each period.

Results: Pre-weaning mortality was reduced from 14.2% in Group A to 10.9% in Group B, a 22.8% reduction consistent with results observed in multiple previous studies.

The mean birthweight of Group B pigs was 1.56 kg, and 1.49 kg for Group A (P = 0.007). For weaning and subsequent weight analysis, any variability due to age and birth weight were neutralized by including those factors as covariates in the model. The mean weaning weight was not significantly different between groups. At the end of nursery, Group B pigs were 0.41 kg (1.7%) heavier than Group A pigs (P= 0.10). At the end of the finisher stage, pigs from Group B were 3.08 kg (3.2%) heavier than Control pigs. This difference was highly significant at P = 0.002. Group B also had less pigs finishing at <91.6 kg, and more pigs finishing at >103.4 kg (Figure 1).

Figure 1. Comparison of Pig Finishing Weight



Conclusions: This new study further supports previous reports demonstrating the ability of the isotonic solution to stimulate the intestine in that early life window of opportunity and to provide the potential for reduced pre-weaning mortality and accelerated growth in the post nursery phase.

References:

[1] Torow N. et al., 2017. J. Immunol. 198:557-563

APVS 2019
Asian Pig Veterinary Society Congress 2019

Poster Abstracts

ASF

Enteric Bacterial Diseases [Ent]

Immunology & Vaccine [Imm]

Major Bacterial Diseases (Enteric) [Bact(Ent)]

Major Bacterial Diseases (Respiratory) [Bact(Res)]

PCVAD

Practical Line (etc.) [Prac]

Production & Animal Welfare [Prod&Wel]

PRRS

Respiratory Bacterial Diseases [Respi]

Swine Enteric Coronavirus Diseases [Corona]

Transboundary Diseases (CSF, FMD, SIV etc.) [Trans]

ASF-001

Biosecurity risk assessment of ASF in China by ASF COMBAT

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Introduction: This study shows the first survey on the biosecurity situation of pig farms in China in the face of ASF outbreaks.

Materials and Methods: The survey tool used is the ASF COMBAT which is an online- management biosecurity assessment software (https://prevent-asf.com), which includes external risk assessment and location risk assessment. All questions were developed as an online questionnaire and sent to customer's mobile phone by WeChat App, and then were filled and submitted by themselves. This tool enables pig producers to evaluate areas for biosecurity improvement on their farms by completing a short questionnaire based on ASF virus scientific literature and practical experience, intended to help reducing the ASF introduction risk level. The survey started in Oct and ended in Dec 2018. Finally, 1554 valid questionnaires were collected, and they came from 31 cities and provinces of China, 9 come from the other countries.

Results: The results of the survey showed that 15.7% farms brought pork products into farms, 6.05% farms used swill to feed pigs, providing evidence that feeding/food with contaminated pork could be an infection risk.
In 13.71% of the farms proximity to wild boars and in 12.25% free range pigs other pigs were another possible risk of infection - Pig density and distance to main transportations roads, pig processing plants represented the biggest risk for ASF infection as outlined in table 1

Location	Proportion affected (%)
Farms within 5 km to main road	49.68
Farms between 5-10 km to main road	26.5
Farms within 5 km to the other farm	56.76
Farms between 5-10 km to the other farm	25.23

At that time, the proportion of the farms were outside 3 km, 10 km and 50 km of the epidemic area was 7.72%, 8.7% and 17.57% respectively.

Table 2 summarizes the risk associated with transport vehicles

Transport/Vehicle	Proportion (%)
Farm not restricted to trucks	19.31
Trucks used at different sites with washing/Disinfection	50.77
Trucks washed before used for transport	78.31
Trucks disinfected before used for transport	75.68
Drying before transport	47.94
Heat assisted technology for drying	8.69
Truck driver restrictions	23.49
Unattached animal transfer station located away from the farm	27.61
Physical barriers to restrict drivers	48.91

Table 3 summarizes the risk associated with feed and management

Feeding/Management	Proportion (%)
Feed may come from epidemic area	21.69
Visitors entering farm with previous isolation	25.29
Visitors entering farm without previous isolation	13.71
No visitors allowed in farm	9.38
No biosecurity training	24.07
Biosecurity training	33.72
Gilt isolation less than 7 days	8.94
Gilt isolation 7-30 days	28.7
Gilt recruitment within farm	62.36
Using external boars	4.83
Using external semen	14.29
Internal AI or boars on site	59.01

Conclusion:
This survey showed the most important risks parameters related to biosecurity of ASF in China. We can find that there are huge risks in many biosecurity aspects such as truck, feed, swill and loading. We need to improve biosecurity related measures systematically and strengthen staffs training to reduce the prevalence of ASF.

Keywords: ASF, COMBAT, biosecurity, China

ASF-002

Detection of African swine fever virus for border inspection in Taiwan

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Introduction: African swine fever (ASF) is a large, complex and multi-enveloped DNA virus, classified as the sole member of the genus *Asfivirus* within family *Asfarviridae*. ASF is a serious viral disease of pigs that can cause fever, internal bleeding and high death rates. It is highly contagious and can spread rapidly through both direct and indirect contact with infected pigs or pig products. This happens through contact with the blood, tissues, secretions and excretions from infected pigs. In August 2018 the first case of ASF was detected in China. This disease is now widespread across a number of continents and poses a major threat to pig producing, including Taiwan. For these reasons, Animal Health Research Institute (AHRI) has been routinely using the real-time polymerase chain reaction (real-time PCR) and nested-PCR assay to seized pork and pork products from airports and harbors in Taiwan since the 27th August 2018.

Materials and Methods: Various seized pork and pork products were collected from each airport and harbor between the 27th August 2018 and 24th April 2019. Viral DNA was extracted from each vaccine using the QIAamp DNA Mini Extraction Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The specific real-time PCR and nested-PCR for ASF were performed as OIE terrestrial manual 2018 [1, 2]. Assign a threshold cycle (CT) value to each real-time PCR reaction from a scan of all amplification plots. Negative test samples, negative or extraction blank controls should have a CT value >40.0. Positive test samples and controls should have a CT value < 40.0 [1]. The nested-PCR products were analysed by electrophoresis using 2% agarose gel containing 0.5 mg/ml SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA). The nested-PCR amplified DNA fragments were sequenced by the direct sequencing method using the BigDye^{TM} Terminator Cycle Sequencing kit and the ABI 3730 DNA sequencer (Applied Biosystems, Foster City, California, USA).

Results: A total of 1371 seized pork and pork products were analyzed for ASF between 27t^{h} August 2018 and 24th April 2019. Fourty two seized pork and pork products were ASF positive using real-time PCR and nested-PCR. The sequences of nested-PCR amplified DNA fragments from the 42 ASF positive seized pork and pork products were aligned with ASF VP72 showing high degrees of 100% similarity (data not shown). Among the 42 ASF positive seized pork and pork products, 40 from China, and 2 from Vietnam.

Table 1. Detection of ASF in seized pork and pork products from airports and harbors in Taiwan between 27th August 2018 and 24th April 2019.

Country	Years				Total
	2018		2019		
	negative	positive	negative	positive	
China	619	7	488	33	1147
Vietnam	20	0	82	2	104
other	68	0	52	0	120
Total	707	7	622	35	1371

Conclusions: Both real-time PCR and nest PCR were rapid, specific and sensitive diagnostic methods for the detection of ASFV in clinical samples. The preliminary results indicated that a combination of the real-time PCR and nest PCR could be used to detect ASFV in seized pork products on border control. It is helpful to detect the contamination of pork product in border inspection.

Acknowledgement: This work was supported by a grant 107AS-8.1.1-HI-HD to Chun Wang from the Council of Agriculture (COA), The authors are grateful to Dr. Jen-Chieh Chang, Dr. Fan Lee, Dr. Chien-Yuan Huang and Dr. Tsung-Wen Hsu at Animal Health Research Institute, Council of Agriculture, Tamsui, Taipei, Taiwan, for their excellent technical help.

References:

[1] The World Organization for Animal Health (OIE) terrestrial manual 2018.
[2] King et al., 2003. J. Virol Methods. 107, 53-63.

ASF-003

Development of Lab-on-a-Chip (LabChip)-based real-time PCR assay for the detection of African Swine Fever Virus (ASFV)

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Introduction: African swine fever (ASF) is an OIE (World Organization for Animal Health)-listed, highly contagious and devastating disease of domestic pigs and wild boar that causes significant economic losses to the pig industry in affected countries [1]. The virus typically causes hemorrhagic fever with high mortality in domestic pigs and wild boar, whereas infections in African wild suids, such as warthogs and bushpigs, run a nonpathogenic course [2]. Due to the current unavailability of vaccines or treatments for ASF, which is caused by African swine fever virus (ASFV), rapid and reliable detection of the virus is essential for timely implementation of emergency control measures and differentiation of ASF from other swine diseases with similar clinical presentations. In this study, a novel Lab-on-a-Chip (LabChip)-based real-time PCR assay was developed for detection of ASFV.

Materials and Methods: Three ASFV DNAs obtained from the outbreaks in Armenia in 2007 (Arm07), Ukraine in 2012 (Ukr12/Zapo), and Lithuania in 2014 (LT14/1490) were used. The specificity of the novel PCR was evaluated by testing other porcine viruses, including CSFV, PRRSV, PRV, PCV2 and PPV. In order to determine the detection limit of the test, the PCR assay was performed on serial tenfold dilutions of the prepared ASFV genomic DNA standard (from 10^5 HAU/ml to 10^0 HAU/ml). The novel PCR assay was further examined by comparison with two OIE PCR tests following the procedures as described previously. It takes a about 10-20 min for 45 cycles of real-time PCR for DNA of real-time PCR (RT-qPCR) using our Veri-Q PCR316 system (Fig. 1).

Results: To confirm the specificity of the novel PCR, other swine viruses were tested with the assay. As expected, the new PCR assay amplified all 3 ASFV DNA preparations and did not give any positive results when several non-ASFV swine viruses were examined, including CSFV, PRRSV, PRV, PCV2 and PPV.
The novel PCR assay detected a minimum of 10^1 HAU/ml of the ASFV DNA standard. Comparison tests with two OIE PCR assays using $PPA_{1/2}$ primers or OIE-F/R primers showed that the novel PCR yielded stronger signals than the others .

Fig. 1. LabChip_16CH is made of transparent polycarbonate material. It is a lab-on-a-chip (LabChip) product that combines micro mold, mass film bonding technology and microfluidic technology.

Conclusions: In summary, a novel LabChip-based real-time PCR assay was developed to enable the detection of all possible circulating ASFV strains. The novel PCR shows higher sensitivity than the OIE-validated PCR assays, and a high level of agreement with the highly sensitive UPL-based real-time PCR. We believe that this novel real-time PCR could provide reliable diagnosis and surveillance of ASF.

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[2] Luo Y, et al., 2017. Arch. Virol. 162: 191-199

ASF-004

Evaluation of a Rapid Chromatographic Strip Test for The Pen-side Detection of African Swine Fever Virus P30 Antibodies

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Introduction: Pen-side detection of the African swine fever virus (ASFV) infection is an important part of African swine fever surveillance. However, the samples have to be sent back to laboratories for testing. This is time consuming and may effect control. To solve these problems, Excelsior Bio-System has developed a rapid chromatographic strip test (Sentinel® ASFV Ab RT) for detection of specific antibodies against the ASFV early expressed P30 antigen [1]. To examine the detection efficacy of EBS Sentinel® ASFV Ab RT, we had a test evaluation conducted in an OIE reference laboratory (The Pirbright Institute, UK). The results of our rapid test were compared with those of a competitive ASFV antibody ELISA.

Materials and Methods: The serum samples are collected from minipigs inoculated with ASFV OURT88/3 strain (genotype I) on day 0, challenged with OURT88/1 (genotype I) on day 21 and then re-challenged with Georgia 2007/1 (genotype II) on day 42. The standard antibody level of serum was determined by the competitive ASFV antibody ELISA. Percent inhibition (PI) $\leq$ 40% indicate a negative result. PI $\geq$ 50% indicates a positive result. To evaluate the specificity of ASFV Ab RT, 130 negative sera were collected from field pigs of Taiwan. For the ASFV Ab RT, 10 μ L serum sample was added into a sample well of ASFV Ab RT and the result was determined at 10 minutes. A colored T-line indicated a positive result.

Results: The average percent inhibition (PI) values of 0, 5 and 8 days post-vaccination (DPV) of OURT88/3 were 9.15%, 9.36% and 13.46%, respectively. For the 0 and 5 DPV samples, there was no color development at the T-line of ASFV Ab RT, indicating a negative result. Day 0 and Day 5 samples also tested negative for antibodies in the ELISA. However, one 8 DPV sample which tested negative in the ELISA (PI = 13.65%), showed an inconclusive result on ASFV Ab RT. Additionally, one 14 DPV sample which show an inconclusive result from ELISA (PI = 48.67 %), also showed an inconclusive result on ASFV Ab RT. The average PI value on 21 DPV was 64.14%. After the pigs were challenged with the OURT88/1 and Georgia 2007/1, the average PI values became 83.05% on 28 DPV and 102.28% on 63 DPV. All samples from 21 to 63 DPV developed clear positive signals on ASFV Ab RT. The specificity of ASFV Ab RT is 96.92 % (126/130).

Table 1. Comparative results of ASFV Ab RT and ASFV Ab ELISA

		ASFV Ab RT	ASFV Ab ELISA	
Pig	DPV	Result	Result	Average PI %
C926	0	-	-	14.22
	5	-	-	8.76
	8	+/-	-	13.65
	14	+	+	78.22
	21	+	+	75.72
	28	+	+	86.63
	42	+	+	95.56
	49	+	+	100.40
	63	+	+	103.05
C927	0	-	-	4.08
	5	-	-	9.96
	8	-	-	13.27
	14	+/-	+/-	48.67
	21	+	+	52.56
	28	+	+	79.49
	42	+	+	101.25
	49	+	+	103.45
	63	+	+	101.50

+/-, inconclusion, +, Positive., Negative.

Conclusions: Sentinel® ASFV Ab RT is a highly effective kit for the detection of anti-ASFV antibodies. Sentinel® ASFV Ab RT kit is comparable to current competitive ASFV antibody ELISA. Sentinel® ASFV Antibody Rapid Test is reliable, easy-to-use and quick to detect the anti-ASFV specific antibodies. The ASFV Rapid Test has high potential to be used as a surveillance tool for pen-side and laboratory testing.

Acknowledgement: We gratefully acknowledge Dr. Carrie Batten, Dr. John Flannery and Mr. Matt Tully from The Pirbright Institute, UK for evaluation of the Sentinel® ASFV Ab RT.

References:

[1] Afonso CL et al., 1992. Virology. 189(1), 368-373.

ASF-005

Evaluation of VDx® ASFV qPCR in Poland

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Introduction: African swine fever virus (ASFV) is a large double stranded DNA virus with lipoprotein envelop and belongs to a genus Asfivirus and a family *Asfaviridae*. ASFV is highly resistant in the natural environment just like other DNA viruses. ASFV can infect all *Suidae* by biting of the infected tick, direct contact with sick animals or by ingesting by products derived from the infected animals. The characteristic symptoms of ASF is high fever, loss of appetite, hemorrhages in skin and internal organs, and high mortality in pigs. ASF is also one of the economically important diseases and has become a real threat in eastern Europe and China. In this situation, many countries near by China were highly concerned about the risk of transmission and the fast and exact diagnosis of ASF is of importance. In this study, the performance of ASFV qPCR kit was evaluated by testing standard panel and field samples of Poland in 2018.

Materials and Methods: VDx® ASFV qPCR Kit is a commercial kit for detection of viral DNA of ASFV by real time PCR method and this kit can measure simultaneously the p72 gene of ASFV and an exogenous Internal process control (IPC) quantatively by using TaqMan probe. The viral DNAs were extracted from 60 test samples (blood, serum and tissue homogenates from pigs and isolated virus) by using QIAmp DNA Mini Kit (Qiagen) and real time PCR was conducted by using 7500AB capable of reading Fluorescence Dyes FAM and HEX (or VIC). All of the samples used for the evaluation were previously tested by the real time PCR UPL tests recommended by EURL in Valdeolmos in Spain and by the International diagnostic procedures, sampling methods and criteria Animal Health Organization (OIE). In addition, the compliance of the obtained results with the EY requirements included in the decision of the European Union commission No. 2003/422 EC of May 26, 2003 evaluation of laboratory tests results to confirm ASF.

Results: A set of 6 ASFV strains of different genotypes (I, II, V, VIII, IX, X) isolated in 2016 and 34 ASFV-infected samples extracted from wild boars and pigs' tissue in Poland, 2018 were tested. All strains and field samples were detected and the result displayed 100% identity between VDx® ASFV qPCR kit and Fernandez- Pinero method (UPL-162 probe Table1).

Table 1. Sensitivity and specificity of VDx® ASFV qPCR kit

Real time PCR UPL		VDx® ASFV qPCR kit		Total
		Positive	Negative	
Positive (n=40)	Virus (n=6)	6	0	40
	Tissues (n=34)	34	0	
Negative (n=20)		0	20	20
Total (n=60)		40	20	60
Sensitivity	**100**	**Specificity**	**100**	

The limit of detection (LOD) of VDx® ASFV qPCR kit was estimated to be 1 copy of nucleic acid per PCR when evaluating a quantified ASFV plasmid. The LOD of VDx® ASFV qPCR kit, evaluated on ASFV's DNA with known viral titer, is estimated to be $10^{1.16}$ HAD_{50} per mL.

Conclusion: The sensitivity and specificity, evaluated on different genotypes of ASFV and 54 field samples of Poland in 2018 (positive and negative) displayed 100% sensitivity and 100% specificity. The LOD of the PCR was 1 copy of synthesized DNA per PCR and the experimental LOD was $10^{1.16}$ HAD_{50} of ASFV genotype II per mL. VDx® ASFV qPCR Kit could be a useful tool for early detection of ASFV in various materials from infected animals in order to identify the free state of pigs and it's derived materials for trade.

References:

[1] FAO Animal production and health Manual, African Swine Fever: Detection and Diagnosis, 2017
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[3] Fernandez-Pinero et al., 2011, Molecular Diagnosis of African Swine Fever by a New Real time PCR using Universal Probe Library, Transboundary and Emerging Diseases

ASF-006

Evolutionary phylodynamics of foot-and-mouth disease virus serotypes O circulating in 2006-2017

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease of domestic and wild cloven hooved animals across the world [1]. FMD virus (FMDV) is a single stranded positive sense RNA virus (genus Aphthovirus, family Picornaviridae) and exists as seven immunologically distinct serotypes, O, A, C, Asia 1, SAT (Southern African Territory) 1, 2 and 3, each with a wide spectrum of antigenically distinct subtypes[1,2]. Although worldwide most outbreaks are caused by viruses of serotypes O and A, any strain might emerge either by accidental or deliberate actions. Since the large scale of FMD outbreak occurred in Korea in 2010-2011, nationwide vaccination policy has been launched for the prevention and control of the disease. The FMD vaccines used in Korea consists of inactivated FMD virus (FMDV) antigens with oil-based w/o/w or w/o emulsion. Antigen formulation of the vaccines have been diversified according to epidemiological trends in Korea and neighboring countries. In this study, we investigated outbreaks occurring during 2006-2017 in the worldwide using complete sequence analysis of the FMDV serotype O VP1 coding region. Its designed to carry out a systematic study to select and appropriate serotype O vaccine strain for FMD control.

Materials and Methods: This investigation of phylodynamics in WRLFMD report was based on 112 FMD serotype O VP1 coding region sequences from the available FMDV sequence in the Genbank database. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 software using complete VP1 coding region sequence. Briefly, MEGA 6 software was used for the alignment of the sequence and the Kimura 2-parameter model was used for evolutionary analysis of serotype O viruses.

Results: Our results showed the nucleotide sequence identity among 112 FMDV serotype O diversity at a level of 79.1-100%. Moreover, phylogenetic analysis demonstrated that the VP1 coding gene of the FMDV serotype O was clustered into four distinct viral topotypes, ME-SA, SEA, EA and CATHAY

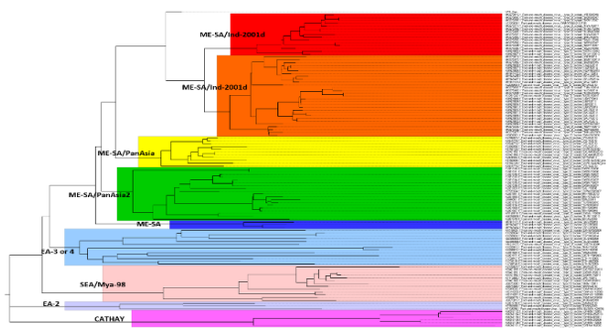

Figure. 1. Maximum likelihood phylogenetic tree generated using nucleotide sequence of the VP1-coding region of FMDV serotype O from WRLFMD report. Sequences with circles were obtained during this study in 2006-2108

Figure. 2. Signature amino acid residues in VP1 of serotype O FMDVs in this study. Only sequences different from the consensus are shown.

Conclusion: In this study also suggest that more FMDV surveillance studies will be necessary in order to evaluate the genetic relationship and efficacy of the current used vaccines against FMDV serotypes circulating in Korea.

Acknowledgement: This study was supported by a grant (N-1543386-2018-22-01) from the Animal and Plant Quarantine Agency's National Animal Disease Research Project.

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[1] Mana Mahapatra et al., 2017. Vaccine. 35, 7147-7153.
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ASF-007

Surveillance of African Swine Fever Virus in wild boar in Republic of Korea

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Introduction: African swine fever (ASF) is highly contagious viral disease for pigs which currently has an impact on many countries. The first case in China, which accounts for more than 50% of the pig population worldwide, was reported on 3 August 2018. To date, ASFV spread to Vietnam, Mongol and Cambodia in Asia. In addition, China and European countries have reported ASF in wild boar. The wild boar has been implicated in the spread and persistence of ASF. Therefore, for prevention of the introduction, early detection of the virus in wild boar is also important. This study presents the results of the surveillance of ASF in wild boars in ROK between January 2019 and April 2019.

Materials and Methods: For the test, a total 325 samples of wild boars were collected in the Northern part of Gyeonggi Province and Gangwon Province near the Military Demarcation Line (MDL). DNA was extracted using Maxwell RSC instrument (Promega) in whole blood (EDTA) for virological test. Real-time polymerase chain reaction (RT-PCR) assay was conducted on the Bio-Rad CFX96 systems. Detection of ASF antigen was conducted using PCR assay according to OIE(World Organisation for Animal Health) manual. When the test result was positive for ASFV by Real-time PCR, we amplified four independent regions from the ASFV genome using conventional PCR. For antibody test, serum samples of wild boars were tested using ID Screen® African Swine Fever Indirect Screening Test ELISA Kit(ID.Vet, France).

Results: All tested samples were negative (Table 1).

Table 1. The virological and serological surveillance results of wild boars for ASF in ROK

Area		No of Samples	Virological	Serological
			Real time PCR	ELISA
Gangwon province	Gangneung	5	Negative	Negative
	Donghae	14	Negative	Negative
	Yangyang	22	Negative	Negative
	Wonju	28	Negative	Negative
	Inje	9	Negative	Negative
	Cheorwon	10	Negative	Negative
	Chuncheon	86	Negative	Negative
	Hongcheon	30	Negative	Negative
	Hwacheon	3	Negative	Negative
	Hoengsuong	2	Negative	Negative
Gyeonggi province	Dongducheon	8	Negative	Negative
	Pocheon	52	Negative	Negative
	Yangpyeong	11	Negative	Negative
	Yeoncheon	24	Negative	Negative
	(Seoul)	21	Negative	Negative
Total		325		

Conclusions: These results collectively suggest that there have not been any infection by ASFV in wild boar population in ROK. Although Korea is known to have very effective sanitary regulations for pork and live animal imports and waste food disposal, the current situation of ASF especially in China convinces us the need to continuous and stricter monitoring of the disease.

Acknowledgement: This work was supported by a grant (No. B-1543085-2019-20-01) from Animal and Plant Quarantine Agency, the Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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ASF-008

Surveillance of African Swine Fever virus on confiscated pork products in Republic of Korea

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Introduction: African swine fever (ASF) is a fatal viral disease that affects pigs of all ages and breeds. ASF virus (ASFV) is highly virulent and remains a global threat because of the lack of a effective vaccine and the ability of the virus to survive in various environmental conditions. Since 2007, ASFV has been spreading across Europe and Russia. In August 2018, China reported the first outbreak of ASF in Asia. Since then, ASFV has been reported in Vietnam, Mongol, Cambodia and Hong Kong. Although ASF has never occurred in the Republic of Korea (ROK), ASFV could be introduced through various routes. The risk for ASF introduction into ROK increases with the continuous spread of the disease across Asia. Pork products contaminated with ASFV is one of the main risk factors for spreading the disease. Hence, we have been conducting surveillance on pork products confiscated at airports and ports from travelers coming from countries affected by ASF. In this study, we report the results of ASF surveillance on confiscated pork products between January and April 2019 in ROK.

Materials and Methods: Samples were randomly selected among the confiscated pork products from travelers from China and neighboring countries. A total of 74 pork products (26 sausages, 4 hams and 44 other products containing pork) were tested by real-time PCR. We homogenized these samples and extracted nucleic acids using High Pure PCR Template Preparation Kit (Roche) in a biosafety level 3 laboratory at the Animal and Plant Quarantine Agency in Gimcheon, To amplify the ASFV *B646L* gene, we performed TaqMan real-time PCR according to OIE(World Organisation for Animal Health) manual.

Results: We detected ASF virus genes from 11 pork products that were confiscated by travelers from China (Figure 1). All 11 genes detected are grouped to ASFV genotype II.

Figure 1. The results of virological surveillance for ASFV on confiscated pork products in ROK

Country	No.of samples	Real time PCR		P72 Genotype
		positive	negative	
China	32	11	21	II
Vietnam	18	-	18	-
Thailand	15	-	15	-
Cambodia	3	-	3	-
Laos	3	-	3	-
Myanmar	1	-	1	-
Russia	1	-	1	-
Mongol	1	-	1	-
Total	74	11	63	

Conclusions: Contaminated pork products fed to pigs have often been the source of ASFV outbreaks and introduction into previously unaffected areas. Thus, detection of ASFV in these confiscated products highlights the importance of surveillance at points of entry to mitigate the risk of ASFV introduction.

Acknowledgement: This work was supported by a grant (No. B-1543085-2019-20-01) from Animal and Plant Quarantine Agency, the Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Ent-001

Antimicrobial resistance patterns of *Clostridium perfringens* isolated from pig feces in Korea

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Introduction: *Clostridium* (*C.*) *perfringens*, a spore-forming anaerobic bacillus, is widely distributed in the environment and the intestinal tracts of domestic animals. Diarrhea caused by pathogenic *C. perfringens* is one of the ongoing problems in pig industries. Although antimicrobials have been used to prevent and reduce diarrhea, the emergence of antimicrobial resistant bacteria is regarded as the major obstacle. Therefore, the understanding of antimicrobial resistance patterns is the first step to set up a strategy of the prevention and treatment. The aim of the present study was to investigate the prevalence of antimicrobial resistance in *C. perfringens* isolated from pigs in Korea.

Materials and Methods: *C. perfringens* (n = 75) was isolated from fecal samples (n = 814) at 10 different pig farms. Antimicrobial susceptibility of the isolates was assessed according to the disc diffusion method, using the following antimicrobial discs (BD Biosciences): ampicillin (10㎍), bacitracin (10㎍), ceftiofur (30㎍), enrofloxacin (5㎍), erythromycin (15㎍), gentamicin (10㎍), kanamycin (30㎍), oxytetracycline (30㎍) and streptomycin (10㎍). And, antimicrobial resistance patterns were determined according to the standard procedures recommended by Clinical Laboratory Standards Institute [1].

Results: The isolates of *C. perfringens* were highly resistant to gentamicin, kanamycin, streptomycin (100%) and oxytetracycline (93.3%). On the other hand, they were highly susceptible to bacitracin (100%).

Conclusions: In this study, antimicrobial resistance rate of aminoglycoside family (gentamicin, kanamiycin, streptomycin) were higher compared to other countries [2, 3]. However, susceptibility of bacitracin was similar to those observed in the previous study [2]. These results could be useful for the treatment of diarrhea caused by *C. perfringens*.

Table 1. Antimicrobial resistance patterns of *C. perfringens* isolated from pig feces

Antimicrobials	No. of *C. perfringens* (%)		
	Susceptible	Intermediate	Resistant
Ampicillin	37 (49.3)	0	38 (50.7)
Bacitracin	75 (100)	0	0
Ceftiofur	45 (60.0)	10 (13.3)	20 (26.7)
Enrofloxacin	2 (2.7)	17 (22.7)	56 (74.7)
Erythromycin	0	40 (53.3)	35 (46.7)
Gentamicin	0	0	75 (100)
Kanamycin	0	0	75 (100)
Oxytetracycline	2 (2.7)	3 (4.0)	70 (93.3)
Streptomycin	0	0	75 (100)

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

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Ent-002

Antimicrobial resistance profiles of pathogenic *Escherichia coli* isolated from piglet feces in Korea

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Introduction: Pathogenic *Escherichia (E.) coli* is one of the pathogens of diarrheal diseases in piglets [1]. Antimicrobial resistance of pathogenic bacteria makes it difficult to prevent infection and treat disease [2]. Therefore, it is important to study the antimicrobial resistance profiles of pathogenic *E. coli.* The purpose of this study was to investigate antimicrobial resistance in pathogenic *E. coli* isolated from suckling and postweaning piglet feces in Korea.

Materials and Methods: From 2017 to 2018, 47 *E. coli* isolates were selected from suckling and postweaning piglet feces (n = 430) by using fimbriae (F4, F5, F6, F18, F41) and toxin (STa, STb, LT, STX2e) PCR, respectively. Antimicrobial resistance was determined by the standard disk diffusion methods according to the guidelines of the Clinical and Laboratory Standards Institute [3]. Isolates were tested for susceptibility to the following 11 antimicrobial agents: enrofloxacin, neomycin, colistin, ceftiofur, amikacin, amoxicillin/clavulanic acid, ampicillin, gentamicin, kanamycin, tetracycline, trimethoprim/sulfamethoxazole.

Results: Most of *E. coli* isolates were resistant to ampicillin (97.9 %) and tetracycline (91.5 %). On the other hand, amikacin (14.9 %) and colistin (4.3 %) showed relatively low resistance. Among 47 isolates, 42 isolates (89.4 %) were resistant to three or more antimicrobial classes.

Conclusions: In this study, we showed antimicrobial resistance profiles and the prevalence of multi-drug resistant of pathogenic *E. coli* isolated from Korean piglets. Therefore, proper use of antimicrobials was recommended for the control of antimicrobial resistance. These results could be used as a strategy to treat and prevent pathogenic *E. coli* infection.

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

Table 1. Antimicrobial resistance of pathogenic *E. coli* isolated from suckling and postweaning piglet feces

Class and antimicrobial agents	Resistance (%)
Penicillin	
Ampicillin	97.9
β-lactam/β-lactamase inhibitor combinations	
Amoxicillin/clavulanic acid	40.4
Cephems	
Ceftiofur	23.4
Fluoroquinolones	
Enrofloxacin	23.4
Aminoglycosides	
Amikacin	14.9
Gentamicin	42.6
Kanamycin	70.2
Neomycin	55.3
Folate pathway inhibitors	
Trimethoprim/sulfamethoxazole	68.1
Tetracyclines	
Tetracycline	91.5
Polypeptides	
Colistin	4.3

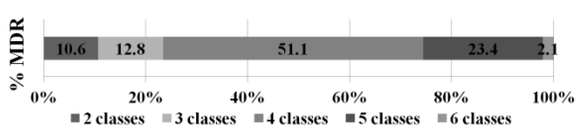

Figure 1. Multi-drug resistance patterns of pathogenic *E. coli* isolated from suckling and postweaning piglet feces

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Ent-003

Antimicrobial susceptibility of *Escherichia coli* isolated from diarrheal suckling and nursery pigs in Central Thailand

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Introduction: *Escherichia coli* is still one of the major causes of economic losses in suckling and nursery piglets in Thailand. To mitigate the problem, antimicrobial uses for prophylaxis and treatment are still the main option chosen by swine farmers. In recent years, regular uses of antimicrobial drugs in farm animals have raised concerns that this practice is likely to promote antimicrobial resistances (AMR) of bacteria that will possess harms to humans and rendered the effectiveness of antimicrobial treatment in the future. The aims of this study were to investigate antimicrobial susceptibility and a prevalence of extended-spectrum beta-lactamases (ESBL) phenotype of *E. coli* in pigs that suffered from diarrhea from farms with antimicrobial use within Central Thailand.

Materials and Methods: A total of 158 *E. coli* isolates were obtained from fecal samples of diarrheal pigs, including suckling pigs (n= 77) and nursery pigs (n=81), from five commercial pig farms in central provinces of Thailand in 2018. Depended on farms management system, the age ranges of suckling pigs were from 1-day-old to weaning period which approximately 4 weeks old, and nursery pigs were from the period after weaning to around 8-10 weeks old. Every farm regularly administered antimicrobials to the pigs for both prophylactic and therapeutic purposes. All *E. coli* strains were tested for antimicrobial susceptibility using VITEK-2 system (Bio-Mérieux, Marcy l'Etoile, Craponne, France) excepted for colistin susceptibility test which was performed by broth microdilution method (CLSI, 2013). All isolates were screened for potential ESBL production using cefodoxime (10µg), ceftazidime (CAZ) (30µg), and cefotaxime (CTX) (30µg) discs. The *E. coli* isolates tested positive from screening assay were then examined for ESBL production by combination disc method according to CLSI guidelines, 2013 [1]. A $\geq$ 5 mm increase in a diameter of a clear zone of either the CAZ or CTX disc in combination with clavulanic Acid (CA) (BD®) compared to a diameter of a clear zone of each drug without CA was indicated ESBL production.

Results: The antimicrobial susceptibility test revealed that all (100%) of the *E. coli* isolates were resistant to ampicillin, and 96% of the isolates also resisted to tetracycline in both suckling and nursery pigs. Interestingly, the *E. coli* isolates that exhibited resistance to all other antimicrobial drugs were equal to, or higher than 50% (Fig 1.). The ESBL-positive *E. coli* were detected in all of the farms in this study. The ESBL examination revealed that 37 (48%) and 54 (67%) isolates from the suckling and the nursery pigs were ESBL-positive, respectively.

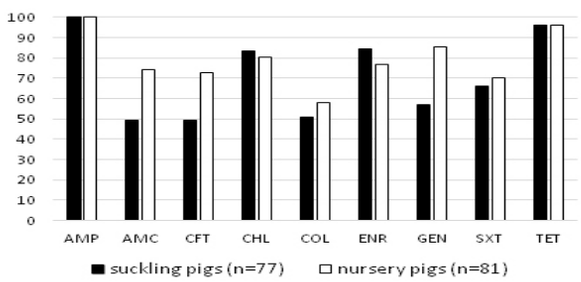

Fig 1. The percentage of antimicrobial resistance of *E. coli* isolated from suckling and nursery pigs. AMP-Ampicillin, AMC-Amoxicillin/Clavulanic Acid, CFT-Ceftiofur, CHL-Chloramphenicol, COL-Colistin, ENR-Enrofloxacin, GEN-Gentamicin, SXT-Trimethoprim/Sulfamethoxazole, TET-Tetracycline

Conclusions: The results indicated high prevalence of antimicrobial resistant and ESBL *E. coli* in neonatal and nursery pigs with diarrheal signs. The higher percentage of ESBL *E. coli* in older nursery pigs needed further study on a correlation with the increased exposure to antimicrobials by *E. coli* over time as the animal aged. This study also demonstrated the need for a monitoring program for AMR in *E. coli* in pig farms to assist in a prudent use of antimicrobials in a Thai pig industry.

Acknowledgement: The authors would like to thank pig farms, field veterinarians, and Kamphaengsaen Veterinary Diagnostic Unit for their support.

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Ent-004

Combination efficacies of avilamycin/tiamulin and avilamycin/tilmicosin against enterotoxigenic *Escherichia coli* (ETEC) and *E. coli* containing *mcr* 1 gene from pigs in Thailand.

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Introduction: Use of antimicrobial combination regimen is an alternative tool to enhance of the bactericide efficacy especially during the global crisis of antimicrobial resistance. Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of diarrhea in neonatal and nursery period. Moreover, plasmid mediated colistin resistant *E. coli* (*mcr-1*) has been emerged in pigs and pork in several countries with public health concern (1). Thus, to observe their susceptibilities, it could be helpful for recommendation into a guideline in antimicrobial usage in pig farms. The objectives were to determine the single susceptibilities to avilamycin, tiamulin and tilmicosin and the combination efficacies of avilamycin/tiamulin and avilamycin/tilmicosin against plasmid mediated colistin resistant *Escherichia coli* (*mcr*-1+) and enterotoxegenic *E. coli* isolated from pigs.

Materials and methods: *Bacterial strains:* A total of 29 *E. coli* strains were determined the minimal inhibitory concentration (MIC) by agar dilution technique. Eleven colistin resistant *E. coli* strains containing *mcr-1* gene were (MIC > 2 μ g/ml) (1) and 18 strains of enterotoxegenic *E. coli* (ETEC) containing heat-labile toxin subunit encoding gene (*ltb*) by multiplex PCR (3) were used.
Antimicrobial susceptibility test: The individual susceptibility procedure for avilamycin, tiamulin and tilmicosin against all strains were performed by agar microdilution method (4).

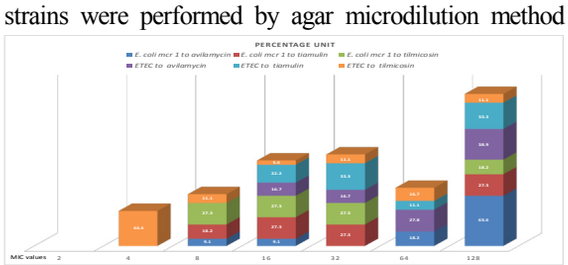

Fig 1. Distribution of MIC values of all tested *E. coli* to avilamycin, tiamulin and tilmicosin.

Combination effect determination and interpretation: The combination of both antimicrobials was performed by checkerboard microdilution technique. The cut-off interpretation was detected by being transparent within well representing inhibition by antimicrobial combination. The fractional inhibitory concentration (FIC) index for combination was calculated follows the recommendation (5).

Results and discussion: The individual of MIC level to the antimicrobials ranged from 4-128μg/ml. Unfortunately, the interpretation breakpoint of CLSI recommendation is not available, thus only trend of MIC fluctuation is showm in Fig 1. Overall, most of *E. coli* were highly resistant to avilamycin followed by tiamulin. Tilmicosin was the most effective agent and individual MIC50 was only 8 and 16 μg/ml for ETEC and *mcr1* positive *E. coli*, respectively. While, the MIC50 to tiamulin was 32 μg/ml for both groups. Overall, the combined MIC50 values were reduced by 2-4 times of single MIC_{50}. The results indicated that the combination regimens enhanced antimicrobial efficacy against *mcr-1 E. coli* and ETEC.

Conclusion: The two combination regimens enhanced the antimicrobial efficacies to colistin resistant *E. coli* and ETEC without antagonistic observation.

Acknowledgements: We thank Elanco (Thailand) Ltd. for funding and antimicrobial agent supports.

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Table 1. MIC50 values of antimicrobials in single and combined reactions and the outcome of synergistic efficacies.

Bacteria	Avilamycin		Tiamulin		Tilmicosin		Avilamycin/tiamulin			Avilamycin/tilmicosin		
	Single	Combined	Single	Combined	Single	Combined	S	I	A	S	I	A
E. *coli mcr*+	128	16	32	16	32	4	2/11	9/11	-	2/11	9/11	-
ETEC	128	32	32	16	8	8	2/18	16/18	-	1/18	17/18	-

S, synergistic; I, indifferent; A, antagonist

Ent-005

Concurrent Detection of Antimicrobial Resistant Salmonella and *Arcobacter* in Retail Pork in Khon Kaen Municipal Area, Khon Kaen Province, Thailand

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Introduction: Pork is a popular meat and a notorious source of foodborne infections. *Salmonella* is a common contaminant of retail foods in Thailand [1]. *Arcobacter* was recognized as a human health hazard (ICMSF, 2002). The first outbreak in Thailand caused by contaminated drinking water [2]. As reviewed by [3], Three species of *Arcobacter, A.butzleri, A. cryaerophilus, and A. skirrowii* cause significant clinical cases. *Arcobacter* was detected in various species and environment, yet its pathogenicity in pigs is disputed [3]. Here we investigated the presence of above mentioned bacteria in pork sold at retail outlets.

Materials and Methods: Raw pork (n=12) was collected from supermarket (S) and fresh market (F) in Khon Kaen municipal city to detect Salmonella and Arcobacter. During May 2018, sampling at S and F markets was done on alternate days 6 times. Ten grams of pork was pressed by stomacher after adding 90 ml of *Arcobacter* enrichment broth with CAT supplement (Oxoid®, United Kingdom). Culture [4] and multiplex-PCR detection of Arcobacter species was done as previously described [5].

Isolation and identification of *Salmonella* was done according to ISO 6579:2002 method. Twenty-five grams of pork was added with 225 ml of BPW and mixed by stomacher, pre-enriched, sub-cultivated in Rappaporte Vassiliadis medium with soya and Müllere Kauffmann tetrathionate broth (Oxoid®). The enriched cultures were streaked on XLD and Hextoen agars (Oxoid®). *Salmonellae* were identified by biochemical tests, Kauffmanne White Scheme serotyping, and slide agglutination test against specific O and H antigens (S&A, Thailand).

Antimicrobial resistance (AMR) was determined by disk diffusion method (CLSI, 2012). Nine antibiotic disks (Oxoid®, USA); gentamicin (GN, 10μg), tetracycline (TE, 30μg), ciprofloxacin (CIP, 5μg), sulphamethoxazole-trimethoprim (SXT, 25μg, erythromycin (E, 15μg), nalidixic acid (NA, 30μg), amoxycillin (AML, 25μg), cefquinome (CEQ, 10μg), and colistin (CT, 10μg). Since *Arcobacter* has no clear zone standard, it was compared with that of *Campylobacter* (CLSI, 2017). Quality control tests were performed using *E. coli* ATCC 25922 and *S. aureus* ATCC 259223. The susceptibility was carried out in duplicates.

Results: Six serotypes of *Salmonellae* and only *A. butzleri* were identified in raw pork. *A. butzleri* was isolated from all samples (100%), both *Salmonellae* and *Arcobacter* from (91.7%), and *Arcobacter* only was isolated from (8.3%) samples. Pork sold at S & F markets had similar bacterial contamination. AMR rates of *Salmonellae* and *A. butzleri* were 90.9% (10/11) and 75% (9/12), respectively (Table1).

Table 1. Species and AMR patterns of *Salmonellae* and *Arcobacter* isolated from raw pork.

No.	Market Place	*Salmonella spp.*		*Arcobacter butzleri*
		Serovar	Antimicrobial resistance	
1	S	Agona	TE-SXT	CEQ
2	S	Augustenborg	susceptible	CEQ
3	S	Derby	TE-SXT	NA-CEQ
4	S	Derby	TE-SXT	NA-CEQ
5	F	Derby	TE-SXT-CT	NA-SXT
6	S	Kedougou	TE-SXT	NA-SXT
7	F	Rissen	NA	TE
8	F	Rissen	TE-SXT	NA-SXT
9	F	Rissen	TE	susceptible
10	S	Typhimurium	TE-SXT	NA
11	F	Typhimurium	SXT	susceptible
12	F	Not detected	-	susceptible

Conclusions: The study reveals heavy widespread contamination of retail pork by *A. butzleri* and *Salmonellae*. To prevent infection it is crucial that cross contamination be avoided and food cooked thoroughly. AMR of both bacteria is alarming and therefore antimicrobial usage should be monitored.

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Ent-006

Detection of different pathogenicity factors of enterotoxigenic escherichia *coli* in 25 commercial thai herds

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Introduction: Neonatal diarrhea caused by enterotoxigenic *E. coli* (ETEC) remains a critical disease that limits production in pig farms worldwide, with a recent cost estimate of >5,000 baht per sow per year [1]. The etiology of diarrhea signs in suckling pigs is associated with at least two established virulence factors such as specific fimbriae (K88 [F4], K99 [F5], 987P [F6], F41) and heat-labile enterotoxin (LT) [2]. Generally, F4-positive ETEC is the most prevalent fimbrial antigen causing neonatal diarrhea in piglets, whereas F5, F6, and F41 strains are less often founded [2]. In Thailand, ETEC-positive farms had a prevalence of 75%, whereas the F6 gene was not found [3]. The aim of this study was to estimate the prevalence of fimbrial antigens (F4, F5, and F6) and LT-positive farms that cause neonatal diarrhea in Thailand.

Materials and Methods: Twenty-five swine farmys from several parts of Thailand were tested during 2017-2018. The prevalence of *E. coli* fimbrial antigens was identified in 94 pooled swab samples collected from 1-14-day-old suckling pigs. Some farms showed no evidence of prior exposure to porcine reproductive and respiratory syndrome virus (PRRSV) through strict biosecurity and all-in/all-out by site and single-source pigs. Three rectal swab samples from piglets of the same litter were pooled using FTA ELUTE cards and sent to DIAGNOS® Laboratory. A multiplex real-time polymerase chain reaction (PCR) test, adapted from previous studies, [3,4] was performed, to detect genes encoding F4, F5, F6 adhesion factors, the heat-labile toxin (LT) of *E. coli*, and β -toxin of *Clostridium perfringens* type C.

Results: The overall prevalence of ETEC represented 80% of positive samples (20/25), whereas *Clostridium perfringens* type C (β toxin) was not found. Furthermore, fimbrial genes for F4-, F5-, F6- and LT-positive ETEC isolates were identified in 51.06% (48/94), 2.13% (2/94), 0% (0/94) and 10.64% (10/94), respectively. Some samples carried both virulence genes, fimbriae and enterotoxin ($F4^+LT^+$) in 14.89% (14/94), or more than one fimbriae ($F4^+F5^+$) in 1.06% (1/94), $F4^+F6^+$ in 1.06% (1/94) and $F4^+F5^+LT^+$ in 2.13% (2/94). The remaining samples were ETEC-negative up to 17.02% (16/94) (Figure 1).

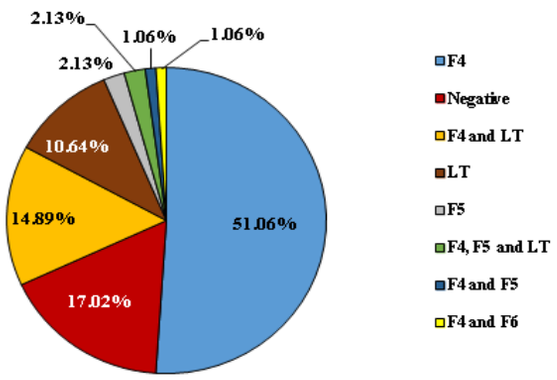

Figure 1. Analysis of a multiplex real-time PCR test results divided by pathogenic factors.

Conclusions: The F4 gene was the most prevalent contributor to diarrhea in piglets in Thailand, showing similar results to those obtained in other studies [5]. It is clearly accepted that the presence of *E. coli* is an underlying problem across many herds and is likely a substantial cause of diarrhea in suckling piglets. Using a multiplex real-time PCR assay is the best diagnostic tool to prove the presence of the bacteria and the disease. This study may provide a key database for field surveillance in Thailand, which in turn can have an impact on the selection of suitable vaccines containing ETEC main adhesion factors and toxins.

Acknowledgements: The authors would like to thank Dr. Sithipon Jongpattanasombut for his helpful advice on various technical issues analyzed in this paper and the farm owners that contributed to the rectal swab sampling of piglets across Thailand.

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Ent-007

The Economic Benefits Of Vaccination With Porcilis Ileitis In A Commercial Farm In Luzon, Philippines

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INTRODUCTION Porcine Proliferative Enteropathy (PPE) caused by the obligate intracellular bacterium *Lawsonia Intracellularis* is a common enteric disease worldwide affecting grower pigs between 6 to 20 weeks of age inducing decreased weight gain and feed efficacy, and sometimes death.

MATERIALS AND METHODS Weaned piglets of 3 weeks and above were weaned and divided into two Groups. Group 1 (N=156) was vaccinated with Porcilis Ileitis at 3 weeks of age and maintained on current antibiotic regimen of farm. Group 2 (N=156) was not vaccinated group and maintained on the current antibiotic regimen of farm. Vaccinated and non-vaccinated groups were housed in separate pens from nursery to slaughter to allow for feed consumption measurement and ear-tagged so that no comingling between vaccinated and non-vaccinated groups occurred. Average daily gain, % survivability and final weight gain were computed. On establishing the economic benefit of having an intact control program for Ileitis, current prices and cost assumptions were applied. Data on initial weights and final weights were subjected to statistical analysis using T-test.

RESULTS AND DISCUSSION Final weights between the two groups were significantly different with the treatment group having an advantage of 11.3 kilos per head on the average over the control group. ADG of the treatment group from wean to finish was 100grams more per day compared to the control group. This may be associated with the integrity of villi intestines which aids the pigs in nutrient absorption that enables optimal growth rates. Subclinical PPE may degrade the integrity of the intestine which may makes the unvaccinated group grow 100grams less per day. The treatment group has 2 more pigs sold compared to the control. Based on mortality recorded, the control group also had at least 1 mortality directly related to PPE based on necropsy at post mortem. With the 11.3 kilos advantage earned by the treatment group, the result clearly will cover a vaccination program to control PPE. In this particular study, the treatment group garnered a total of 162,301.50 pesos net benefit over the control that is less the cost of the vaccine.

The study conducted proves that Porcilis Ileitis can protect pigs from PPE and also reduce economic losses associated. Significant differences in terms of final weights proves that having Porcilis Ileitis in the health program of the farm can have a beneficial impact on the farm economics. It is possible that this calculation is an under-estimate of the true economic benefit that Porcilis Ileitis brings, because we were not able to directly measure FCR. Adding in FCR measurements will bring another dimension of economic benefit to the farm, because of improved feed conversion efficiency, which will directly lead to less feed being used to raise pigs.

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Ent-008

Effects of Baytril Max® Injection on the Treatment of Colibacillosis in Piglets

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Introduction: Colibacillosis is mainly occurred in piglets of post-natal or weaning period infected with pathogenic *E. coli*. Major symptoms are diarrhea and edema causing economic damage. Enrofloxacin is a fluoroquinolone antibiotic that inhibits bacterial DNA gyrase and blocks DNA and RNA synthesis, thereby killing bacteria.
In this study, we investigated the antibacterial effects of Baytril Max®, which contains enrofloxacin(100 mg/mL) as an active ingredient, on GI tract disease by pathogenic *E. coli* in piglets.

Materials and Methods: Eighteen male weaned piglets (5~6 weeks old, 10~15kg bw, Landrace) were allocated six piglets per group to three groups (Untreatment (Control), *E. coli* challenge (Challenge) or *E. coli* challenge+ Baytril treatment (Treatment)). Pathogenic *E. coli*(express STa and F4) was orally inoculated twice with 40 mL of bacterial culture solution (4.35 to 5.88 × 10^9 CFU/mL) in the challenge group and the treatment group to induce colibacillosis. In the treatment group, Baytril Max® was subcutaneously administered once at a dose of 7.5mL per 100kg bw after observance of the clinical symptoms.
Body weight was measured before the induction of GI tract disease and on days 1, 4, 7, 10, and 14 after administration of the test substance. Daily weight gain and clinical signs were observed for 14 days of the experiment. On the 14th day, piglets of all groups were euthanized, and gross examination was performed.

Results: In the challenge group, weight gain was decreased and soft stool was consistently observed during the experiment period. Rough skin and dull behaviors were significantly observed in most piglets until the end of the experiment period (Fig.1). Pathogenic *E. coli* was detected in the feces until the end of the test period. The small intestinal wall was thinner, and intestinal detention and congestion were observed. Villus epithelial defoliation and atrophy were also observed.
In the treatment group, Table 1 indicates that the administration of the drug resulted in inhibition of decrease in body weight gain due to GI tract diseases. Soft stool was observed in most piglets until the day of the test drug administration, but the symptom was disappeared from the 4th day after the administration, and normal stool was observed until the end of the experiment (Fig.1). Pathogenic *E. coli* was detected only up to the 4th day of the drug administration in the feces, and was not detected until the end of the test period. No pathologic findings were observed.

Table 1. Changes in feed efficiency after *E.coli* challenge and administration of test substance.

Group	Daily weight gain	Feed efficiency
Control	0.80±0.10	0.49±0.06
Challenge	0.40±0.19***	0.27±0.13***
Treatment	0.55±0.10**	0.35±0.06**

** p<0.01, *** p<0.001, significantly different from Control group.

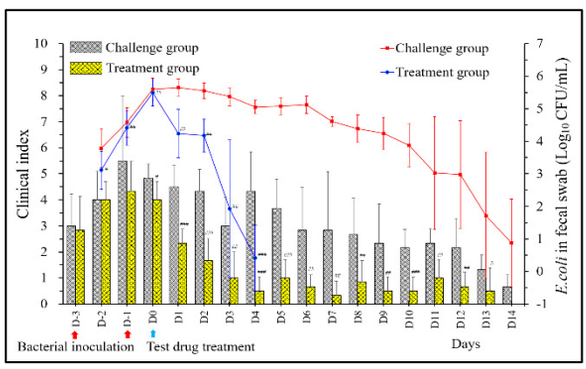

Fig. 1. Clinical sign index (Bar) and the mean number of *E.coli* in feces (Line) of each group after bacterial challenge with or without Baytril Max®.

* p<0.05, ** p<0.01, *** p<0.001, significantly different from challenge group

Conclusions: Baytril Max® was confirmed to have bactericidal effect against pathogenic E. coli and to be effective in treating GI tract diseases of piglets by reducing clinical signs, improving daily weight gain and preventing GI tract damage.

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Ent-009

Efficacy and safety of SUISENG® against neonatal diarrhoea under field conditions

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Introduction: Neonatal porcine diarrhoea (NPD) is a disease with productive and economic costs owing to the high mortality rate, the increased number of weak piglets and all the treatment costs associated with the clinical outbreak[1]. However, maternal immunity is essential to protect piglets against infections during the suckling period, and vaccines help to enhance the protective effect of colostrum[2]. The aim of this study was to compare the efficacy and safety of a novel vaccine against NPD as well as the impact of vaccination on production parameters during the lactation period.

Materials and Methods: A commercial farm in Guangdong province with 500 sows was selected for this study. The farm was negative for pseudorabies, PRRS-stable, and showed clinical signs of neonatal diarrhoea within 14 days after farrowing. A total number of 92 sows were selected and randomly divided into two groups. The experimental group received an intramuscular injection of SUISENG® and the control group received a phosphate-buffered saline solution (PBS). Both vaccinations were performed, 6 and 3 weeks before farrowing, in accordance with the manufacturer's instructions. Parameters such as clinical signs of diarrhoea, mortality, morbidity and ADWG were recorded to evaluate the productive performance of the piglets. On the other hand, side effects were also daily monitored until two days after vaccination.

Results: After vaccination and revaccination, no side effects were observed in any of the animals in the study (Table 1). Regarding the production performance, statistical analysis showed that the total number of weak piglets at weaning ($P<0.01$), the percentage of piglets suffering diarrhoea ($P<0.05$) and the average daily weight gain during lactation (ADWG) ($P<0.05$) in the vaccinated group is significantly better than the control one (Table 2).

Table 1. Safety results after vaccination

	SUISENG®	Control
Number of sows (n)	46	46
Local reactions (%)	0	0
Pyrexia (%)	0	0
Abdominal breathing (%)	0	0
Vomiting (%)	0	0
Mortality (%)	0	0
Lack of appetite (n)	0	0

Table 2. Overview of piglet production performance of sows vaccinated with SUISENG® vs PBS

	SUISENG®	Control
Number of litters	46	46
Weaned piglets	464	462
Weak piglets at weaning	7	16
Weak piglets at weaning (%)*	1.5%	3.5%
Litters with diarrhoea	7	16
Litters with diarrhoea (%)	15.2%	34.8%
Piglets with diarrhoea	15	101
Piglets with diarrhoea (%)*	3.2%	21.9%
Affected piglets / litter	0.3	2.2
Dead piglets	0	2
Dead piglets (%)	0%	0.4%
Average weight at farrowing (kg)	1.39	1.44
Average weight at 28 days (kg)	7.51	7.23
ADWG (g)*	218.57	206.79

* Statistically significant differences

Conclusions: These results show that SUISENG® is a safe and effective vaccine against NPD under field conditions, since no side effects were observed when compared to the control group. On the other hand, the productive performance of vaccinated sows was also significantly better. This is of special significance if we consider that this farm is PRRS-positive and the piglets are much more susceptible to diseases such as NPD during the first weeks of life.

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Ent-010

Infection status of gastrointestinal parasites in pigs in Korea

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Introduction: In domestic pigs, the damage caused by diarrhea continues to occur and affects the production efficiency of pig farms. The research on the distribution of parasitic diseases in diarrhea has been rarely carried out, so it is very difficult to understand the situation in Korea. The purpose of this study was to investigate the prevalence of gastrointestinal parasites in domestic pigs and to examine the relationship between farm environment, parasitic disease, and diarrhea.

Materials and Methods: Fecal samples were collected from 882 pigs at 29 farms (Chungnam, 2; Gyeongbuk, 5; Gyeongnam, 18; Jeju, 2; Jeonnam, 2) in association with pig farms and veterinarians. Identification of gastric parasites in pig stool samples was carried out by using a flotation method with a saturated sodium chloride solution [1] and observed under a microscope on the basis of morphological characteristics. For the samples infected by parasites, eggs/oocysts per gram of feces (EPG/OPG) were obtained for quantitative analysis.

Results: Of 882 pig feces, 72 (8.2%) were infected by at least a gastrointestinal parasite, including *Isospora* spp. (n = 35; 4.0%), *Balantidium coli* (15; 1.7%), *Ascaris suum* (17; 1.9%), and *Oesophagostomum dentatum* (7; 0.8%). Two samples were coinfected with *Isospora* spp. and *O. dentatum*. *Trichuris suis* and *Strongyloides ransomi* were not detected in this study. Of the 72 positive fecal samples, 45 (62.5%) had less than 1,000 EPG/OPG, meaning light infection.

Conclusions: Since the overall infection intensity is low, it seems that the breeding environment and the anthelmintic management of pigs are well conducted by farm owners. However, the relation between parasite infection and fecal type will be continuously analyzed. Particularly, in case of EPG/OPG of 1,000 or more, it is recommended to anthelmintic treatment for pigs.

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

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Table 1. Prevalence of gastrointestinal parasites in pigs according to age group and fecal type

Age	Fecal type	No. tested	No. Infected (%)	Isospora spp.	Balantidium coli	Ascaris suum	Oesophagostomum dentatum
Piglet	diarrheal	87	5 (5.7)	5	0	0	0
	normal	22	5 (22.7)	5	0	0	0
Weaner	diarrheal	214	20 (9.3)	11	7	0	2
	normal	129	16* (12.4)	10	3	1	4
Grower	diarrheal	64	3 (4.7)	1	0	1	1
	normal	81	6 (7.4)	0	0	6	0
Fattener	diarrheal	57	4 (7.0)	1	1	2	0
	normal	89	11 (12.4)	2	2	7	0
Sow	diarrheal	6	0	0	0	0	0
	normal	133	2 (1.5)	0	2	0	0
Total	diarrheal	428	32 (7.5)	18	8	3	3
	normal	454	40 (8.8)	17	7	14	4

* Two samples were coinfected with Isospora spp. and O. dentatum.

Ent-011

Modified method for the Minimal Inhibitory Concentration (MIC) determination of *Lawsonia intracellularis* without isolation based on PMA-qPCR

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Introduction: Proliferative hemorrhagic enteropathy (PHE) and proliferative intestinal adenomatosis (PIA) caused by *Lawsonia intracellularis* (LI) resulting in diarrhea, a rough hair coat, anorexia, and growth retardation in finisher and grower pigs. Up to now, antimicrobial therapy remains the only treatment available. Tiamulin, tylosin, lincomycin, and chlortetracycline have been commonly recommended and used in the field and oxytetracycline, valnemulin, doxycycline, josamycine, and leucomycin were also known as effective according to field experiences, not from the exact *in vitro* antimicrobial susceptibility testing (AST). However, AST could not be easily performed for LI because it requires complicated cell culture system and particular atmosphere for its growth and proliferation. Propidium monoazide (PMA) is a photoreactive DNA-binding dye that inhibits PCR amplification by DNA modification. PMA intercalates into the DNA and can be covalently cross-linked to it, which strongly inhibits PCR amplification. Therefore, the aim of this study was to modify method for the Minimal Inhibitory Concentration (MIC) determination of LI without cell culture system using propidium monoazide (PMA)-qPCR.

Materials and Methods: The four novel *L. intracellularis* field isolates were obtained from hemorrhagic region of the small intestine from a finisher pigs with PHE (CBNU001, CBNU002, and CBNU006) and lactating piglets (CBNU004) in 2013 to 2017. The isolates were prepared in IEC-18 cells and harvested and then, the AST was conducted by determining conventional extracellular minimum inhibitory concentrations (ExMIC)s, intracellular minimum inhibitory concentrations (InMIC)s and PMA-qPCR against isolates.

Results: Our results showed that CT value of PMA-qPCR assay was correlated with patterns of conventional eMIC in LI antimicrobial activity test. Therefore, Modified method for the MIC determination of LI without cell culture system using PMA-qPCR were useful to evaluate antimicrobial susceptibility and to save the time to detect.

Table 1. MICs and PMA-qPCR for Tylosin and Tylvalosin against CBNU006 strains of *Lawsonia intracellularis* isolated from Korea

Extra MIC*/Ct		MIC (ug/ml)†											
		0.25	0.5	1	2	4	8	16	32	64	128	256	512
Macrolides													
Tylosin	ExMIC	R	R	R	R	R	R	R	S	S	S	S	S
	InMIC	R	R	R	R	R	R	S	S	S	S	S	S
	Ct	17.18 ±0.45	16.82 ±0.12	16.80 ±0.11	16.78 ±0.38	16.42 ±0.16	16.28 ±0.28	16.58 ±0.11	18.30 ±0.02	19.33 ±0.13	19.97 ±0.06	20.29 ±0.04	21.33 ±0.28
Tylvalosin	ExMIC	R	R	R	R	S	S	S	S	S	S	S	S
	InMIC	R	R	S	S	S	S	S	S	S	S	S	S
	Ct	17.28 ±0.10	18.44 ±0.03	16.35 ±0.25	16.61 ±0.28	18.10 ±0.23	18.79 ±0.62	19.04 ±0.42	20.04 ±0.12	20.88 ±0.78	21.40 ±0.18	22.96 ±0.07	23.85 ±0.17

† MIC is defined as the minimum antimicrobial concentration necessary to inhibit 99% of the growth of *L. intracellularis* relative to a drug-free control.
* Control Ct=16.68±0.11.

Conclusions: We need to new and rapid antimicrobial susceptibility test against *Lawsonia intracellularis* isolated from pigs. Ct value of PMA-qPCR assay is correlated with patterns of conventional ExMIC in *Lawsonia intracellularis*. Although, there is a limitation to the number of antibiotics and field isolates, this modified method using PMA-qPCR for determining the ExMIC of *Lawsonia intracellularis* without cell culture system was useful to evaluate antimicrobial susceptibility.

Acknowledgement: This work was supported by a grant from the Next-Generation BioGreen 21 Program (Project No. PJ01181601), Rural Development Administration (RDA), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Ent-012

Multilocus sequence typing and antimicrobial susceptibility testing of *Brachyspira hyodysenteriae* isolates from pig in Taiwan: 2015-2018.

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Introduction: *Brachyspira hyodysenteriae* is the most important causal agent of swine dysentery (SD). This disease usually occurs in grower-finisher pigs and causes severe economic losses. Antimicrobial agents like tylosin, lincomycin, and tiamulin are widely used to control the infection of SD in Taiwan. However, there is not any studies about epidemiology of B. *hyodysenteriae* in Taiwan, and information about antimicrobial susceptibility testing is rather scarce. This study aims to evaluate the antimicrobial susceptibility by using agar microdilution (AD) and to analyze epidemiology by using multilocus sequence typing (MLST) approach. This study also investigated the associations between antibiotic-resistance phenotype and sequence types (STs).

Materials and Methods: In this study, *B. hyodysenteriae* (n = 49) isolated from diarrheic pigs between 2015 and 2018 in Taiwan were confirmed by PCR targeting the *nox*-gene. Antimicrobial susceptibility testing of *B. hyodysenteriae* to tylosin, lincomycin and tiamulin was evaluated by the AD method as described previously [1]. MLST was performed according to previously published protocols [2], and seven conserved housekeeping genes including alcohol dehydrogenase (*adh*), alkaline phosphatase (*alp*), esterase (*est*), glutamate dehydrogenase (*gdh*), glucose kinase (*glpK*), phosphoglucomutase (*pgm*) and acetyl-coA acetyltran ferase (*thi*) were used as MLST loci. Isolates were assigned to STs according to the allele profiles provided on the *Brachyspira* MLST website (http://pubmlst.org/brachyspira/). New STs and alleles were submitted to the curator of the website for assignments.

Results: The antimicrobial susceptibility results showed that the 50% and 90% minimum inhibitory concentration (MIC) values of the tested macrolides were all >256 μ g/mL. The MIC50 of lincomycin, and tiamulin were 32, and 1 μ g/mL. MLST revealed only three STs in Taiwan. 95.91% isolates belonged to only two STs, namely NST-1 (63.26%), and NST-2 (32.65%) which were new STs. Another one is ST56 which has been observed in North America. The proportion of strains that showed resistance to both tylosin and lincomycin (89.79%) varied little among all the STs ranging from 50% (1/2 isolates resistance) in ST56 isolates to 90.32% (28/31), and 93.75% (15/16) in isolates belonging to NST-1, and NST-2, respectively.

Comprehensive data of the B. hyodysenteriae including antimicrobial susceptibility and STs are provided in Table 1.

Table 1. Sequence types and minimum inhibitory concentrations for B. hyodysenteriae isolates.

Sequence types	Isolates	MIC (μg/mL)		
		tylosin	lincomycin	tiamulin
NST-1 (n = 32)	n=17	128 - >256	32 - >256	0.125 - 64
	n=12	128 - >256	8 - 16	0.125 - 4
	n=3	8 - 16	0.5 - 4	0.125 - 2
NST-2 (n = 15)	n=14	128 - >256	32 - >256	≦0.125 - 64
	n=1	32	16	≦0.125
ST56 (n = 2)	n=1	>256	32	32
	n=1	16	4	0.125

Conclusions: The *B. hyodysneteriae* in Taiwan is characterized by a low genetic diversity, with macrolide-lincosamide-resistant isolates of NST-1 and NST-2 being predominant.

Acknowledgement: We thank the National Chiayi University of Veterinary Medicine-Bacterial Laboratory for providing us with *B. hyodysenteriae* field isolates originating from the Taiwan. We also thank Prof. H.C. Kuo and his team for technical assistance and statistical evaluation.

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Ent-013

Plasmid-Mediated Polymyxin E Resistance Gene of *Escherichia coli* in Diseased Pigs

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Introduction: *Escherichia coli* is a zoonotic pathogen that causes enteric diseases or extraintestinal infections in animals and humans. *E coli* is an important pathogen for a wide range of swine diseases, including diarrhea, edema disease, septicemia, polyserositis and urogenital tract infection. Colistin is widely used in food-producing animals to treat alimentary infections caused by *Enterobacteriaceae*. However, resistance to colistin has been discovered in *Salmonella, Klebsiella, Acinetobacter, Pseudomonas* and *E.coli* from human and animals. Bacteria have developed acquired mechanism of polymyxin resistance involving PhoP/PhoQ and PmrA/PmrB two-component systems and plasmid-mediated colistin resistance gene, *mcr* gene. Since Liu discovered the plasmid-media colistin resistance gene—*mcr-1* in 2015, the prevalence and distribution of *mcr-1* have been published in various countries. To date, eight *mcr* variants have been described: *mcr-1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7 and mcr-8* [1].

Materials and Methods: Antimicrobial susceptibility testing and prevalence of plasmid-mediated colistin resistance for 810 *E.coli* isolated strains from diseased swines during 2016 and 2017 were analyzed in this study. According to the European Committee on Antimicrobial Susceptibility Testing. The breakpoint for polymyxin B and colistin are > 2 μ g/mL, when using microbroth dilution method. The prevalence of *mcr-1* to *mcr-5* was studied using multiplex polymerase chain reaction. Strains with colistin resistance but without *mcr1* to *mcr-5* gene were detected, and they were then investigated by using bacterial conjugation to find whether there is other colistin resistance gene or drug resistance mechanism. Multiplex PCR was performed as follows: denaturation at 94 °C for 15 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 90 s and elongation at 72 °C for 60 s, and a final cycle of elongation at 72 °C for 10 min [2]. The amplification was visualized by electrophoresis using 2% agarose gel at 110V followed by staining in ethidium-bromide.

Results: The results showed that 49.1% and 44.4% of strains were resistant to polymyxin B and colistin, respectively. And MIC_{50} and MIC_{90} were both 2 μ g/mL and 8 μ g/mL. (Table. 1) Source of strains is divided into intestinal isolates (79.5%) and extraintestinal isolates (20.5%) and compared by age. Most strains were from suckling pigs (35.1%) and nursery pigs (54.8%).Of the 409 strains, 365 strains were found carrying *mcr* gene by using multiplex PCR. Only *mcr-1* and *mcr-3* were detected in this study, and the results indicated that prevalence of *mcr* gene increased year on year (Table 2). The proportions of strains carrying only *mcr-1*, only *mcr-3* and both *mcr-1* and *mcr-3* were 354 (97.0%) of 365 strains, 7 (1.9%) of 365 strains and 4 (1.1%) of 365 strains, respectively.

Table 1. MIC_{50}, MIC_{90} and resistance percentages of *E. coli* isolated from diseased swines.

Antibiotics	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	R (%)
Polymyxin B	2	8	49.1
Colistin	2	8	44.4

Table 2. Annual tendency of colistin-resistant *E. coli* and *mcr* gene positive isolates from diseased swines.

Year	colistin resistant (%)	*mcr-1* positive (%)	*mcr-3* positive (%)	*mcr-1* and *mcr-3* positive (%)
2016	34.4	87.4	0	0.8
2017	50.6	86.2	2.4	1.0
2016-2017	44.4	86.6	1.7	1.0

Conclusions: Colistin-resistance was common in pathogenic *E. coli* isolated from diseased pigs in Taiwan and this is probably owing to the presence of plasmid- mediated colistin resistance.

Acknowledgement: The author thanks professor Kuo and his laboratory for the technical assistance.

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Ent-014

Prevalence and virulence of *Clostridium perfringens* from pigs in Korea

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Introduction

Clostridium(C). perfringens was pathogen that induced digestive diseases, such as diarrhea and hemorrhagic and necrotic enteritis in pigs and were divided into five types (A, B, C, D and E) based on their major toxins (alpha, beta, epsilon and iota toxins). In South Korea, *C. perfringens* type A (alpha toxin) was common isolated from pigs, whereas a novel toxin encoded by the *cpb2* gene, called by beta2 toxin has been also implicated in pathogenesis of enteritis in pig. In the present study, we investigated that the prevalence and virulence of beta2 toxin gene of *C. perfringens* in pig in Korea from 2018.

Materials and Methods

A total of 231 fecal samples (diarrhea = 132, non-diarrhea = 99) were collected from all aged of pig. Samples were taken from 10 different pig farms in South Korea. All of fecal samples were inoculated into blood agar plate and incubated anaerobically at 37℃ for 24 hr. Presumptive colonies with characteristic dual hemolytic zones were picked up. Total DNA was extracted from the isolates using DNA extraction kit. Multiplex PCR was performed to detect the major four and beta2 toxin genes.

Results

Seventy four (20.3%) of *C. perfringens* were isolated in fecal samples and all of isolates were confirmed as type A: 42 (32.1%) *C. perfringens* were originated from diarrhea samples and 32 (32.0%) were from non-diarrhea samples, respectively. 34 (80.9%) of *C. perfringens* isolates from diarrhea were possessed with beta2 toxin gene, 20 (62.5%) of the isolates from non-diarrhea were detected with beta2 toxin gene.

Conclusion

C. perfringens (n = 74) were isolated from both diarrhea and non-diarrhea in pigs, and all isolates were confirmed as type A. Although 80.9% of the *C. perfringens* isolates from diarrhea and 62.5% of the isolates from non-diarrhea were detected with beta2 toxin gene. However, the association between cpb2 and type A *C. perfringens* in pig has not been published. This result suggest that the need for further studies related to the cpb2 and type A of *C. perfringens*.

[This work was supported by a research grant from the Animal and Plant Quarantine Agency (APQA)]

Table 1. Detection of virulence factor in C. perfringens from diarrhea and non-diarrhea pig in Korea, 2018

Group	Type of feces	No. of isolation /samples (%)	No. of virulence gene detection*				
			cpa	cpb	ext	iap	cpb2
Suckling	Diarrhea	23/30 (76.7)	23	0	0	0	22
	Non-diarrhea	1/1 (100.0)	1	0	0	0	1
Weaning	Diarrhea	17/80 (21.3)	17	0	0	0	12
	Non-diarrhea	4/27 (14.8)	4	0	0	0	2
Growing/ finishing	Diarrhea	2/20 (10.0)	2	0	0	0	0
	Non-diarrhea	8/47 (17.0)	8	0	0	0	6
Sow	Diarrhea	0/1 (0.0)	0	0	0	0	0
	Non-diarrhea	19/125 (15.2)	19	0	0	0	11
Total	Diarrhea	42/131 (32.1)	42	0	0	0	34
	Non-diarrhea	32/100 (32.0)	32	0	0	0	20

Ent-015

Prevalence of virulence genes of *Escherichia coli* isolated from piglet feces in Korea

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Introduction: *Escherichia (E.) coli* is known as one of the normal gastrointestinal flora found in human and animals [1]. However, pathogenic *E. coli* is a common agent responsible for a variety of intestinal disorders, such as diarrhea and edema disease in animals [2]. In this study, we investigated the prevalence of virulence genes of *E. coli* isolated from piglet feces.

Materials and Methods: From 2017 to 2018, fecal samples (n = 430) were collected from suckling and postweaning piglets in 22 pig farms. All fecal samples were inoculated on blood agar and MacConkey agar plates for the isolation of *E. coli*. The isolates were tested with PCR for the determination of the prevalence of pilus genes (F4, F5, F6, F18 and F41) and toxin genes (STa, STb, LT and Stx2e), respectively.

Results: Among the tested *E. coli* isolates, 71 (16.5%) were positive for the pili and/or toxin genes (Table 1). Although the prevalence of virulence genes in postweaning piglets was higher from diarrhea samples compared to non-diarrhea samples, the difference was not significant ($p > 0.05$). The most prevalent toxin gene was STb (n = 16), followed by Stx2e (n = 15) and LT + STb (n = 13), respectively. On the other hand, F18 (n = 19) and F4 (n = 16) were predominant pilus types in piglets in Korea. F6 and F41 were not detected in this study (Table 2).

Conclusions: *E. coli* with virulence genes was more frequently detected from postweaning piglets than suckling piglets. The predominant toxin types were STb, Stx2e and LT+STb, respectively. And, F18 and F4 were also highly detected in this study. The present study contribute to understanding the characteristics of *E. coli* isolated from diarrhea and non-diarrhea samples in Korea.

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

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Table 1. Prevalence of *E. coli* with virulence genes according to the fecal samples

Age	Fecal sample	No. samples	No. virulence gene positive (%)
Suckling	Diarrhea	117	6 (5.1)
	Non-diarrhea	22	0
postweaning	Diarrhea	191	43 (22.5)
	Non-diarrhea	100	22 (22.0)
Total	-	430	71 (16.5)

Table 2. Distribution of pilus and toxin genes in *E. coli* isolated from diarrhea and non-diarrhea samples

Pilus	No. of positive with indicated toxin genes							Total
	None	STa	STb	Stx2e	LT + STb	Stx2e + STb	STa + STb	
None	0/0[a]	0/0	10/3	9/4	0/0	1/0	2/2	22/9
F4	0/0	0/0	3/0	0/0	7/2	0/0	4/0	14/2
F5	0/0	4/1	0/0	0/0	0/0	0/0	0/0	4/1
F18	5/7	1/0	0/0	0/2	3/1	0/0	0/0	9/10
Total	5/7	5/1	13/3	9/6	10/3	1/0	6/2	71

[a]Isolates from diarrhea/non-diarrhea

Ent-016

Seroprevalence Study of *Lawsonia intracellularis* in the Philippines

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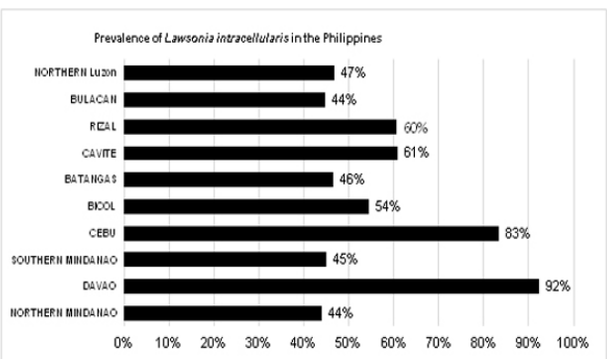
Introduction: *Lawsonia intracellularis* is an obligate intracellular bacterium that is the etiologic agent of ileitis in pigs[1,2]. Ileitis is considered as an economically-important disease in pig production and is responsible for considerable losses worldwide[3]. Its hallmark pathologic finding is thickened mucosal lining primarily of the small intestine due to infected crypt cell proliferation and thickening. A prevalence report in 2004 presented an 86% positive farms in the Philippines for *L. intracellularis*, and even higher in neighboring countries in Asia[4]. Since more up-to-date seroprevalence data on porcine ileitis in the Philippines is limited, this study was conducted to examine the current status of the disease locally.

Materials and Methods: Serum samples from all age groups were obtained from 32 commercial farms all around the country. A total of 1560 sera were collected from weaning pigs (n=104), nursery pigs (n=94), starter pigs (n=18), grower pigs (n=325), finisher pigs (n=253), breeder gilts (n=234), breeder sows (n=500), and breeder boars (n=32). Samples were tested for *Lawsonia intracellularis* antibodies with blocking ELISA using Ileitis-ELISA kit in accordance to the manufacturer's instructions (Svanova Biotech AB, Sweden).

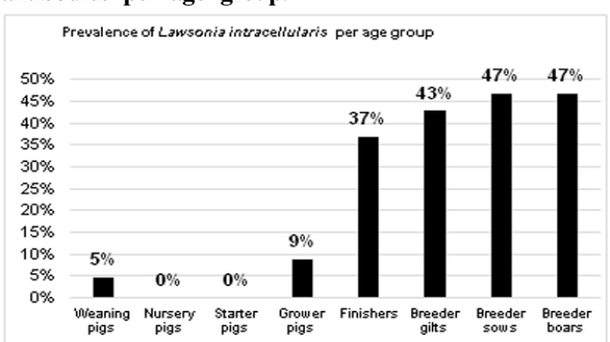
Results: All regions in the Philippines where serum samples were collected and tested for *L. intracellularis* have shown varying degrees of percent positivity relative to sample size. Davao presented the highest positive samples at 92%, followed by Cebu with 83% (Fig. 1).

Figure 1. Prevalence of *Lawsonia intracellularis* in farms from different Philippine regions.



In terms of age group, there were 5% positives in weaning pigs while no positives were detected during the nursery to starter stage. Positive rates started to re-emerge during the growing stage at 9% and continued to rise to 37% at the finishing stage, suggesting seroconversion and exposure to the pathogen. The rate persisted to rise in breeder animals at 43-47% (Fig. 2).

Figure 2. Positive samples (%) to *Lawsonia intracellularis* antibodies per age group.



Conclusions: Based on the seroprevalence data, the causative agent of porcine ileitis *Lawsonia intracellularis* remains to be widespread all throughout the Philippines. Seroconversion, which is the change from a seronegative to a seropositive condition, starts at the growing stage. Positive rates continued to rise with age. This denotes exposure to the pathogen and can be indicative of health challenge in the herd.

Acknowledgement: MSD Animal Health wishes to thank the staff of all farms involved for participating in the study and contributing toward data collection.

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Ent-017

The efficacy of guava leaf extract on treatment diarrhea for piglets

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Introduction: Diarrhea is a common disease of weaning piglets. In Vietnam, farmers usually use antibiotic to control this disease for piglets. This may lead to the abuse of antibiotic using and antibiotic resistance. Therefore, antibiotic alternatives should contribute to the change in the habit of the farmer for a better sustainable agriculture. One of these alternatives is using herbal medicine. Guava leaves are very popular in Vietnam. We can collect them from the garden of intensive farm in the Mekong Delta with reasonable price. Guava leaves contain quercetin as an antibacterial agent and flavonoid, triterpene, tannin as antispasmodic. The aqueous extract of guava leaves inhibits the bacteria which causing diarrhea in piglets such as *Escherichia coli, Salmonella.* The objective of this experiment was to compare the efficacy of guava leaf extracts to treat diarrhea for piglets.

Materials and Methods: Syrup of guava leaves has been manufactured in the laboratory of Nong Lam University with a good taste for the free drink of the nursery piglets or weaning piglets. The trial using this product was conducted from April to July 2018 at swine farm located in Binh Duong province. Total of 322 diarrheal piglets were randomly assigned into 3 treatments. Piglets in the Treatment 1 (T1), the control treatment, followed the routine procedure of the farms, were provided enzyme and oral antibiotic. In treatment 2 (T2), piglets of about one week old, were supplied orally at 2ml of extract per day until recovery. Piglets of more than one week old, in the treatment 3 (T3), were provided of 5ml of extract per day, orally until recovery. The piglets were followed up in 30 days. Percentage of diarrhea day, average treatment day, recovering rate after 3, 4, 5 and 6 days were recorded.

Results: The results of the study were shown in the Table 1. The data has proved that the aqueous guava leaf product has effectively treated piglet diarrhea and the extract do not have any negative effect in the performance of the pigs.

Table 1. The mean of experimental criteria among treatments

Criteria	T1	T2	T3	p
No of diarrhea pigs	117	109	96	
Body weight at beginning (kg)	1.42	1.37	2.69	
Percentage of diarrhea (%)	19.50	16.8	13.40	
Average treatment day (day)	5.60	5.00	4.80	0.217
Average daily gain (g)	0.191	0.199	0.28	<0.001
Recovering rate after 3 days (%)	27.35	41.28	38.54	0.068
Recovering rate after 4 days (%)	62.39	74.31	88.54	<0.001
Recovering rate after 5 days (%)	78.36	95.41	97.91	<0.001
Recovering rate after 6 days (%)	94.01	98.16	100	0.023

Conclusions: The aqueous guava leaf extract has effectively treated piglet diarrhea. As a result, it may be a potential alternative for antibiotic to control diarrhea in the piglet.

Acknowledgement: The authors wish to acknowledge the support of the Department of Veterinary Biosciences, Faculty of Animal Science and Veterinary Medicine, Nong Lam University Ho Chi Minh City, Vietnam for this study.

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Ent-018

The reduction of proliferative hemorrhagic enteropathy after mass vaccination with Enterisol® Ileitis in Korean Sow farm

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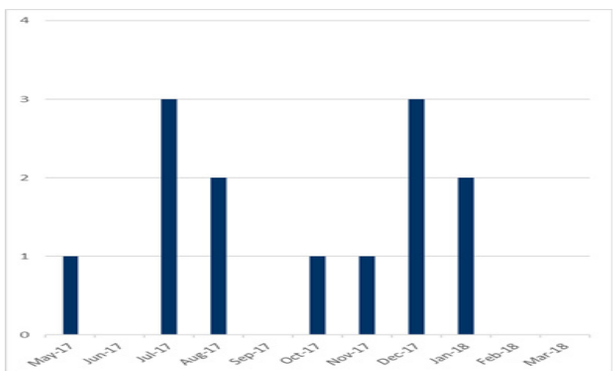
Introduction: Proliferative enteropathy (ileitis) is an infectious enteric disease caused by Lawsonia intracellularis and characterized by thickening of the mucosa of the intestine due to hyperplasia of the crypt enterocytes [1]. The disease in pigs includes several acute and chronic clinical manifestations, including proliferative hemorrhagic enteropathy (PHE) and acute hemorrhagic diarrhea with sudden death of pigs close to market age, and porcine intestinal adenomatosis, a chronic mild diarrhea with reduced performance of growing pigs. Some cases of sudden death occur in replacement gilts seen with bloody diarrhea and PHE. The present paper describes the reduction of PHE cases by mass vaccination in a sow farm.

Materials and Methods: This field case was recorded in a sow farm managed 3-site system with 800 sows. 4-weks old piglets are transferred to the nursery farm. Around 70 days old pigs are moved to the fattening farm. The sow herd is PRRS positive. After entry of gilt from GP farm on Jun. 2016, some dry sows showed hemorrhagic diarrhea and died suddenly. From May 2017 gestation sows' death increased rapidly. Some dead sows' were 3rd or 4th parity. During this period, fattening farm manager complained about fatteners' diarrhea and slow growth. From a dead sow with hemmorrhagic diarrhea, we detected *Lawsonia intracellularis* antigen by PCR. From that time, to prevent sow's sudden death we treated by using antibiotics (tiamuline, tylosine, licomycine) in the feed. After checking all antibiotics were used up, we determined to vaccinate Enterisol® Ileitis to all pigs in the herd. After that routine vaccination was implemented to the sow herd and gilt every 3 month.

Results: After mass vaccination mortality of sows and selected gilts ceased. In addition, symptoms like hemmorrhagic diarrhea also disappeared.

Figure 1. Symptoms of hemorrhagic diarrhea which occurred in this sow herd.

Figure 2. PHE case every month from May 2017. Mass vaccination conducted Mar. 2018. After mass vaccination no further PHE cases were detected.



Conclusions: PHE case often occurred 3rd to 4th parity sows. In an acute ileitis outbreak, mass vaccination to sow herd was effective in reducing mortality. Mass vaccination against ileitis is rarely used, but it is worth considering during a severe outbreak.

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IMM-001

A commercial modified live porcine reproductive and respiratory syndrome vaccine confers cross-protection against heterologous North American (type II) lineage 3 high virulent isolate challenge

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) is one of the most common diseases in global swine industry. PRRSV consists of two genotypes, PRRSV-1 and PRRSV-2, which are further composed by several sub-lineages [1]. With broad divergence of PRRSV, the reliability of global universal vaccine to confront divergent PRRSV field strains remains questionable for decades [2]. This study was aimed to evaluate the cross protective efficacy of Fostera® PRRS MLV, an attenuated lineage 8 PRRSV strain, against virulent heterologous lineage 3 PRRSV field isolate challenge.

Materials and Methods: The PRRSV field strain (TSYM-142575) used for pig challenge in this study was originally isolated from a farm with severe post-weaning respiratory distress and continuous high mortalities in Taiwan, which shared 85.7% of ORF5 identity with the vaccine strain. Eighteen Landrace-Yorkshire cross-bred, male, conventional pigs were introduced from a PRRSV-free farm and randomly divided into Mock, MLV and unvaccinated (UnV) groups. MLV group was administrated with Fostera® PRRS vaccines at 3 weeks old. Meanwhile, both of MLV and UnV groups were inoculated with 10^6 $TCID_{50}$ of TSYM-strain PRRSV inocula (IM+IN) at 7 weeks old. All pigs were then sacrificed for pathological examination at 21 days post-challenge (DPC). During experimental period, blood samples were taken for virological analysis and the clinical performances were measured.

Results: Following TSYM-strain challenge, UnV group presented persistent fever (≥ 40℃) from 1 to 13 DPC. In contrast, MLV group showed slightly raised body temperature after challenge and then gradually recovered from 4 DPC. The average daily weigh gain (ADWG) of UnV group was significantly retarded than MLV group, which still had 70% preservation of ADWG compared to the Mock pigs. In the pathological examination, the severity of interstitial pneumonia (IP) induced by PRRSV infection of MLV group was milder than UnV group in both macro- and microscopic evaluations. Furthermore, the PRRSV viremia data also indicated that the viral titers of MLV group were consistently 10^1 to 10^2 less than that in UnV group at 4, 7, 10 and 14 DPC.

Table 1. Summary of measuring parameters in Mock, MLV and UnV groups after challenge.

Parameter	Group		
	Mock	MLV	UnV
Mortality (%)	0	0	16.6
Fever days (≥ 40℃)	0.0±0.0	3.0±0.5	6.2±0.5
ADWG (g)	744±29	524±46	325±65
IP area (%)	1.9±0.4	19.6±6.1	37.8±11.3
IP score1	0.7±0.1	1.4±0.1	2.0±0.2

[1]Histopathological score of interstitial pneumonia ranging from 0 (normal) to 4 (severe).

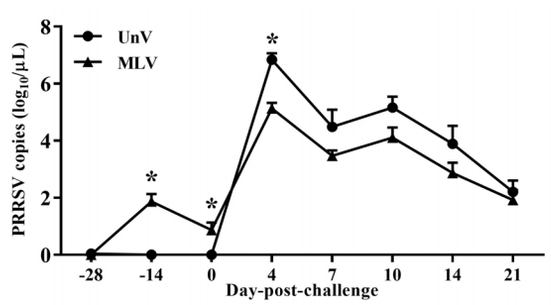

Figure 1. PRRSV viremia titers of Mock, MLV and UnV groups after vaccination and challenge.

Conclusions: The present study demonstrates that the vaccination with Fostera® PRRS MLV confers partial cross-protections against the heterologous challenge of a virulent lineage 3 PRRSV isolate.

Acknowledgement: This research was supported by Zoetis Taiwan Limited through contract research funds (Grant number 106-D-638) in NCHU.

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IMM-002

A novel inactivated *Actinobacillus pleuropneumoniae* cells by GI24 induce robust immune responses and provide effective protection in piglets

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Inrroduction : Porcine pleuropneumonia (PP) is a severe, contagious, swine pulmonary disease caused by *Actinobacillus pleuropneumoniae* (APP). This disease affects pigs of all ages and has a major impact on economics, ecology and animal welfare in the pig-rearing industry. For control of PP, vaccination is crucial. However, the many serotypes of APP have made effective vaccination difficult. This study was carried out to examine whether intramuscular (im) immunization of post weaning piglets with the novel vaccine candidate could effectively protect the piglets from porcine pleuropneumonia.

Materials & Methods : *Actinobacillus pleuropneumoniae* (APP) serotypes 2 or 5 strain was lysed by GI24. The mixture of two types lysed cells were used as APP vaccine candidate in post weaning piglets. All piglets were primed at 4 weeks of age and were boosted at 6 weeks of age. Group A piglets were im inoculated with PBS. Group B piglets were im immunized with 2×10^9 of the mixture of formalin-inactivated APP type 2, and type 5 cells. Group C piglets were im immunized with 2×10^9 the mixture of the APP type 2 lysed cells, and the type 5 lysed cells. Seral IgG titers from all piglets were evaluated using ELISA kit. All piglets were challenged with the mixture of wild-type APP types 1, 2, 5, 7, and 12 strains at 4 weeks post prime immunization. All piglets were monitored daily for mortality for 7 days after challenge. The signs of porcine pleuropneumonia (PP) were evaluated from all piglets after challenge.

Results : The Seral IgG titers from group B-C piglets were significantly higher than those of group A piglets. After challenge with APP types, mortality and signs of PP in group C piglets was not observed. However, mortality was observed in 60% of groups A-B piglets, and the signs was observed in 100% of group A and in 80% of group B.

Conclusion & Discussion : These findings indicate that im immunization of piglets with the mixture of the GI24-inactivated vaccine cells can effectively protect the piglets from PP.

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IMM-003

A summary of production diseases on Malaysian Swine Farms

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Introduction

Production diseases such as Porcine Circovirus 2 (PCV2), *Actinobacilus pleuropneumoniae* (APP), Porcine Reproductive and Respiratory Syndrome (PRRS) and *Lawsonia intracellularis* (LI) are present in the Malaysian swine industry. This study aims to study the epidemiological patterns associated with the above diseases in Malaysian swine farms and provide recommendations with regard to the types of vaccines needed.

Material and methods:

Serum samples were collected from 10 farrow to finish swine farms and tested at a diagnostic laboratory in the Netherlands. The samples were collected from gilts, pregnant sows and swine at age 3, 6, 9, 12, 16 and 24 weeks Sample were tested for PCV2 Alphalisa (MSD Animal Health In-house), PCV2 ORF2 qPCR, APP APX4 ELISA (IDEXX), APX 1,2 and 3 ELISA (MSD Animal Health In-house), PRRS ELISA (IDEXX) and *Lawsonia intracellularis* ELISA (Svanova)

Conclusions:

PCV2 antibody titer : High titers were usually present in sows and gilts. These are likely the reservoirs of the virus. Piglets at 3 weeks of age had high levels of maternal antibody, from this age onwards there was a steady decline in antibody level till around average week 16 (Range 12-24 weeks) when antibody level begins to rise (Chart 1), indicating that this is the common period of virus challenge.

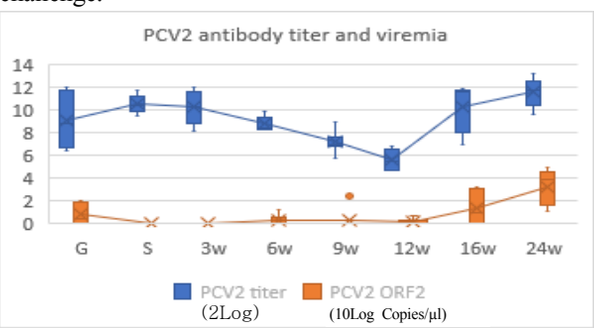

Chart 1. PCV2 antibody titer and viremia in different age group in a farm.

PCV2 ORF2 qPCR : Sow and gilt displayed seropositivity to antibodies but viremia was not present or present in only low levels. Viremia began to increase in some farms around 12-16 weeks of age (Range as high as 10^4 - 10^5) Given the presence of late stage viremia in some herds, a vaccine with an adequate duration of immunity till market is necessary to prevent late infection.

APP : Gilts and sows were found seropositive in all herds for APX4, indicating that APP was present in all 10 farms (Chart 2). The breeder herd is most commonly the reservoir for the piglets and most mature swine are exposed to field APP by the time they enter the breeding herd. They potentially then serve as transmission reservoirs for their piglets. Most herds were seropositive for APX 1 and APX 2. Given that multiple serotypes can produce APX 1 and 2 simultaneously, it is not possible to ascertain which serotypes are present on these farms. A vaccine which contains multiple APX antigens is indicated.

PRRS : The majority of herds exhibited seroconversion in late or early nursery, coinciding with an increase in respiratory infections reported. The majority of herds were not vaccinating piglets for PRRSv. Given the farrow to finish nature of the sampled farms, vaccination may be indicated in these circumstances as an aid to control PRRSv infection.

LI : All sows and gilts profiled were seropositive, suggesting that these are the reservoir in the herd. Subsequently, all herds showed increasing seroconversion suggesting exposure to field infection from 12 weeks onwards, suggesting that LI is present in most farms in Malaysia and that control methods may be required to prevent production losses.

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IMM-004

Administration with a commercial recombinant Verotoxin 2e vaccine induces serum neutralization antibody and prevents Edema disease in conventional pig farms in Korea

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Introduction: Edema disease(ED) is caused by an *Escherichia coli*(*E.coli*) producing Stx2e which is also termed as Verotoxin 2e(VT2e) due to its cytopathic effect(CPE) on Vero cells. ED is characterized by edema, neurological disorders and sudden death in weaning pigs. Due to antibiotic resistance and pork safety issue, extensive research is ongoing to develop safe and effective ED vaccines to avoid antibiotics. The frequencies of VT2e-producing *E.coli* isolated from pigs in Korea increased over the recent years, but there has been no commercialized ED vaccine in Korea until now. The purpose of this study was to evaluate whether a commercial recombinant VT2e vaccine(VEPURED®, Hipra, Spain) induces serum neutralization antibody against VT2e and prevents ED outbreaks in conventional pig farms in Korea.

Materials and Methods: This study was conducted in three conventional pig farms with high mortality problems associated with ED. A total of 186 two days old(do) piglets were used, 95 were administered with VEPURED(VAC group) and 91 were administered with PBS(CON group). Until slaughter, dead pigs suspected or confirmed as death caused by ED were recorded, and the mortality rate was calculated. For seroneutralization assay, total 5 times of bleeding were performed; at 2 do, the start of weaning, growing and fattening, and the end of fattening, respectively. 1:2 diluted serum was mixed with same volume of Korean VT2e having concentration of 50% CPE and added to 96-well plate with Vero cell monolayers. After incubation and removal of dead cells, the percentage of live cells using optical density at 595nm was calculated to determine whether the serum neutralized 50% CPE caused by VT2e. The percentage of pigs showing VT2e neutralizing antibodies in each group was calculated.

Results: Until slaughter, total 0% of ED mortality was observed in VAC group whereas 13% was observed in CON group. In VAC group, all pigs were seronegative at 2do, seroconversion took place during suckling period, 89.5% of pigs turned to be seropositive of VT2e neutralizing antibodies at the start of weaning and the percentage remained consistent until the end of fattening; 89.9%, 79.5% and 74.7% at the start of growing and fattening, and the end of fattening, respectively. In contrast, significant seroconversion was not observed until the end of fattening for CON group. For ED mortality and percentage of pigs showing VT2e neutralizing antibodies, there were statistically significant differences between VAC and CON groups($p<0.05$).

Table 1. The percentage of pigs showing VT2e neutralizing antibodies to VT2e (%)

Group	Bleeding time				
	1st	2nd	3rd	4th	5th
VAC	0	89.5	89.9	79.5	74.7
CON	0	2.2	10.3	8.9	1.3
*p**	-	<0.001	<0.001	<0.001	<0.001

VAC, vaccinated; CON, control;
* *p-value for chi square test. Results are statistically significant if p<0.05*

Conclusions: Vaccination with HIPRA VEPURED induced high percentage of VT2e neutralizing antibodies persisting until slaughter date and demonstrated reduction of mortality associated with ED, resulting in prevention of ED outbreaks in three conventional pig farms in Korea.

Acknowledgement: This work was supported by Hipra Korea, Inc., Republic of Korea.

References:

[1] Do, Kyung-Hyo, et al., 2019, J Vet Sci, 20(1), 43-5.

IMM-005

Algal polysaccharides to improve gut health

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Introduction: Supporting gut barrier function and the gut-associated lymphoid tissue is determinant to ensure gut health in animals. Recent research has highlighted the potential of in-feed marine macroalgal polysaccharides to strengthen animal's defenses. The cell wall of marine algae is mainly composed of water soluble sulfated polysaccharides with several biological activities such as modulation of the immune response [1] and reinforcement of gut barrier function via induction of mucin secretion in-vivo in the intestinal mucosa [2].

Materials and Methods: A research project in collaboration with INRA (France) using the intestinal epithelial cell line IPEC-1 led to the demonstration of the ability of a specific extract from green algal Ulva sp to upregulate the expression of immune mediators (cytokines and chemokines) and the molecular mechanisms underlying this immunomodulatory activity by identifying the cell receptor and the signaling pathways involved [3;4]. The potential enhancement properties of a red algal extract from Solieria Chordalis on intestinal integrity were assessed using in vitro cell models HT-29 MTX and Caco-2 cells for mucin and tight junction evaluation respectively, under physiological and inflammatory conditions (Intestinal Biotech Development, 2017). The gene expression of several mucins (MUC1, MUC2, MUC4, MUC5AB and MUC5AC) and tight junction proteins (ZO-1, ZO-2, claudin-2) was evaluated by qRT-PCR.

Results: *Ulva sp* extract has the capacity to upregulate the expression of immune mediators: TNFα , CCL20, IL-1α , TGF-β that are involved in cell differentiation and proliferation, recruitment of immune cells and anti-inflammatory activities. *Ulva sp* extract interacts with TLR4 and TLR2 and leads to rapid activation of transcription factors PI3K and NF-κ B. The red algal extract was shown to upregulate the expression of different target genes related to transmembrane (claudin-2) and scaffolding proteins (ZO-1 and ZO-2). Furthermore, the red algal extract was shown to upregulate the expression of mucin targeted genes: membrane-bound mucins (MUC4) and secretory mucins (MUC2, MUC5B and MUC5AC).

Figure 1. Genetic expression of immune mediators, expression in presence of Ulva sp. compared to control.

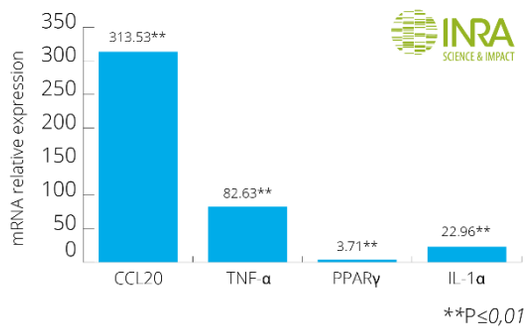
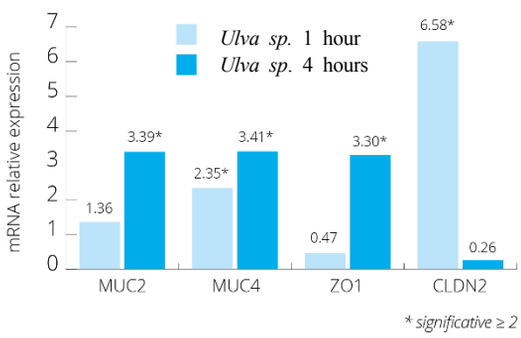


Figure 2. Genetic expression of gut integrity biomarkers in presence of Ulva sp. compared to control.



Conclusions: The combination of the tested algal extracts can reinforce gut health targeting barrier function which is the first line of mucosal defense and via modulation of local innate and adaptive immune responses and induction of anti-inflammatory activities. The use of macroalgal sulfated polysaccharides can play an important role within the reduction of antibiotics in farms.

References:

[1] Kim SK et al., 2011. Adv Food Nutr Res. 64:391-402.
[2] Barcelo J., 2000. Gut. 46:218–224.
[3] Berri M et al., 2016 J Appl Phycol
[4] Berri M et al., 2017 Algal Research 28, 39-47

IMM-006

Antibody responses and pork carcass defects after vaccination with Smartvac® compare to traditional syringe and needle injection under field conditions

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Introduction

Smartvac® is an autonomous electric vaccine injector developed by Ceva Desvac Campus for both intramuscular and subcutaneous injections with a needle for pigs. It causes less stress and pain, reduces leakage at the injection site and guarantees the compliance of the expected volumes and the quality of injections by recording and analysis of the data obtained during the vaccination sessions with a total traceability. The aims of this study were to measure the antibody responses and detecting of injection site reactions at slaughter after vaccination with Smartvac® compare to the traditional syringe injection, under field conditions in Thailand.

Materials and Methods

In total 240 pigs were randomly selected and divided into four equal groups T1, T2, T3 and T4 of 60 pigs each. All groups were injected with classical swine fever vaccine (Coglapest®, Ceva) intramuscularly at 6 weeks of age with different methods. Group T1 pigs were injected with Smartvac in the neck behind the base of the ear. Group T2 pigs were injected with Smartvac® at the ham of the hindleg. Group T3 pigs were injected with a traditional syringe at the neck. And group T4 pigs were injected with a traditional syringe at the ham. All vaccinations were performed on the right side of the pigs and no other injections were permitted. Blood samples were collected from the same 16 pigs for each groups at 6 (before vaccination), 8, 12, 16, and 20 weeks of age. Antibody titers were measures by neutralizing peroxidase-linked antibody assay (NPLA). Meat abscesses were evaluated when the pigs reached market weight.

Result

Antibody titers of T1 and T2 groups were slightly higher than T3 and T4 groups at all time points. However, all pigs had higher SN titers than the threshold of vaccine protection. At 8 weeks of age, the T1 group SN titer was significantly higher than T3 and T4 group. There were no statistically significant at other time points. There were 5.0% and 1.7% meat abscesses detection in group T3 and T4 respectively. However, no meat abscess detection from T1 and T2 groups.

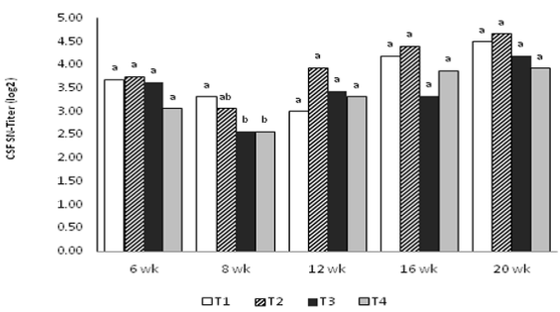

Figure 1 Serum neutralizing antibody levels (log2) from 16 same pigs for each groups

Table 1 Meat abscess detection from 60 pigs each groups (n=240).

Group	No. of meat abscess detection	Percentage
T1	0/60	0.0%
T2	0/60	0.0%
T3	3/60	5.0%
T4	1/60	1.7%

Conclusion

The result showed that Smartvac® group T1 induces a higher antibody response against CSF compared to traditional syringe application 2 weeks post-vaccination. Nevertheless, SN titers in all groups reached the protective level. Also, under the conditions of this study, the use of Smartvac® reduced number of abscesses in both neck and ham. This study confirms that Smartvac® can improve quality of vaccination and enhances animal welfare.

Reference

[1] King., 2010., Journal of Swine Health and Production: 290-293
[2] Smartvac® User Manual
[3] Terpstra et al., 1984. Veterinary Microbiology Volume 9, Issue 2: 113-120

IMM-007

Comparative field efficacy study between FLEXcombo® and ready-to-use vaccine in a Korean grow-out farm

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Introduction

Porcine circovirus type 2(PCV2), Mycoplasma hyopneumoniae (M. hyo) are two major pathogens causing Porcine respiratory disease complex (PRDC). These two pathogens result in serious economic losses in Korean swine industry (1). To mitigate economic losses, two different kinds of commercial combination vaccines are available for the less injection and better efficacy. In this study, the efficacy like mortality, weight gain and medication cost were evaluated for the improvement of farm productivity.

Material & Method

Table 1. Trial Design

Group	Treatment	Number of pigs (days / weight)
A	FLEXcombo® 2ml	2317 78.3 days / 33.5kg
B	Fostera™ PCV MH 2ml	2365 81.5 days / 31.8kg

The field observation was conducted on the grow-our farm. Piglets were weaned at 25 days of age and transferred to the grow-out farm at around 80 days of age. To determine infection timing of PCV2, SerELISA PCV-2 was analyses with serum from different ages. The seroconversion was shown at 130days of age (data now shown). Farm performance parameters after movement to grow-out farm were recorded from 2,317 pigs from Feb 27 to May 22, 2017 (FLEXcombo®, treatment group A) and from 2,365 pigs from June 5 to September 18, 2017 (FosteraTM PCV MH treatment group B). Animals of both treatment groups were vaccinated at 21 days of age according to label. There were no other changes except PCV2 vaccination program. To evaluate the performance of the pigs in the two different treatment groups, mortality rate, average market weight, average daily weight gain (ADG), FCR and medication cost were recorded for each group. Fisher's exact test was used to test differences of mortality between two vaccination groups.

Results

Table 2. Production parameter

		A	B	Diff
Pigs	(n)	2317	2365	
Mortality	(%)	3.2	7.3	-4.1
Antibiotic Medication Cost	(won/pig)	665	1,559	-894
FCR		2.89	3.11	-0.22
ADG	(g)	807	797	+10
Average market day	(Day)	174.0	184.5	10.5
Grow-out period	(Day)	95.7	103.0	-7.3
Market weight	(kg)	110.7	113.9	-3.2

< 1 $ = 1,150 Korean won >

Conclusions and discussion

Fostera™ PCV MH vaccinated pigs (Group B) showed higher mortality than FLEXcombo® vaccinated group (Group A). The grow-to-slaughter mortality was 3.2% for group A and 7.3% for group B ($p<0.0001$). All parameters pointed in favor of the FLEXcombo® vaccinated group. The findings of this study are in line with other studies that show that FLEXcombo® more efficacious compared to other PCV2 vaccines (2).

Acknowledgement and references

[1] National swine disease surveillance 2016. KPPA
[2] Payne et al. (2013). Proceedings of the 22nd IPVS, Jeju, South Korea, VP-575

IMM-008

Comparative safety and efficacy in sows and piglets of a PCV2 vaccine in Philippine field conditions

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Introduction: Objective of these studies were to assess safety and efficacy of a newly developed PCV2 vaccine based on the PCV2d genotype now predominant in many regions [1].

Materials and Methods: Trial 1 was performed in a farrow to finish farm owning 500 sows. Twenty-four pregnant sows were allocated according to parity to 3 groups of 11 sows each (groups T and C) and 2 sows (group NV). Sows in group T received the tested vaccine twice around 6 and 3 weeks before expected farrowing (Suigen® PCV2, Virbac, 2 ml for each IM injection). Sows in group C received a booster injection of the PCV2 vaccine they were vaccinated with in previous parities (2 ml by IM route). Sows in the NV group did not receive any PCV2 vaccine during the study. Piglets issued from each sow received the same PCV2 vaccine (1 ml in group T and 2 ml in group C by IM route) around 6 weeks of age while piglets from NV sows were not vaccinated against PCV2. Trial 2 was performed in a farrow to finish farm owning 300 sows. One hundred twenty piglets were allocated to 2 groups of 60 each. Piglets were vaccinated against PCV2 around 6 weeks of age by the tested vaccine in group T (Suigen® PCV2, Virbac) and by the usual PCV2 vaccine of the farm in group C (1 ml by IM route in both groups). Pigs were individually weighed at birth, weaning and finishing in trial 1 and at weaning/finishing in trial 2. Local and general reactions were checked after vaccination in both trials as well as mortality cases till finishing. In trial 1, PCV2 DNA extracts from lungs and lymph nodes samples (from 2 wasting pigs) were sequenced for PCV2 genotyping. Categorical data were compared between groups using Fisher's exact test, discrete data using Kruskall-Wallis test. Pre-wean mortality was analyzed using a GLM with the number of born alive as covariate in trial 1. Quantitative data were compared between groups by GLM with the number of born alive as covariate for pre-wean ADG in trial 1.

Results: Neither local nor general reactions were noticed after injection of the tested and references vaccines in sows and piglets. In trial 1 the number of weak born piglets per litter and pre-wean mortality were the lowest in T group. These differences should be interpreted cautiously since the mean number of born alive was numerically higher in NV group (only 2 sows). ADGs were not significantly different between groups. However the rate of finishing pigs weighing less than 80 kg was numerically higher in C group and mean FCR was higher in NV group than in vaccinated ones. In trial 2 performances were similar between both groups. PCV2 DNA extract was identified as d genotype in trial 1.

Table 1.Farrow to finish mean performances (trial 1)

Group	T	C	NV
Weak born/litter	0^{a}	$1.1^{a,b}$	2.5^{b}
Pre-wean mortality	$7.3\%^{a}$	$21.2\%^{b}$	$13.3\%^{a,b}$
Weaned/litter	9.2^{a}	9.5^{a}	13^{a}
Pre-wean ADG (g/d)	151^{a}	145^{a}	157^{a}
Wean to finish mortality	$1.0\%^{a}$	$2.9\%^{a}$	$3.9\%^{a}$
Wean to finish ADG (g/d)	636^{a}	634^{a}	619^{a}
Finish weight<80 kg	$5.0\%^{a}$	$11.9\%^{a}$	$4.0\%^{a}$
Feed conversion ratio	1.99	2.09	2.54

[a, b]:Different superscripts indicate significant differences

Table 2. Wean to finish performances (trial 2)

Group	T	C
Weaning weight (kg)	6.3 ± 0.3^{a}	6.4 ± 0.2^{a}
Finishing weight (kg)	86.1 ± 4.6^{a}	86.1 ± 4.2^{a}
Wean to finish ADG (g/d)	573 ± 31^{a}	572 ± 27^{a}
Wean to finish mortality	$8.3\%^{a}$	$8.3\%^{a}$

Conclusions: Safety and efficacy of the tested vaccine was confirmed in sows and piglets. PCV2d genotype was isolated for the first time in the Philippines.

References:

[1] Karuppannan AK et al., 2017. Viruses 9, 99.

IMM-009

Comparative safety in piglets of a porcine circovirus type 2 (PCV2) vaccine in field conditions

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Introduction: PCV2 vaccination of piglets around weaning is widely implemented due to the return on investment regarding wean to finish performances (control of mortality and growth losses due to PCV2 infection). Objective of this study was to compare safety of a newly developed PCV2 vaccine to an existing commercial one in field Taiwanese conditions.

Materials and Methods:
Two hundred 3-week old piglets from a farrow to finish commercial farm were individually identified and weighed before inclusion. They had to be clinically healthy and to weigh at least 4 kg. They were then randomly allocated to 3 groups of 90 (group T), 90 (group C) and 20 (group P) piglets each. Randomization was balanced on sow parity and piglet sex and body weight at inclusion. Group T received a whole inactivated PCV2d vaccine (Suigen® PCV2, Virbac, 1 ml by IM route), group C received a subunit commercial PCV2 vaccine (2 ml by IM route) while group P received a saline serum (2 ml by IM route). All piglets were daily checked for local and general reactions during 3 weeks following injection (one week before weaning then 2 weeks in the nursery unit). Body weights at inclusion and sex ratio were compared between groups by the Kruskal-Wallis and Chi-square tests, respectively. Local and general reactions frequencies were compared by the Fisher's exact test followed by pairwise comparisons.

Results: Distribution of sex and body weight at time of vaccination was not significantly different between groups (mean body weight between 6.3 and 6.6 kg). Local reactions were noticed as injection site swelling or erythema on 31 piglets within the 3 weeks following injection. Their frequencies were significantly higher in C than in T and P groups. General reactions were noticed in 2 pigs from group C only. They consisted in weakness and panting on the day of vaccination (no significant difference between groups).

Table 1. Local and general reactions frequencies after vaccine or placebo injection

Group	T	C	P
Local reactions	4.4%[a]	28.9%[b]	5.0%[a]
General reactions	0.0%[a]	2.2%[a]	0.0%[a]

[a, b]:Different superscripts indicate significant differences

Conclusions: The difference of local reactions frequency between both vaccines may be due to difference of formulation. Regarding the whole inactivated PCV2 vaccine, formulation consists in a water in oil in water (W/O/W) emulsion due to its specific adjuvant well known for other pig viral vaccines [1-2]. These types of emulsion have a low viscosity and are able to induce short and long term immunity [3]. In conclusion, safety of the tested vaccine was confirmed in field conditions, lack of local reactions being relevant for animal welfare and performances.

References:
[1] Park JH et al., 2013. Clin Exp Vaccine Res 2, 8-18.
[2] Gu Z et al., 2015. Virus Research 195, 57-63.
[3] Aucouturier J et al., 2001. Vaccine 19, 2666-2672.

IMM-010

Compatibility of Fostera® PRRS with a PCV2 vaccine adjuvanted with MetaStim

Jose Angulo[1], *Lucina Galina Pantoja*[1], *Marcia L. Keith*[2], *Lucas P. Taylor*[2], *James R. D. Allison1*[*]

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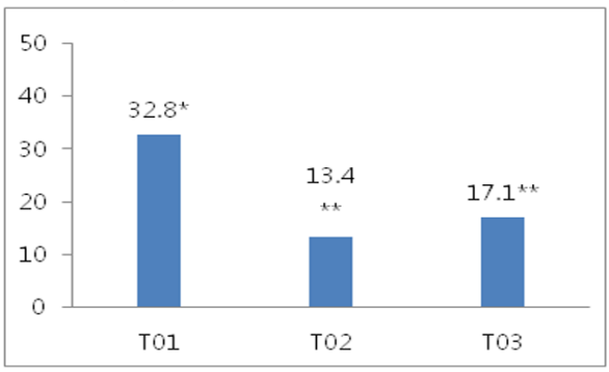
Introduction: Porcine Circovirus type 2 (PCV2) and Porcine Reproductive and Respiratory Virus (PRRSV) are two important components of PRDC in growing pigs and their control through the right vaccination strategies is critical to minimize economic losses. The objective of this study was to evaluate the possibility of antigen interference when a live PRRSV vaccine (Fostera® PRRS) was rehydrated with a Metastim-adjuvanted PCV2 vaccine (Fostera® Gold PCV) in place of the normal diluent.

Materials and Methods: Sixty healthy three-weeks of age PRRSV-negative pigs (by IDEXX ELISA) were housed in a BSL-2 containment facility. In order to detect whether common vaccines could be mixed without a detrimental effect on efficacy, a non-inferiority study was implemented using two treatments groups, T02 and T03 (N=24 pigs per group), with lung lesions as the primary variable and a margin of non-inferiority delta of 5%. T02 received a Fostera® PRRS vaccine reconstituted with sterile diluent and T03 received Fostera® PRRS reconstituted with Fostera® Gold PCV (containing PCV2a, PCV2b and MetaStim adjuvant). An additional group, T01 (N=12 per group) was used as a non-vaccinated-challenged control group. The vaccine was administered intramuscularly (2ml) once to pigs at 21 days of age. Pigs were then challenged with virulent PRRSV isolate NADC20 at approximately 7 weeks of age (four weeks after vaccination). Upon arrival pigs were housed in three separate rooms by treatment with T01 pigs in one room and T02/T03 in 2 additional rooms (one treatment per room). Prior to the challenge phase all pigs (T01, T02 and T03) were rehoused into 1 room. Serum was harvested from all pigs at various time points pre-and post-challenge. Body weights were recorded prior to challenge and the day of necropsy. On study-day 38 (10 days post challenge), pigs were euthanized, and lung lesions were scored. The primary variable in determining non-inferiority was percent lung lesions in treatment group T03 in relation to T02. Secondary variables included clinical signs attributable to respiratory disease as well as level and duration of viremia (RT-qPCR) and body weights.

Results: Treatment group T03, Fostera PRRS rehydrated with Fostera Gold MetaStim (PCV 2a/2b MetaStim) was non-inferior to treatment group T02, Fostera PRRS rehydrated as labeled using a delta of 5% (Non-inferiority decision passed as the lower confidence limit of the comparison of the test to the standard at -4.45% was above -5%). Additionally, the least squares mean percent lung with lesion for both treatment groups (T02 and T03), was significantly less ($P \leq 0.05$) than the level of lesions observed in control group (T01) (figure 1). When comparing secondary variables, clinical signs attributable to respiratory disease were not biologically different between treatment groups, in addition, level of viremia (RT-qPCR), and body weight including ADG showed significant differences ($P \leq 0.05$) between vaccinated groups (T02 and T03) compared to non-vaccinated controls (T01) post-challenge supporting efficacy against a virulent PRRSV challenge in both vaccinated groups (T02, T03) when compared to controls.

Table 1: Percent lung lesions following a virulent challenge 28 days post-vaccination with PRRS-MLV rehydrated with sterile diluent (T02) or PCV2a/2b MetaStim (T03)



[**]significant difference to non-vaccinated control (T01) at p value <0.05

Conclusions: This study provides evidence of efficacy against a virulent PRRSV challenge in both vaccinated groups (T02: Fostera PRRS alone and T03: Fostera PRRS rehydrated with PCV 2a/2b MetaStim) when compared to controls.

IMM-011

Comprehensive metabolomics characterization of different pseudorabies virus strains infection in porcine alveolar macrophages

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Introduction: Emerging pseudorabies virus (PRV) variant has led to frequent outbreaks of PRV infection among Bartha-K61 vaccinated swine population in Chinese swine farms [1]. Despite the widespread use of vaccines, classical PRV strain (PRV-EA) caused high mortality in pigs of all age since late 1998, and emerging PRV virus variant strain (PRV-HNX) was a serious threat to swine industry since 2011. However, the mechanisms underlying the interaction between different pseudorabies virus strains infection and host immune cells are not fully understood. Here, we stimulated immortalized porcine alveolar macrophages (iPAMs) [2] with PRV-Bartha K61, PRV-EA, and PRV-HNX, and performed a metabolomics assay. This study aims to investigate the metabolomics changes in different PRV strains infected iPAM cells.

Materials and Methods: iPAMs cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS). These cells were equally seeded in 75 cm^2 cell culture flask to 90% confluent, and stimulated with PRV-Bartha K61, PRV-EA and PRV-HNX (multiplicity of infection [MOI]=0.05) for 24 h. Metabolomics analyses followed LC-MS-based untargeted metabolic profiling strategy.

Results: The PCA scores plots showed an almost complete separation between iPAMs infected with different PRV strains and control cells. The difference between PRV-HNX and PRV-EA/PRV-Bratha K61 were significant. A total of 408 metabolites differed significantly between the PRV-HNX infection and control cells, while 411 different metabolites in PRV-EA infection and 413 different metabolites in PRV-Bartha K61 infection. Venn diagram showed the overlap among the metabolites altered by stimulation of PRV-HNX, PRV-EA and PRV-Bartha K61. To further investigate the possible pathway change, different metabolites were analyzed by MBRole pathway analysis assay. A total of 75 metabolic pathways changes were forecasted between the PRV-HNX infection and control cells, while 54 metabolic pathways were forecasted in PRV-EA infection and 63 metabolic pathways in PRV-Bartha K61 infection.

Figure 1. PCA scores plots corresponding to pairwise comparisons of the data obtained from iPAMs cells infected with PRV-Bartha K61, PRV-EA and PRV-HNX (A). Venn diagram showed the overlap among the metabolites altered by stimulation of PRV-HNX, PRV-EAand PRV-Bartha K61 (B).

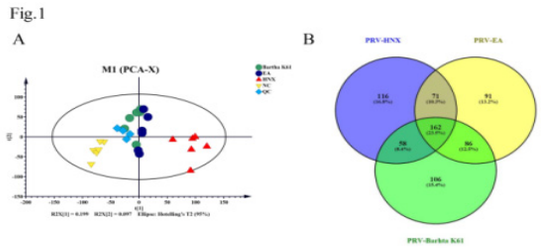
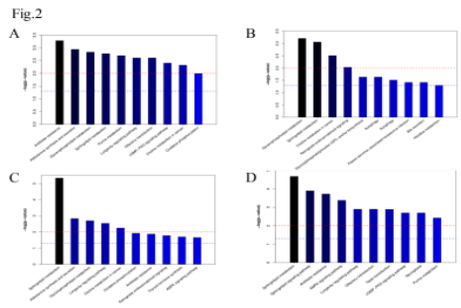


Figure 2. Metabolic pathway changes were forecasted between the PRV-HNX infection and control cells(A), PRV-EA infection and control cells (B), PRV-Bartha K61 infection and control cells (C), PRV-HNX and PRV-EA infection(D).



Conclusions: In iPAMs, Metabolites show a great difference of different pseudorabies virus strains infection. Compared with vaccine strain (Bartha-K61), PRV-HNX and PRV-EA can cause significant changes of metabolites and metabolic pathways in iPAMs.

Acknowledgement: This research was supported by China Agriculture Research system (No.CARS-35)

References:

[1] Yin Y et al, 2017. Virus Genes; 53(4):565-572.
[2] Wang TY et al, 2018. Oncotarget; 12174-12185

IMM-012

Correlation of Serum Neutralization Test (SNT) and ELISA Tests as Diagnostic Tool for Classical Swine Fever (CSF)

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Introduction:

Classifcal Swine Fever (CSF) is an endemic disease in Malaysia, which can lead to immunotolerance, immunosuppression and subsequently huge mortality loses in pig farms. Control of the disease in Malaysia had mainly relied on vaccination using a live attenuated C-strain vaccine, as cell mediated immunity (CMI) plays a vital role in providing protection against CSF for sows and piglets. Serum Neutralization Test (SNT) is acknowledged globally as the gold standard serology test for CSF (Brown, 2018). Nevertheless, due to lack of access to SNT services, commercial Enzyme-Linked Immunosorbent Assay (ELISA) test kit remained as the main serology diagnostics for CSF. The objective of this trial is to investigate the correlation between the SNT and ELISA titer for vaccinated pigs.

Materials and Methods:

Serology samples from 7 farms across Peninsular Malaysia were collected as part of routine monitoring diagnostic tests. A total of 101 serum samples from the breeding herd and 168 serum samples from the porker herd were collected. The breeding herd is consisted of sow and gilts while the porker herd is consisted of pigs from 4 weeks old up to 20 weeks old. All the samples were equally split for ELISA test using IDEXX CSFV Ab Test in the Faculty of Veterinary Medicine, Universiti Putra Malaysia and then sent for SNT test in the Faculty of Veterinary Science, Chulalongkorn University in Thailand. Correlation test was then conducted, comparing the blocking % results from the ELISA test, and the SNT value from the SNT test. The Spearman correlation test was done using Minitab version 18.

Results:

Results are summarized in table 1.

Table 1: Spearman correlation coefficient for both samples

Serum samples ID	Number of samples	Spearman correlation, rho value
Sow	101	0.396
Porkers	168	0.329

The rho value for both groups of samples are lesser than 0.4, which is suggestive of a weak correlation. Thus, from this trial it is indicated that the CSF ELISA titers has a weak correlation with the CSF SNT titers. With that, SNT would still be the preferred serology diagnostic for CSF as compared to the ELISA in circumstances requiring an accurate diagnosis. Nevertheless, SNT is more laborious and costly than ELISA, as porcine cell line culture is required.

Using serology antibody titer as an evaluation tool of live attenuated CSF vaccine efficacy has its limitations as only the humoral immunity is assessed, whereas CMI is more vital in providing protection against this disease. It was also proven that complete clinical protection against CSF can be achieved as early as 5 days post vaccination with live attenuated C-strain CSF vaccine, despite significant increment in SNT titers were only detected about 10 days post vaccination (Graham, 2012).

Conclusions:

ELISA for CSF has a weak correlation with the CSF SNT test.

References:

[1] Brown VR and Bevins SN, 2018. Vet. Sci. 5:31
[2] Graham SP, et al., 2012. PLoS ONE 7(1): e29310

IMM-013

Deletion of *opvAB* operon in *Salmonella* Typhimurium isolated from swine and its quantitative analysis using DOC-PAGE

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Introduction: *Salmonella* Typhimurium (ST) is pathogenic Gram negative bacteria that cause salmonellosis in swine. It is one of our most common zoonosis that can be transmitted by ingestion of processed meat. Among the diverse virulence factors in ST, *opvAB* operon is reported to modulate O-antigen chain length in lipopolysaccharide (LPS) as epigenetic regulator. It was also reported that alteration of O-antigen length leads to reduce serum resistance and macrophages proliferation. [1] In this study, we constructed *opvAB* deletion mutant and the LPS quantitation was analyzed by DOC-PAGE and LAL kit. The mutant did not show any significant difference in O-antigen fraction in band ladder when compared to the wild type ST. Further experiments would be required to analyze *opvAB* deletion in the pathogenicity of ST.

Materials and Methods: 1. Bacterial strains and condition. Bacterial strains were cultured at 37°C in Luria Broth(LB). Wild type *Salmonella* Typhimurium (ST WT) and ST *Δopv*AB were derived from swine.

2. Construction and Identification of ST WT, ST *Δopv*AB mutant by PCR. ST *Δopv*AB mutant was constructed using Lambda red recombination system as described principally by Datsenko and Wanner. Samples (ST WT, ST *Δopv*AB mutant) were mixed to master mixture. PCR was operated to followed condition (Denature 1x 95°C 5min, amplification 34x, final step 1x 72°C 6min), and 10ul of PCR product was loaded on 0.8% agarose gel. 3.Preparation of Lipopolysaccharide (LPS). LPS was extracted from ST WT and ST *Δopv*AB mutant using the hot phenol-water extraction method as described previously.

ST standard was obtained from Sigma-Aldrich (*S.enterica* serotype Typhimurium). 4. LPS pattern analysis of the mutant. Extracted LPS samples were boiled for 10 min and then separated by sodium deoxycilate-polyacrylamide gel electrophoresis (DOC-PAGE). It was stained by silver staining.

Results: DNA size of the ST WT is 1,218 but that of ST *Δopv*AB mutant is 1,174 bp as expected and this indicates ST *Δopv*AB mutant was successfully constructed (Fig 1). The amount of endotoxin was estimated to be 0.28 EU/ml in ST WT and 0.25 EU/ml in ST *Δopv*AB mutant (Fig 2). The degree of endotoxin was measured by using the LAL quantitation kit. DOC-PAGE was showed no significant differences in ST WT and ST *Δopv*AB mutant.

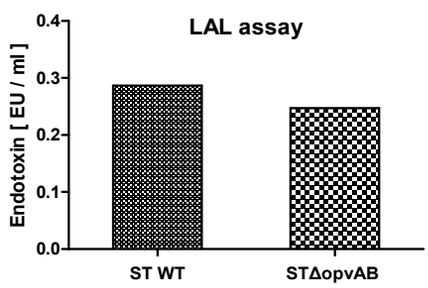

Figure 1. Endotoxin unit (EU / ml) of ST WT, ST *Δopv*AB LPS sample using LAL Kit assay. Extracted LPS samples were diluted 10^7times to adjust in standard concentration range. Detection range of the LAL-assay: 0.1 to 1 EU/ml.

Conclusions: From the results, it was indicated that *Δopv*AB deletion did not affect the LPS quantity in ST. The size of band ladder is similar between ST WT and ST *Δopv*AB mutant, however, the intensity band ladder in ST *Δopv*AB mutant was less than the that of ST WT in DOC-PAGE. Difference in band intensity would be analyzed in future study. It is summarized that the *opvAB* mutation did not affect on the endotoxin function and LPS pattern.

Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF-2017R 1A2B4003834).]

References:

[1] Bravo D et al., 2008. J. Med Microbiol. 57, 938-946.
[2] I. Cota, 2012. PLoS One, 7, p. e36863
[3] I. Cota, 2015. PLoS Genetics, 11, e1005667

IMM-014

Development of a liquid-phase blocking ELISA based on foot-and-mouth disease virus O/Jincheon/SKR/2014 for the use of post-vaccination sero-surveillance

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Introduction: A liquid-phase blocking ELISA (LPBE) has been standardized to be used for serological assessment of herd immunity against foot-and-mouth disease virus (FMDV) because this assay is the relatively simple and high-throughput procedure compared to virus-neutralization test (VNT). In this study, we developed LPBE to measure the level of antibodies against serotype O FMDV and validate its performance using an extensive range of sera from pigs. The developed LPBE used inactivated purified 146S particles of FMDV O/Jincheon/ SKR/2014 (O-JC) as an antigen because the O/SEA/Mya-98 lineage that was normally major endemic in Korea since 2010.

Materials and Methods: FMDV O-JC cultivated in BHK21 cells and then, viral activity was chemically inactivated by BEI. The 146S particles of the inactivated O-JC were purified by sucrose gradient ultracentrifugation and then used for an ELISA antigen and the production of two hyperimmune sera, one for capture antibody (α -rabbit) and the other for detector antibody (α -guinea pig). After optimization of ELISA reaction, the developed LPBE was applied to antibody titration against O-JC using the field-collected pig sera.

Results: To evaluate the diagnostic performance of the developed LPBE, a total of 136 sera obtained from pigs vaccinated with serotype O monovalent vaccine were screened in parallel by the VNT. When test sera at a 1:20 dilution with a cut-off point of 50% inhibition of reaction, LPBE exhibited proper analytic performance with a high diagnostic sensitivity (88.7%) and specificity (90.5%) in comparison with the VNT. In addition, the developed LPBE has a high correlation with the VNT (r2 = 0.8782). Using 100 pig sera randomly collected from farms with the history of FMD vaccination, sero-monitoring was carried out by the developed LPBE demonstrating that the overall seropositive rate were 64.3% against FMDV O/SEA/Mya-98 lineage.

Conclusions: Antigenic variants continue to emerge within each serotypes of FMDV, causing a major problem in FMD diagnosis. Because the currently approved and used FMD vaccines in Korea consist of varying formations of inactivated viruses of different serotypes, a more precise diagnosis is required to detect specific FMDV antibodies raised against existing vaccine viruses. Therefore, to incorporate the appropriate FMDV strains in ELISA should be considered to increase diagnostic accuracy of the LPBE. In this regard, the developed LPBE used 146S particles of FMDV O-JC as an ELISA antigen because many field virus in the past outbreaks and the vaccine antigen of some FMD vaccine in Korea belong to the O/SEA/Mya-98 lineage. The test performance of the developed LPBE was comparable with VNT quantitatively, emphasizing that this methods will be helpful to fulfill the requirement for testing large number of serum samples under post-vaccination sero-surveillance

Table 1. Sensitivity and specificity of the LPBE developed in this study

		OJC LPB ELISA				
		Positive	Negative	Total number of serum	specificity (%)	sensitivity (%)
VNT	Positive	67	7	74	90.5	88.7
	Negative	7	55	62		

Acknowledgement: This study was supported by a grant (B-1543386-2018-20-0201) from the Animal and Plant Quarantine Agency's National Animal Disease Research Project.

References:

[1] Basagoudanavar SH et al., 2013. Arch Virol. 158, 993-1001.

IMM-015

Effect of a commercial vaccine containing recombinant Verotoxin 2e on growth performance in conventional pig farms with Edema disease in Korea

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Introduction: Pig Edema disease(ED) is caused by *Escherichia coli(E.coli)* which colonizes the small intestine and produces Verotoxin 2e(VT2e), which enters the bloodstream and damages endothelial cells resulting in edema in targeted tissues. ED mainly occurs in newly weaned piglets, although it can also be observed throughout the growing and finishing phases. Some affected pigs become inappetent and may have a decreased growth rate, resulting in financial losses on commercial farms due to delayed slaughter age. The frequencies of VT2e-producing *E.coli* isolated from pigs in Korea increased over the most recent years, but there has been no commercialized ED vaccine in Korea until now. This study was performed to evaluate whether a commercial recombinant VT2e vaccine(VEPURED®, Hipra, Spain) is effective to control ED in Korean conventional pig farms.

Materials and Methods: This study was conducted in three conventional pig farms with high mortality problems associated with confirmed ED. A total of 186 two days old(do) piglets were used, 95 were administered with VEPURED(VAC group) and 91 were administered with PBS(CON group). Until slaughter, mean body weight(BW) of each group was recorded to calculate average daily gain(ADG) according to specific rearing phases(suckling, weaning, growing and fattening). ADG was calculated using a following formula; ADG=[(BW at the end point-BW at the start point /observation period(day)]. Additionally, slaughter age of each experimental pig was individually recorded.

Results: In all three farms, an average body weight of VAC group was higher than CON group at the end of fattening; 8.71, 1.32 and 7.28kg higher in farm A, B and C, respectively. Comprehensively, an average daily gain of VAC group from vaccination to the end of fattening was 0.033kg higher than CON group, and it had statistically significant difference($p<0.05$). The higher mean body weight and average daily gain observed in VAC group resulted in an earlier average slaughter age of VAC group; 9.7 days earlier than CON group.

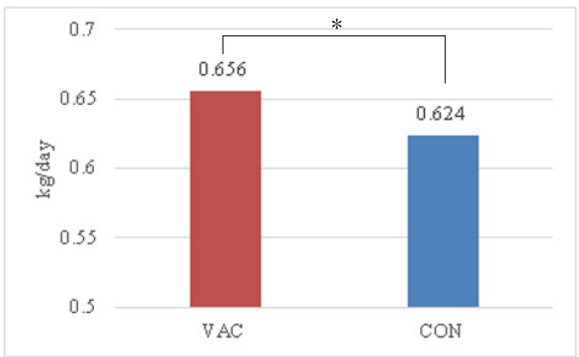

Fig 1. Average daily gain from vaccination to the end of fattening. *$p<0.05$

Table 1. Average slaughter age of experimental pigs in each farm

	VAC	CON	VAC - CON
Farm A	168do	179do	11 days
Farm B	176do	182do	6 days
Farm C	185do	197do	12 days
Average	176.3do	186do	9.7 days

VAC, vaccinated; CON, control; do, days old

Conclusions: Vaccination with HIPRA VEPURED resulted in faster growth and shortened slaughter age; higher mean body weight at the end of fattening and higher average daily gain were observed in vaccinated pigs. It demonstrates that prevention of ED with vaccination might result in better growth performance.

Acknowledgement: This work was supported by Hipra Korea, Inc., Republic of Korea.

References:

[1] Do, Kyung-Hyo, et al., 2019, J Vet Sci, 20(1), 43-5.

IMM-016

Effect of optimizing the vaccine program on a Pseudorabies virus infected farm to avoid interference from maternal immunity

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Introduction : Pseudorabies (PR) is a disease with high economic costs for the swine industry worldwide, owing to its clinical effects on the nervous, respiratory or reproductive system of pigs and the commercial restrictions it causes[1]. Reducing the PR virus (PRV) infection rate in fattening pigs is a key element for the control of the disease on a single farm. The aim of this study was to produce PR-free pigs by optimizing the current vaccination program on a PRV infected farm in China to avoid interference from maternal antibodies.

Materials and methods : One PRV infected farm with 1,500 sows (farrow to finish) in Shandong Province, China, was selected for this study. PR status before and during the study was determined by serology of gE antibodies (Fig 1, Fig 2, Fig 3) using a commercial ELISA kit (Civtest, HIPRA, Spain). Table 1 represents the immunization programs that were used before and during the study. A commercially available vaccine with a modified live Bartha-k61 strain (AUSKIPRA® GN) was used for vaccination throughout this study. The immunization procedure for newborn piglets was intranasal (I.N.) spraying with AUSKIPRA® GN and red diluent on their first day of life; while the rest of the animals received an intramuscular (I.M.) vaccination with AUSKIPRA® GN and A3 diluent.

Table 1. PRV Immunization programs

Group		Before study	During study (July 2015)	During study (Dec 2015)
Boars		I.M. GN, 4 times/year	I.M. GN, 4 times/year	I.M. GN, 4 times/year
Sows		I.M. GN, 4 times/year	I.M. GN, 4 times/year	I.M. GN, 4 times/year
Gilts		22/26W, I.M. GN	22/26W, I.M. GN	22/26W, I.M. GN
Piglets	Day 1	I.N. GN	I.N. GN	I.N. GN
	Day 56	I.M. GN	None	None
	Day 70	None	I.M. GN	None
	Day 84	I.M. GN	None	I.M. GN
	Day 98	None	I.M. GN	None
	Day 112	None	None	I.M. GN

Results : The first serological screening showed that the farm had a very high PR prevalence before the study, with a gE positive rate of over 70% in sows and gilts; and almost 100% in fattening pigs (Fig 1). After changing the immunization program, the gE positive rate gradually decreased (Fig 2) and at the end of the study period the farm was producing PR-free pigs and gilts (Fig 3). These results suggest that maternal antibodies from infected sows interfered with the efficacy of piglet vaccination. By postponing the first and second shots, we successfully avoided interference from maternal antibodies and improved piglet protection, optimizing the benefits of vaccination.

Figure 1. PRV gE antibody rate in June 2015

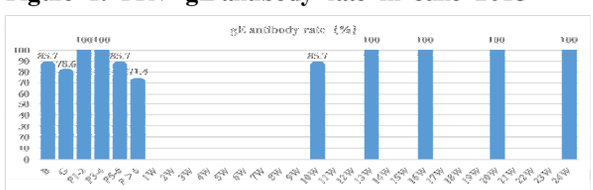


Figure 2. PRV gE antibody rate in April 2016

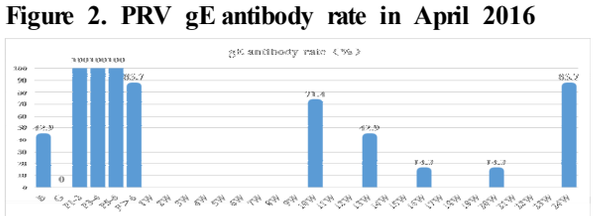
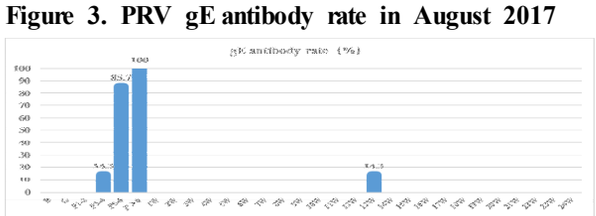


Figure 3. PRV gE antibody rate in August 2017



Conclusion : An optimized vaccination program with AUSKIPRA® GN that successfully avoids interference from maternal antibodies can be used to produce PR-free animals on a commercial pig farm.

References

[1] Lowell A. et al. 2008. Pseudorabies eradication report. Tech. Bu. 1923

IMM-017

Efficacy in reduction of Actinobacillus pleuropneumonia related lung lesions by vaccination against Actinobacillus pleuropneumoniae and optimized vaccination against Mycoplasma hyopneumoniae co-infection

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Introduction: Due to the demand on reduction in antimicrobial use, the optimisation of farm vaccination protocols of ever-increasing importance to farms in the United Kingdom.

Interaction of disease-causing pathogens is well documented inside porcine respiratory disease complex (PRDC) where Mycoplasma hyopneumoniae (Mhp) and Actinobacillus pleuropneumoniae (Ap) are major players together with viral agents.

The aim of this study was to reduce Ap related pleurisy at the time of slaughter as the indicators of reduced growth and decreased feed efficiency in an Ap endemic farm co-infected with Mhp via optimal vaccination.

The study design comprises two steps: 1) implementing Ap prophylaxis, 2) optimising Mhp vaccination, to reveal the contribution of each intervention.

Materials and Methods: An Ap and Mhp endemic 350 sow unit, not previously vaccinating against Ap, but vaccinating with a competitor Mhp two shot vaccine at one and three weeks of age (woa) experiencing suboptimal PRDC control was enrolled.

The Ceva Lung Program (CLP) was used to assess Ap related lung lesions measured on extension by the dorsocaudal pleurisy (DCP) percentage and severity on the Ap Pleurisy Index, the APPI.

At least three batches of ≥ 100 pigs each for each vaccination protocol were CLP investigated and evaluated.

Group 1: Three batches of pigs over a 3 months period, prior to Coglapix® implementation.

Group 2: Three batches of pigs over the last 3 months of 7 months running a Coglapix® vaccination protocol at 7 and 9 woa due to the farms decision.

Group 3: Four batches of pigs over the last 3 months of 7 months running a Hyogen® vaccination protocol once at 4 woa and continuing the Coglapix® protocol implemented in group 2.

Group 4: One batch receiving same vaccine protocol as group 3 but one year after implementation to see how far results could be taken (see details below).

Kruskal-Wallis one-way analysis of variance used for statistical evaluation amongst groups.

Results: Group 1 & 2 CLP's varied very little between the 3 test batches each group, where as the values in Group 3 consistently and seemingly linear declined. For that reason, at the time of data evaluation, it was decided to create a Group 4 on an CLP investigation one year following implementation of the combined Coglapix® + Hyogen® protocol.

DCP and APPI were equally significant between all groups. Each group 2, 3 and 4 were showing significant reduction in both Ap related lung lesions scores ($p<0.001$) compared to Group 1. In Group 4 the Ap related lung lesion scores were significantly lower ($p>>0.001$) than Group 1 (Table 1).

Table 1

Vaccination protocol	Average DCP (%)	Average APPI
Pre-Ap prophylaxis /3	64.3^{a}	1.72^{a}
Coglapix® /3	33.0^{b}	0.84^{b}
Coglapix® + Hyogen® /4	24.4^{bc}	0.60^{bc}
Coglapix® + Hyogen® /+1y	10.9^{c}	$0,25^{c}$

Conclusions: The results of this study demonstrate the significant importance of optimal control of both Ap and Mhp infections in co-infected farms, due to their mutually increased impact.

Also, it confirms field experience of farms stabilising further over the first year or more following implementation of better control strategies.

References:

[1] Del Carmen, P et al., Proc ESPHM 2019
[2] Catelli, E et al., Proc ESPHM 2017
[3] Krejci, R et al. Proc ESPHM 2017

IMM-018

Evaluation of Bayovac® ST-APP vaccine efficacy with low inoculation doses of *Actinobacillus pleuropmenuoniae* serotype 1

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Introduction: *Actinobacillus pleuropneumoniae* (APP) is one of the main respiratory agents causing porcine pneumonia and economic losses in the pig industry. The immunity status is one of the influencing factors for infection severity, but also stress by change of environmental conditions, the degree of exposure to the infectious agent and virulence of the strain(s). Vaccination is a useful tool to control this disease, reduce mortality, and reduce antibiotic treatments. New vaccine development focusses on the addition of Apx toxoid or recombinant Apx as core ingredient to protect against the large range of serotypes prevalent in different countries. The aim of this study was to evaluate the efficacy of a vaccine containing additional recombinant Apx in a bacterial challenge model. Commercially available vaccines with Apx toxoid or recombinant Apx were included as control.

Materials and Methods: Forty piglets were assigned to five groups, ranked within litters by weight and gender, each group contenting eight pigs. Bayovac® ST-APP vaccine (group 1) and three other APP commercial vaccines (groups 2, 3 and 4) were used in this study. Group 1 (Vaccine A - Bayovac® ST-APP: contents included *A. pleuropneumoniae* serotype 1 bacterin, recombinant Apx toxins and OML with oil adjuvant); group 2 (Vaccine B: Apx toxoid and OMP with oil adjuvant); group 3 (Vaccine C: *A. pleuropneumoniae* serotype 1 and 2 bacterin with aluminum hydroxide adjuvant); group 4 (Vaccine D: *A. pleuropneumoniae* serotype 1, 2 and 5 bacterin, and recombinant Apx toxins with aluminum hydroxide adjuvant). One group of 8 piglets was served as unimmunized/unprotected control (group 5). Between week 5 and 7, piglets in vaccination groups were injected intramuscularly with the respective vaccine according to the label recommendation. All piglets were transferred to isolation animal facilities at 11 weeks of age. Antibiotic free feed was used during the whole study period. At 12 weeks of age all piglets were inoculated via endotracheal route with 0.1 minimal lethal doses (1 MLD=10^8 cfu) of *A. pleuropneumoniae* serotype 1. After challenge the clinical signs were recorded daily, including activity, appetite, respiratory syndrome and rectal temperature and scored as: 0- normal, 1- slight, 2- moderate, 3- severe. Pigs were euthanized at 13 weeks of age and necropsied. The ventral and dorsal lung lesions were evaluated according to Bernardy et al., 2007.

Results: All assessed parameters were normal before inoculation. All pigs survived post *A. pleuropneumoniae* serotype 1 inoculation. The main observable clinical signs were prostration and loss of appetite, shown mainly in control group 5 and group 3. The average daily weight gain (ADWG) was calculated for the week post inoculation. The ADWG was 566±252 g/day for group 1 (Bayovac® ST-APP) and 469±169 for the group 2. Those values were superior when compared to the other groups (range from 396±325 to 401±325) but the differences were not significant. The lung lesion score in Bayovac® ST-APP vaccine (group 1) was 8.75±7.96 and was significantly ($p<0.05$) better than other commercial vaccines (group 2 to 4) and the control (group 5) (28.4±20.5, 39.4±31.8, 21.6±15.1, 31.5±25.2, respectively). The bacterial re-isolation rate from lung lesions was ranged from 50% to 87.5% in this study.

Conclusions: Bayovac® ST-APP can reduce the ADWG loss and lung lesion scores of pigs challenged with low doses of *A. pleuropneumoniae*. The oil adjuvanted vaccines including Bayovac® ST-APP and group 2, provided better protection than group 3 and 4 which contained ammonium hydroxide as adjuvant.

Acknowledgement: This work was supported by Bayer CO International Species swine Manager Program (Project No. 500128).

References:

[1] Hensel et al., 2000. Vaccine 18:2945-2955.
[2] Bernardy et al., 2007. Resarch in Pig Breeding. 7-13

IMM-019

Efficacy of the Porcilis® Begonia vaccine in pigs following challenge with the highly virulent ZJ01 pseudorabies strain

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Introduction: Since the re-emergence of new variants of pseudorabies virus (PRV) in 2011 in China, efforts have been made to find novel vaccine strategies to control Aujeszky's disease caused by PRV infection in pig herds. It has been suggested that vaccines based on the Bartha-K61 strain do not provide effective protection against infection with these new PRV strains. Since the Porcilis® Begonia vaccine is based on another PRV strain, it was evaluated whether it is efficacious against problems associated with experimental PRV infection with the contemporary and virulent Chinese ZJ01 strain.

Materials and Methods: For this, piglets were either vaccinated through the intradermal (ID) route or the intramuscular (IM) route and were subsequently intranasally infected with strain ZJ01 and monitored for 14 days.

Results: Vaccinated pigs were healthy during the study compared to the not vaccinated ones, which developed lesions associated to PRV infection and 4 out of 9 pigs died before the end of the experiment. This study has shown that Porcilis® Begonia was safe and protective both via ID or IM against a contemporary PRV strain.

Conclusions: This study has shown that Porcilis® Begonia was safe and protective both via ID or IM against a contemporary PRV strain.
PeerJ 6:e5785 https://doi.org/10.7717/peerj.5785

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IMM-020

Efficacy of two different vaccination programs against PCV2 and *Mycoplasma hyopneumoniae* compared in a large scale field trial

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Introduction: PCV2 and Mycoplasma hyopneumoniae are two of the most frequent pathogens of swine involved in PRDC, causing major economic losses[1]. The efficient prevention requires a long lasting protection, which can be evaluated by scoring lungs at slaughter and by recording pigs' growth rate. The aim of this trial was to evaluate the efficacy of a single shot vaccines, Circovac® & Hyogen® in comparison with a two shot vaccines on protection against PCV2 and Enzootic Pneumonia.

Materials and Methods: Three commercial pigs farms with 2000 sows each were selected for the trial. In total 3200 piglets were vaccinated with Circovac® and Hyogen® at 3 WOA (group G1) and 3200 piglets were vaccinated with combined Mhyo plus PCV2 vaccine A at 3 and 6 WOA (group G2). Pigs' growth performance was recorded and the relevant economic impact was calculated using Respinomics™. Lung scoring was performed at slaughterhouse according to the Ceva Lung Program.

Results: Animals vaccinated with Circovac® and Hyogen® showed 34.28% of Bronchopneumonic lungs (BP) while animals vaccinated with vaccine A presented 41.22% of BP ($p<0.05$). The EP-Index (calculated from the frequency and severity of EP-like lesions) was on average by 0.41 lower ($p<0.05$) in G1 compared to G2. The scar scoring resulted also in statistically significant difference between the vaccinated groups (G1=8.44, G2=17.66 $p<0,05$) Table 1. From the zootechnical point of view, G1 had on average 16gr higher ADG ($p>0.05$) compared to G2; 0.03 better feed conversion than G2 ($p>0.05$) and 0.16% lower mortality ($p>0.05$) Table 2. The profit in G1 was calculated using Respinomics™ application as 4.19€ per pig Table 3.

Tab 1. Enzootic pneumonia like lesions

	Bronchopneumonic lungs (%)	EP Index	Scar scoring (%)
Group 1	34.28	0.80	8.44
Group 2	41.22	1.21	17.66

Tab 2. Dorsocaudal pleurisy and APPI Index

	Dorsocaudal pleurisy (%)	APPI Index
Group 1	3.25	0.12
Group 2	5.03	0.19

Tab 3. Zootechnical summary and economic benefit calculation

	FCR	ADG (g)	Mortality %	Economic evaluation € per pig
Group 1	2.63	923	2.35	+ 4.19 €
Group 2	2.66	907	2.51	-

Conclusions: The single dose vaccinations with Circovac® and Hyogen® improved pigs' lung health and farm's profitability due to better growth performance. Similar results were also found in other studies comparing vaccine protocols[2,3].

Despite zootechnical differences were not significant, the economic impact was extremely relevant, since a high amount of animals was evaluated. Moreover, it was less stressful for the pigs compared to the double shot vaccination.

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IMM-021

Enhanced immunity and safety of foot and mouth disease vaccine with supplementary components

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease and causes economic damage at a national level. The quality of a vaccine depends heavily on the effect of the adjuvants[1,2] that are simultaneously applied with the antigen in the vaccine. The adjuvants[3] enhance the protective effect of the vaccine against viral attack.

Materials and Methods: In this study, we fabricated test vaccines by mixing diverse known oil adjuvants and composites that can act as immunity adjuvants (gel, saponin, and other components) and examined the enhancement effect on the vaccine. In the experiment, water in oil (W/O) and water in oil in water (W/O/W) adjuvants showed better immune effects than oil in water (O/W) adjuvants, which have a small oil component. However, the oil-type adjuvants have a problem in that they leave oil residue inside the bodies of mice. The W/O type left the largest amount of oil residue, followed by the W/O/W and O/W types. We observed long-term immunogenicity of the mice for 90 days after the vaccination and found that ISA206 maintained the best immunity and protective power among the oil adjuvants in the experimental group.

Results: In the mouse model, intramuscular inoculation showed a better protection rate than subcutaneous inoculation, and the protective effect was particularly weak in case of inoculation in inguinal fat. We screened the adjuvant composites aiming at the enhancement of foot-and-mouth disease (FMD) immunity and confirmed that the Mincle agonist most improved the protection rate. The initial immune reaction and persistency of long-term immunity was also confirmed in an immunity experiment on pigs. The formation of an antibody with sufficient FMD protective power was confirmed by the formation of a neutralization antibody.

Figure 1. Immunogenicity and residue-frequency of various adjuvants with foot and mouth disease virus, type A antigen at 21 dpv.

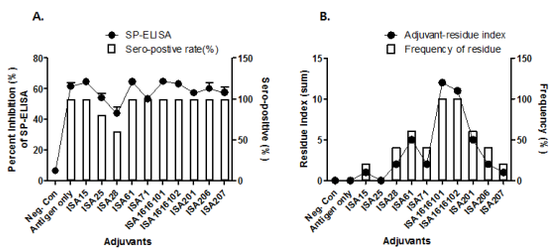
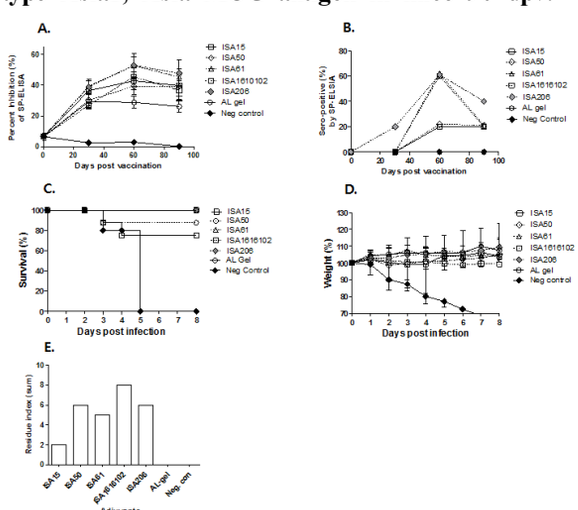


Figure 2. Immunogenicity through vaccination of various adjuvants with foot and mouth disease virus, type Asia1, Asia MOG antigen in mice 90 dpv.



Conclusions: ISA206 containing gel and saponin showed short- and long-term immunity persistency, despite the existence of oil residue. Mincle agonist was effective for improving immunity, demonstrating early antibody formation and long-term immunity.

Acknowledgement: This work was supported by the Animal and Plant Quarantine Agency, Republic of Korea.

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IMM-022

Evaluated efficacy of Bayovac® MH-PRIT-5 one vaccine in field condition

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Introduction: Enzootic pneumonia is a chronic respiratory disease found in pig industry worldwide. The causative agent is *Mycoplasma hyopneumniae*. The disease is characterized by high morbidity but low mortality. It causes the pig growth retardation, poor feed conversion, and increases susceptible to other respiratory disease infectious agents to form porcine respiratory disease complex. Control *Mycoplasma* pneumonia can be achieved by improvement farm management, housing condition, antibiotics treatment and vaccination. Through vaccination can reduce the lost in performance and severity in lung lesion. Bayovac® MH-PRIT-5 ONE is a new marketed single dose vaccine (with 10^{10} ccu of inactivated bacteria combined with oil adjuvant). In this study the vaccine efficacy is evaluated in the field conditions compared with the other commercial vaccine (inactivated bacteria combined with oil adjuvant).

Materials and Methods: Ninety-nine piglets born within 3 days were used in this study. The study is designed as blind test, operation and analysis by different groups. Piglets are assigned to two groups according to the litters. Bayovac® MH-PRIT-5 ONE vaccine group contained 50 piglets and intramuscularly immunized at 3 weeks-old. The compared vaccine group contained 49 piglets and intramuscularly immunized at 6 weeks-old according to the indication. The serum samples were random collected at 3, 13 and 24 weeks-old. The anti-*Mycoplasma* Ab was analyzed by Mycoplasma Hyopneumoniae Antibody Test kit (IDEXX). The individual body weight was recorded at 4, 10, and 24 according to the pig moving schedules. The lung lesion scores were evaluated at slaughter house according to method descripted by Madec *et al*., *1982*. Non-parametric statistical analysis (Mann-Whitney U test) was used to test difference in gross lung lesion scoring among pigs from different treatment groups.

Results: The trial farm is a well management and low population density herd. The *Mycoplasma vaccine* routing used in the farm is inactive bacterin combined with aluminum hydroxide as the adjuvant. Current vaccination program is immunized at 1 week-old and booster at 3 weeks-old. The *Mycoplasma* infection in this herd is of low intensity and sub-clinical. The aim of this study is focus on the performance. No mortality occurred during the trial period. The anti-Mycoplasma Ab was analyzed by ELISA kit. The Ab was not detected at 3 weeks-old in both groups. The Ab was significantly raised at 13 weeks-old. The S/P value (seroconversion rate) in Bayovac® MH-PRIT-5 ONE vaccination group and the compared vaccine group was 0.33±0.30 (33.3%) and 0.19±0.21 (7.7%). All the pigs were seroconverted at 24 weeks-old. The S/P value in Bayovac® MH-PRIT-5 ONE vaccination group and the compared vaccine group was 1.25±0.42 and 1.40±0.33. There was no significantly different in body weight between Bayovac® MH-PRIT-5 one vaccination group and compared vaccine group at 4 and 10 weeks-old. But Bayovac® MH-PRIT-5 one vaccination group had significantly better body weight than compared vaccine group at 24 weeks-old ($p<0.05$). The average daily weight gained in Bayovac® MH-PRIT-5 ONE vaccination group was 34.2 g/day more than the compared vaccine group. Lung lesion scores were no significant difference between two groups ($p>0.05$).

Conclusions: Bayovac® MH-PRIT-5 ONE vaccine in well management and low population density herd can provide better growth performance.

Acknowledgement: This work was supported by a grant from the Bayer Global Marketing Swine Team Program (Project No. 500159).

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IMM-023

Evaluation of Bayovac® ST-APP vaccine efficacy with high inoculation doses of *Actinobacillus pleuropmenuoniae* serotype 1

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Introduction: *Actinobacillus pleuropneumoniae* (APP) is an important agent involved in the swine respiratory disease complex, causing porcine pneumonia and generating performance and economical losses for the swine industry. Among the factors affecting the clinical outcome are the immunity status, stress by change of environmental conditions, level of exposure to the infectious agent and virulence of the strain(s). Vaccination is a useful tool to control this disease, reduce the mortality, and potentially reduce antibiotic treatment. The development of new ideal vaccines focusses on the addition of Apx toxoid or recombinant Apx as core ingredient to protect against the broad range of serotypes prevalent in different countries. The aim of this study was to evaluate the efficacy of a registered vaccine containing additional recombinant Apx in a bacterial challenge model. Commercially available vaccines with Apx toxoid or recombinant Apx were include as controls.

Materials and Methods: Forty piglets were assigned by weight and gender to five groups, each group contenting eight pigs. Bayovac® ST-APP vaccine (group 1) and three other APP commercial vaccines (groups 2, 3 and 4) were used in this study. Group 1 (Vaccine A: Bayovac® ST-APP - Contains APP serotype 1 bacterin, recombinant Apx toxins and OML with oil adjuvant). Group 2 (Vaccine B: Apx toxoid and OMP with oil adjuvant); Group 3 (Vaccine C: APP serotype 1 and 2 bacterin with aluminum hydroxide adjuvant); Group D (Vaccine D: APP serotype 1, 2 and 5 bacterin, and recombinant Apx toxins with aluminum hydroxide adjuvant). One group of 8 piglets served as unimmunized/unprotected control (group 5). Between 5 to 7 weeks of age, piglets (groups 1 to 4) were injected intramuscularly with a dose of the test vaccine and boosted, according to registered product label. All piglets were transferred to isolation animal facilities at 11 weeks of age. Antibiotic free feed was used during the study period. At 12 weeks of age all pigs were inoculated endotracheally with 10 minimal lethal doses (1 MLD=10^8 cfu) of APP serotype 1. Clinical signs were recorded daily, including activity, appetite, respiratory disease symptoms and rectal temperature and scored as: 0- normal, 1- slight, 2- moderate, 3- severe. On week 13 piglets were euthanized and necropsied. The lung lesions were evaluated according to Bernardy et al., 2007.

Results: All assessed parameters were normal before inoculation. Total of 34 piglets died one day post APP serotype 1 inoculation. Only 3, 2 and 1 pigs, respectively from group 1, group 2 (vaccine B) and group 4 (vaccine D), survived. The surviving pigs in the Bayovac ST-APP group did not show significant clinical signs, but pigs in the group 2 and group 4 showed prolonged eating times and coughing. Lung lesions of all pigs that had died one day post inoculation showed severe edema and hemorrhages. The chest cavities of those pigs were filled with brown fluid. There was also white-yellow fibrin attached to the surface of lungs and pleura in some pigs. Lung lesions of surviving pigs in the group 1 and group 4 were minor and restricted to small granulomatous lesions. In contrast, the lung lesions of surviving pigs in group 2 were focal necrosis, haemorrhagic lesions and fibrin/fiber attached to the surface of lungs and pleura. Better lung lesion score was found in Bayovac® ST-APP vaccine group (58.52±49.59), compared to the other tested vaccines (group 2 to 4) and control group (74.5±23.9, 73.3±18.2, 77.8±15.7, 77.6±15.1, respectively).

Conclusions: Bayovac® ST-APP reduced ($p<0.054$) the mortality and lung lesion scores of pigs challenged with high doses of APP. The oil adjuvant vaccines, including Bayovac® ST-APP and group B, provide better protection than group C and D, which contained ammonium hydroxide as adjuvant.

Acknowledgement: This work was supported by Bayer CO International Species swine Manager Program (Project No. 500128).

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IMM-024

Evaluation of live attenuated Japanese encephalitis virus vaccine candidate, KV1899-200P for swine based on the biologic and genetic characterization

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Introduction: Japanese encephalitis (JE) is one of the major vector-borne and zoonotic viral diseases in the Asian-pacific region. Recently, the Japanese encephalitis virus (JEV) antigen was identified in mosquitoes of European countries. The JEV causes encephalitis in human, horses, and leads to reproductive failure in sows. JEV is classified into five genotypes based on the nucleotide sequence of envelope gene. A live attenuated JE vaccine strain, Anyang 300 strain belonging to genotype 3, has been used for swine over 30 years in Korea. However, JEV genotype shift happened in South Korea since 1990 and these changes in these genotypes have required new JEV vaccine that fits in recent epidemiological situation and is effective for the prevention of JE in pigs. The isolation of JEV, KV1899 strain belonging to genotype 1 was reported and inactivated JEV vaccine using the same strain was also reported. The passages of the KV1899 strain have been conducted in several cells to develop a new live JEV vaccine candidate. In this study, we evaluated live attenuated JE vaccine candidate, KV1899-200P for swine based on the biologic and genetic characterization.

Material and Methods: Virus (KV1899-200P strain): The passage of the KV1899 strain isolated was successively conducted in several cells such as Vero, TF104, ST, PK-15 cells until 200th passages. Vero cells were infected with KV1899 parent or -200P in 6-well plates. When the cytophatic effect of the Vero cells occurred, the photograph was taken and then fixed with 80% cold acetone (-20℃) for 20 min. After washing the cells with PBS (pH 7.2) three times, the cells were reacted with a mouse monoclonal antibody and and subsequently stained with FITC-conjugated anti-mouse IgG. After rinsing with PBS, the Vero cells were examined under a fluorescence microscope. **Plaque assay** was performed using monolayers of Vero cells inoculated with viral titer of $10^{5.0}$ $TCID_{50}$/mLin 24-well plates in D-MEM. The wells were replaced with 0.5 mL 1.0% low-melting-point agarose. The second overlay containing 1.0% low-melting-point agarose in D-MEM with 0.1% neutral red was added to all wells. Plaques were counted after 2 days of the second overlay by gross examination. For the electron microscopy, KV1899-200P-infected Vero cells were harvested 96 h post-inoculation and frozen and thawed twice. After centrifugation at 4,000 × *g* for 30 min, the supernatant was treated with polyethylen glyco 8000 and 0.5 M NaCl. After centrifugation at 2,500 × *g* for 30 min, the pellet was resuspended in TE buffer (5 mM Tris-HCl and 1 mM EDTA; pH 7.8) at 5% of the original volume. The suspension was layered on top of sucrose gradient and centrifuged at 100,000 × *g* for 90 min using a SW-41 rotor (Beckman, Danvers, MA). The viral band on top of the 30% sucrose layer was collected. One drop of purified virus was placed on a Formvar-coated grid and negatively stained with 1% uranyl acetate. The viral particles of KV1899-200P were visualized under a Hitachi 7100 electron microscope. Viral RNA extracted from the KV1899-200P strain was sent to an external agency (Macrogen Inc., Seoul, Korea) to confirm the complete genome sequence using a next-generation sequencing (NGS) technique. The whole genome sequence was compared with those of the KV1899 parent and JEV reference strains. In addition, a phylogenetic analysis was performed based on the envelope gene of the KV1899 parent and other JEV strains obtained from the GenBank database.

Results: After conducting passages 200 times in several cells, we investigate biologic feateres of KV1899-200P with CPE, IFA, plaque assay and electron microscopy. The KV1899-200P strain showed specific CPE, cytoplasmic fluorescence, small plaque and typical Flavivirus. In genetic anaysis of KV1899-200P, there were 221 nucleotide and 73 amino acids changes in a total genome compared with that of KV1899 parent strain.

Table 1. Changes in nucleotides and amino acids of KV1899-200P compared to those of KV1899 parent strain

ORF	C	M	E	NS1	NS2	NS3	NS4	NS5	Total
Nucleotide	12	15	28	32	30	37	18	49	221
Amino acid	5	2	8	14	6	11	8	19	73

Conclusions: We evaluated KV1899-200P strain based on the biologic and genetic characterization. The identity of KV1899-200P as JEV genotype 1 was confirmed based on its cytopathic effects, immunofluorescence assays, electron microscopy and NGS. The KV1899-200P strain can be used as attenuated JEV vaccine strain for swine. Acknowledgement: This work was supported financially by a grant (N 1543085-2017-36-01) from the Animal, and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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IMM-025

Evaluation of protective immunity against serotype O foot-and-mouth disease virus in pigs vaccinated with double oil-based emulsion FMD serotype O vaccine

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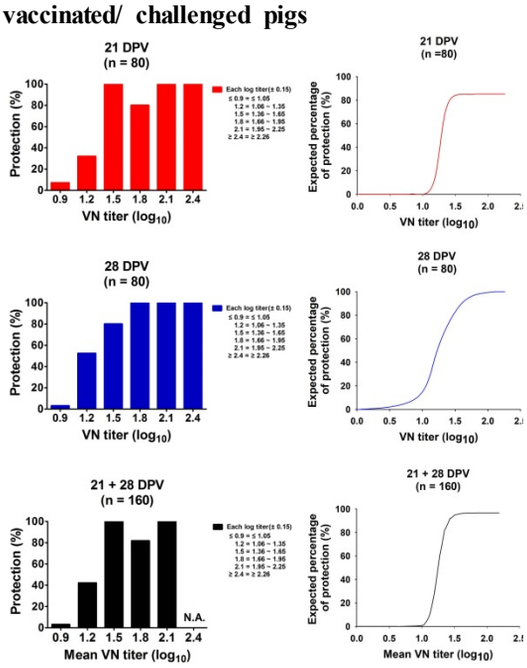
Introduction: Since the foot-and-mouth disease (FMD) outbreaks of 2010-2011 in Korea, nationwide vaccination policy has been launched for cattle and pigs as a prophylactic measure. As Korea has experienced several more FMDs so far, it has become essential to monitor the immune status of vaccinated herds whenever the disease occurs because the level of herd immunity represents the potency of FMD vaccine currently in use. Because in vivo challenge test for evaluation of FMD vaccine potency has several disadvantages from a biosafety point of view, we established an alternative method to the challenge test based on the correlation between serum titers of vaccinated animals and protection against infection. After that, we used this method to estimate the level of antibody titer that can protect against FMD

Materials and Methods: Protection data was collected from in vivo homologous challenge test using 80 pigs vaccinated with FMDV serotype O monovalent vaccine (O/SEA/Mya-98 lineage). The statistical analyses were conducted by using logistic regression in order to find relationship between ratio protected / non-protected species and VNTs. Expected percentage of protection (EPP) for serotype O FMD vaccine was calculated with the logistic regression curve using mean VNTs from sera collected at 21 and 28 days post vaccination (DPV).

Results: Our results demonstrated that the mean VNT measured from sera at 28 DPV were correlated with the percentage of protected animals as compared to that of VNT obtained 21 DPV. It was evident that log10 VNT of 1.51 was associated with >80% percentage of protection against challenge with O-JC FMDV strain.

Conclusions: A close correlation was observed between the VN titers at 28 DPV and the percentage of pigs that were protected from challenge at 35 DPV ($p > 0.99$). In contrast to 28 DPV, less correlation was found in VN titer at 21 and 21 + 28 DPV, in which they showed a low p- value computed by Hosmer-Lemeshow statistic (21 and 21 + 35 DPV were 0.03 and 0.48, respectively). In conclusion, we would like to emphasize that the reliability of serology to assess the protective immunity in individuals and herds as well as its ease of adaptability for many different FMDV strains could help the logical and efficient disease control. Furthermore, it could contribute to the eradication of FMD in South Korea. However, further studies are needed to examine the validity of this novel approach for other subtype of FMDV. In addition, it is important to secure more serological data in order to set the criterion for predicting the protection of FMD based on the level of antibodies in vaccinated target animal species.

Figure. 1. Correlation between protection and VNTs in vaccinated/ challenged pigs



Acknowledgement: This study was supported by a grant (B-1543386-2018-20-0201) from the Animal and Plant Quarantine Agency's National Animal Disease Research Project.

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IMM-026

Evaluation of the Efficacy of a New Chimeric PCV2 Subunit Vaccine CIRCOQ™ at a Commercial Farm in Taiwan

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Introduction: Porcine circovirus type 2 (PCV2) is the causative agent of porcine circovirus disease (PCVD), and it can cause huge economic losses glocally. PCV2 caused PCV2 subclinical infection, PCV2 systemic disease (PCV2-SD), PCV2 lung disease (PCV2-LD), PCV2 enteric disease (PCV2-ED), PCV2 reproductive disease (PCV2-RD) and porcine dermatitis and nephropathy syndrome (PDNS) [1]. Recently, commercial PCV2 vaccines are available in the swine industry. However, the impact of different vaccination programs on the efficacy of PCV2 vaccine for protecting pigs in the fields is still studied [2]. The purpose of this study was to evaluate the efficacy of CIRCOQ™, a recombinant chimeric vaccine in a farrow-to-finish pig farm in Taiwan that is with PCV2 infection.

Materials and Methods: In this study, 450 pigs were randomly divided into 3 groups, including 2 vaccinated groups and 1 unvaccinated group. Group A and Group B were administered with CIRCOQ™ and a commercial PCV2 subunit vaccine at the age of 4 weeks, respectively. Group C was the control group without being given any PCV2 vaccine. Serum samples from 4, 8, 12, 16, and 18 week-old pigs were screened for PCV2 viremia using real-time polymerase chain reaction assay. All serum samples were also analyzed for anti-PCV2 antibody response using enzyme-linked immunosorbent assay. Average daily weight gains (ADWG), and growth performance of all the pigs in this study were also analyzed and compared among different groups.

Results: The results showed that the growth rate of the three groups (A, B, and C) of pigs were 94.6%, 90.5%, 88.5% respectively. The results of ADWG was 601.0g, 597.6g and 590.8g respectively (Table 1).

Viremia

Pigs in all groups were infected with PCV2 during the age of 4-8 weeks, but the viremia didn't exceed the clinical threshold (10^3 copies/ μ L). At the age of 12 weeks, one sample from group C was higher than the clinical threshold (7,067 copies/μ L) suggesting that the pig could have clinical symptoms. At the age of 16 weeks, serum samples each from group A and C showed that 56% and 47 % of samples were higher than the clinical threshold. At the age of 18 weeks, the levels of PCV2 viremia in all groups became lower than the threshold.

Antibody response

At the age of 8 weeks, the antibody titres in group B and C decreased and were with poor uniformity. At the age of 12 weeks, the result in group C was similar to that at the age of 8 weeks, whereas the titre in group A decreased. However, the titre in group B increased significantly and had great uniformity suggesting that pigs in group B might generate antibodies against PCV2 after infection. At the age of 16 week, the antibody titre and the positive rates of antibody increased markedly only in group A.

Table. 1 ADWG and growth rate in three groups.

	ADWG (g)	Growth rate
Group A	601.0 g	94.6 %
Group B	597.6 g	90.5 %
Group C	590.8 g	88.5 %

Conclusions: Based on the results in this study, it demonstrated that CIRCOQ™ not only could improve the growth rate and average weight gain, but also could reduce PCV2 viremia in comparison with the control group.

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IMM-027

Evaluation the immune effect of the same immune schedule in pseudorabies virus gE antibodies positive and negative pig farms

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Introduction: Outbreaks of pseudorabies virus (PRV) were reported across China since 2011. The objective of the study was to evaluate the immune effect of the same PRV immune schedule in pseudorabies virus gE antibodies positive and negative pig farms.

Materials and Methods: Two farrow-to-finish herds were selected: herd A with1,000 sows(PRV gE antibodies positive) and herd B with 800 sows(PRV gE antibodies negative) .The two farms performed the same immune schedule with the same BarthaK-61 vaccine : Breeders herd were vaccinated 4 times a year by intramuscular , neonatal piglets were vaccinated by intranasal, piglets were vaccinated at 10 week and 13week by intramuscular. Each time per dose. 30 piglets were randomly selected for blood samples at 6,8,10,12,14 week from each farm. The blood samples were used to determine gE or gB antibodies by ELISA(IDEXX, USA).

Results: At 6, 8, 10, 12, 14 week, in herd A: the piglets seropositivity rate of gE antibodies were 80%, 86.67%, 60%, 10%, 10%, the seropositivity rate of gB antibodies were 100%, 100%, 70%, 30%, 20%. In herd B:the piglets seropositivity rate of gB antibodies were 100%, 86.67%, 40%, 100%, 100% .

Conclusion: According to the results, the pseudorabies virus positive and negative pig farms shouldn't perform the same PRV immune schedule, for the maternally-derived antibodies in the positive farm are higher than the negative farm which can affect immune responses in piglets.

Disclosure of Interest: None Declared

Keywords: immune effect, pseudorabies virus, gE, positive, negative

IMM-028

High Return on investment following control of Actinobacillus Pleuropneumonia with an Actinobacillus Pleuropneumonia vaccine expression APX toxins I, II and III under field conditions

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Introduction: Actinobacillus pleuropneumoniae (Ap) causes porcine pleuropneumonia (PP), a disease of high dissemination, highly contagious and often lethal in pigs. Ap infections results in production losses, high mortality and decrease in the growth rate, in grower and finishing pigs. Due to reduction in antimicrobial (AM) use in Spain, as in more other countries more PP is seen. Vaccination with an Ap vaccine expressing Apx toxins I, II and III, Coglapix® (Ceva), has previously proven to provide efficient protection and when possible to calculate, high ROI's of 5 to 7 both without and with reduced AM use. The aim of this study was to assess the efficacy and return on investment (ROI) of vaccination with Coglapix® against Ap in comparison with non-Ap-vaccinated controls.

Materials and Methods: The study was performed in pigs from a Spanish 1700 sow herd. Obvious clinical Ap problems were observed at the end of the finishing period. To estimate the moment of optimal Ap vaccination, a cross-sectional serological investigation in pigs from 7 till 16 weeks of age was performed. Average age-group Ap specific Apx IV ELISA titers for generation of maternal antibody regression curve and PRRS ELISA to avoid vaccinating at a time of active PRRSV circulation.

A total of 16632 pigs were vaccinated against Ap with Coglapix at 8 and 11 woa, and productivity parameters were compared to a total of 16431 non-vaccinated controls over a period of two consecutive years.

The following parameters were recorded by groups: mortality, feed conversion ratio (FCR), average daily weight gain (ADG) and production cost/pig as informed by the producer.

Results were analyzed by a parametric test Anova.

Results: Non-Ap-vaccinated group:

Mortality 9,01%, FCR 2,61, ADG 640g and production costs/pig 127€.

Coglapix® group:

Mortality 6,50% ($p=0.011$), FCR 2,49 ($p=0.023$), ADG 680g ($p<0.001$), production costs/pig 122€ ($p=0.003$) and ROI: 2.5x ($p=0.003$)

Conclusions: The productivity parameters of pigs vaccinated with Coglapix® were all clearly and significantly better than those of the non-Ap-vaccinated pigs.

The strongly significant improvement of ADG and FCR in case of Ap outbreaks late in finishing strongly indicate losses at earlier stages of life. As experienced in more other field cases, no awareness to Ap problems on the farm at these earlier stages exist. They may be anticipated subclinical or at least low level, subacute and possibly confused with other disease?

A highly significant improvement of the ROI greater than 2 demonstrates that outside improvement of health and animal welfare, it is very healthy business to control bot acute and subacute Ap with an toxoid vaccine like Coglapix®.

These results confirm data obtained in others field trials.

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IMM-029

Immunogenicity and Safety of *Salmonella* Typhimurium *phoBR* Deleted Gene Mutant.

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Abstract: *Salmonella* Typhimurium(ST) is pathogenic Gram-negative bacteria causing food-borne gastroenteritis in human as well as animals. The gene of *phoBR* is one of the gene encoding two-component replicator (TCR) systems that expresses phosphate regulon transcriptional regulatory protein [1]. Members of a TCR family are likely to play a role in the regulation of processes involved in intestinal colonization, and therefore, induces pathogenesis [2]. However, the virulence of *phoBR* in ST has not been clearly understood.

In this study, we constructed a *phoBR* deletion mutant in ST isolated from pigs, and examined antibody levels in vivo. In addition, the pathogenicity between the wild type and the mutant type was compared. When the *phoBR* gene in *Salmonella* Typhimurium was deleted in ST, it showed to increase immunity and safety. We suggest that ST mutant with *phoBR* deletion can be one of vaccine candidates in the livestock industry.

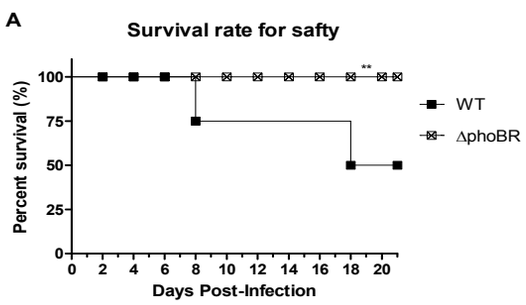
Materials and Methods: The ST mutants were constructed using the λ Red recombinase system [3]. To generate the deletion mutants ST Δ*phoBR*, the primer pairs *phoBR*-long-F/*phoBR*-long-R were used. The primer pairs *phoBR*-con-F/*phoBR*-con-F were used to confirm the mutations by PCR. The chloramphenicol resistance cassette in the *phoBR*: Cm mutant was first removed using flippase (FLP) recombinase encoded in the pCP20 plasmid, resulting in an *phoBR* mutant lacking chloramphenicol resistance.

Table 1. *Salmonella* strains used in this study

Strains	Name	Description
HID1120	WT	isolate from pig
HID2168	Δ*phoBR*	*phoBR*-deleted mutant
HID2171	Challenge strain	ST1120+pBBR1-MCS4 (AMPR)

To evaluate the protection conferred by the *phoBR* mutant against ST infection, 7-week-old BALB/c were divided into three groups. Two groups (n = four mice in each group) were vaccinated at 7 weeks of age intraperitoneal (IP) with 100 μ L of a suspension containing 1×10^3 CFUs of WT and *phoBR* mutant, respectively. The other groups (n = four mice in group) were injected at 7 weeks of age intraperitoneal with 100 μ L of PBS. Mortality was assessed daily for 21 days post-vaccine (dpv). After 3 weeks, all groups were challenged intraperitoneal with 100 μ L of a suspension containing 1×10^3 CFUs of challenge strain. Mortality was assessed daily for 14 days post-challenge (dpc). All surviving mice were euthanized at 14 dpc, and the livers and spleens were collected to measure the CFU counts.

Results: The survival rates of the mice vaccinated wild type ST and *phoBR* deletion mutant. The mice survival rate was daily monitored after vaccination for 21 days (A)



(*, $p < 0.05$; and **, $p < 0.01$).

Conclusions: The Δ*phoBR* mutant showed similar immunogenicity compared with the wild type ST induce Th1-immune response. The mutant showed best protection after challenged with virulent ST. The Δ*phoBR* mutant showed attenuation of virulence by fold because the survival rate is 100% but 50% in WT ST injection group. we suggest that the Δ*phoBR* mutant is safe and therefore, can be as one of the vaccine candidate for preventive vaccine studies.

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IMM-030

Improved foot-and-mouth disease vaccine with O PanAsia-2 strain protect pigs against O/Jincheon/SKR/2014 originated from South Korea

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Introduction: Foot-and-mouth disease(FMD) is an acute livestock epidemic that infects cloven-hoofed animals including cattle, pigs, sheep, and goats. The type O is the most prevalent FMDV serotype in the world. We conducted this study to develop a vaccine expected to be able to protect not only against the FMD SEA topotype viruses occurred in 2014, but also against the viruses belonging to the ME-SA and Cathay topotypes in Asia. In this study, a vaccine strain for O PanAsia-2 was developed for the prevention and protection against the FMD type O virus, and the immunity of the vaccine was evaluated through a neutralization test for various topotypes of FMDV.

Materials and Methods: The pO-Manisa-FG P1 genes were replaced by that of PanAsia-2 amplified from a synthetic gene through polymerase chain reaction(PCR). The virus inactivated with binary ethyleneimine (BEI) for antigen was purified and visualized under a transmission electron microscope (Hitachi H7100FA, Tokyo, Japan). The inoculated antigen was mixed with ISA 206 and 10% aluminum hydroxide gel adjuvant. After seven days post-vaccination, BALB/c suckling mice were challenged with O Vietnam 2013 (ME-SA topotype) virus and the O/Jincheon/SKR/2014(SEA topotype) virus following IP injection with and observed for seven days. The ten-week old pigs (n = 20) and the five-month old cattle (n = 5) were inoculated with the vaccine. The vaccinated pigs were challenged with O/Jincheon/SKR/2014 viruses (each at 10^5TCID$_{50}$/0.1 ml).

Results: In the challenge test of the ME-SA/PanAsia virus (O Vet 2013) in the mice immunized with one dose (1.5 μ g of 146S particle in 0.1 mL), the mPD50 was determined to be 128. In the challenge test of the SEA topotype virus (O/Jincheon/SKR/2014), mPD50 was determined to be slightly lower at 64. The antibody titers of cattle produced significantly higher titers of antibodies (> 1: 100) two weeks after vaccination. After the second vaccination, the antibody titer increased even further, and the neutralizing antibody titers were maintained at the protective levels up to six months after vaccination. In pigs, immunogenicity tended to increase gradually until the fourth week after the first vaccination, and the neutralizing antibody titers increased to a very high level (about 1: 1,000 on average) after the second vaccination. Neutralizing antibody titers were maintained at a high level up to 70 days after vaccination. In the immunological antibody induction test, type O SP-ELISA antibody exhibited a similar immune response as the existing vaccine and in the virus neutralization test using the ME-SA topotype virus, each experimental group showed a similar pattern. A challenge test was performed on immunized pigs four weeks after immunization and all animals in the vaccination group (n = 4) were protected completely. The immunized animals showed an average antibody titer of 1: 100 after the challenge and the neutralizing antibody titers were rapidly elevated after the challenge.

Conclusions: This new experimental vaccine is produces high titers of neutralizing antibodies in cattle in only two weeks. In the O/Jincheon/SKR/2014 virus challenge test, all pigs were protected against the virus, and almost no virus shedding was detected after the virus challenge.

Acknowledgement: This project was supported by the Animal and Plant Quarantine Agency, Republic of Korea.

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IMM-031

Interleukin (IL)-23 Signaling in Dendritic cells and Macrophages is a Key Immune Modulator of Foot-and-Mouth Disease Infection

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Introduction: Innate immune cells, notably dendritic cells (DCs) and macrophages (MΦs), produce proinflammatory cytokines by antigen (Ag)-specific interaction with pattern recognition receptors (PRRs) and play key role in the initiation and amplification of the host immune system as a first line of defense. The most common approach for the prevention of foot-and-mouse disease (FMD) has been vaccination with Ag derived from inactivated (killed) virus, along with various adjuvants, immunopotentiators, or immunostimulants that can potentiate humoral immunity, which is mediated by Ag-specific antibodies (Abs). However, many clinical studies have indicated that humoral immunity alone cannot sufficiently protect the host against FMD infection. These results highlight the innate immunity and T cell-mediated cellular immunity. The foot-and-mouth disease virus (FMDV) studies reported to date have not provided a comprehensive description of Ag-mediated innate and cellular immunity. The relevant action mechanism in animals is also unknown. Thus, the first aim of this study was to verify a thorough comparative analysis of the cytokine profiles and time kinetics induced by the Ags of seven FMDV serotypes, both *in vivo* and in isolated murine DCs and MΦs. A second goal of this study was to identify which cytokines derived from these cells act as key molecules and which PRRs are stimulated to secrete these cytokines. Finally, we investigated whether critical cytokines and PRR ligands play a crucial role in host defense as an adjuvant.

Materials and Methods: Here, we purified different serotypes (O, A, Asia1, C, SAT1, SAT2, and SAT3) of FMDV-derived Ag and investigated an overview of the FMDV Ag-mediated cellular immune response *in vivo*. DCs and MΦs were also isolated from the peritoneum of mice to elucidate the origin of each cytokine and to investigate the Ag-mediated immune responses and cellular/molecular crosstalk between DCs and MΦs. The importance of DCs and MΦs in initiating Ag-mediated innate and cellular immunity and the roles of the cytokines expressed in these cells in a broad spectrum host defense against FMDV infection were investigated using cell depletion and cytokine neutralization methods incorporating specific Abs, as well as the administration of recombinant cytokines and adoptive transfer of Ag-stimulated DCs and MΦs. Finally, we measured Ag-mediated PRRs expression in DCs and MΦs, and confirmed the efficacy of PRR ligands as an adjuvant.

Results: We found that FDMV Ag-mediated proinflammatory cytokine IL-23 and anti-inflammatory cytokine IL-10 were potentially produced by DCs and MΦs at competing levels to form a positive-feedback loop to maintain host homeostasis via the coactivation of PRRs. Moreover, these responses also modulated both cellular and humoral immunity, thereby further tailoring the immune response to a broad spectrum of pathogens. These results underscore the role of IL-23 signaling via PRRs in DCs and MΦs as a key immune modulator in early stages of FMDV infection.

Conclusions: The induction of IL-23 via synergistic activation of PRRs such as TLR-7/8, STING, Dectin-1/2 and Mincle by the O TWN 97-R Ag mediated a powerful boost to the innate cellular immune response and protected the host effectively in the early stages of FMDV infection. These findings may suggest a new strategy for the development of highly efficacious FMDV vaccines.

Acknowledgement: This research was supported by the grant from the Animal and Plant Quarantine Agency, Republic of Korea.

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IMM-032

Intradermal and subcutaneous inoculation of foot-and-mouth disease vaccine into the neck region of pigs reduce the incidence of local lesions and reduce meat disposal due to adverse reaction

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Introduction: Foot-and-mouth disease(FMD) is a highly contagious disease causing vesicular formation in foot and mouth of cloven-hoofed animals, and intramuscular (IM) inoculation of FMD vaccine in the neck region of pigs has been widely used. Other routes of administration such as intradermal and subcutaneous have been described as more suitable to reduce injection site adverse effect at slaughterhouse but adjuvants and formulation available so far were not compatible with this goal. The purpose of this study was to evaluate the effect of a new FMD vaccine, which has incorporated the latest emulsion technology and suitable adjuvants to be used by either ID or SC route, on the discarded meat of the injection site at slaughterhouse.

Materials and Methods: This study was conducted in three conventional pig farms and a total of 96 eight weeks old pigs were used. Polyvalent single-oil emulsion FMD vaccine(Bioaftogen® ID, Biogénesis-Bagó) was used with two administration routes; 42 intradermally(ID), 42 subcutaneously(SC), and 12 were controls without vaccination (CON). Each injection was conducted deliberately with a syringe-needle to make sure the vaccine is injected into exact site. The first and second inoculation were conducted at 8wo and 11-12wo into the left and right side of neck, respectively, with a dose of 0.5ml/pig. Both injection sites of pork were collected from slaughterhouse (12 weeks after the second vaccination), each portion was grossly evaluated, and local lesions, such as abscess, granuloma, and vaccine residue, at the inoculation site were recorded. In order to calculate gross weight of abnormal meat, the abnormal portion of each pork neck where local lesions were developed was cut and weighed.

Results: In both ID and CON group, local gross lesions were not observed at the inoculation site(0/84, 0/24, respectively), so there was no abnormal meat either. In SC group, local lesions were observed in 4 pork neck(2 abscesses and 2 vaccine residues), and the incidence rate was 4.8%(4/84). The total weight of abnormal meat in SC group was 0.01kg and the average weight was less than 0.01kg(2.6g).

Table 1. Incidence rate of local lesion and weight of abnormal meat according to administration route

	Route of vaccination			
	IM*	ID	SC	CON
	Decivac®, Intervet*	Bioaftogen® ID, Biogénesis- Bagó		
Number of pork neck	71	84	84	24
Number of local lesion	19	0	4	0
Incidence rate of local lesion	26.8	0	4.8	0
Abnormal meat (total weight, kg)	10.9	0	0.01	0
Abnormal meat (average weight, kg)	0.57	0	<0.01	0

IM, intramuscular; ID, intradermal; SC, subcutaneous, CON, control
* Ko, et al., 2018

Conclusions: According to a previous study, the incidence rate of local lesions at the inoculation site was 26.8%, and an average of 0.57kg per pork neck had been disposed with IM inoculation of FMD vaccine(Decivac®, Intervet, Ko, et al., 2018). In contrast, Bioaftogen® ID inoculation did not make any local lesions and abnormal meat. With SC inoculation, there was significantly reduced local lesions(4.8%) and an average weight of abnormal meat was ignorable(<0.01kg). It demonstrates that ID and SC administration are reliable substitutive routes for FMD vaccination, preventing the incidence of local lesions and consequential economic losses caused by disposal of meat at IM administration site.

Acknowledgement: This work was supported by CARESIDE, Ltd., Republic of Korea.

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IMM-033

Lactobacillus-Displayed CTA1-PEDSe can Induces Protective Immune Responses against PED Virus

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Introduction: Porcine epidemic diarrhea (PED) causes significant economic impact throughout the swine-raising countries including Korea. PED virus (PEDV), the causative agent of PED belongs to the member of family Coronaviridae, is enveloped RNA virus. Due to the tremendous productivity losses to the pig farms, PED infection has been one of the major concerns of the Korean swine industry. Even though several commercial vaccines have been developed and periodic vaccination strategies have been implemented to control PED in Korean swineherds, PEDV has continually emerged, causing tremendous harm to the swine farms. Failing to produce effective vaccines has been mostly due to the continuous evolving of new subtypes of PEDV, and showing antigenic and genetic differences between spike proteins of vaccines and field strains. Thus, the development of an effective PEDV vaccine that provides broad cross protection against existing PEDV strain and emerging subtypes is urgently needed.

Materials and Methods: In this study, we used PEDSe multi-epitope (Spike multi-epitope, Se=COE, SS2, SS6) gene with or without non-toxic mucosal immunogenic adjuvant, Cholera toxin subunit A1 (CTA1) for the construction and we express PEDSe multi-epitope with CTA1 on the surface of Lactobacillus casei (pgsA-CTA1-PEDSe/L. casei) using the pgsA surface display system. Mice were inoculated with pgsA-CTA1-PEDSe /L. casei orally or intranasally and we evaluated the potent mucosal, humoral and cell-mediated immune responses.

Results: The recombinant L. casei (pgsA-CTA1-PEDSe/L. casei) that express PEDSe with CTA1 on the surface were confirmed and oral or intranasal inoculations of pgsA-CTA1-PEDSe/L. casei into mice induced more potent mucosal, humoral and cell-mediated immune responses. Additionally, we checked the long lasting immune responses to produce neutralizing antibodies against PEDV infection.

Conclusions: The recombinant L. casei (pgsA-CTA1-PEDSe/L. casei) could be a promising mucosal vaccine candidate against currently circulating PEDV.

Acknowledgement: This work was supported by the Ministry for Food, Agriculture, Forestry and Fisheries (Grant No. 315044-3, 318039-3)

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IMM-034

Large comparative field efficacy observation between FLEXcombo® and Fostera™ PCV MH in Korea

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Introduction

Korean swine industry experienced significant losses due to PMWS until 2007. Launching efficacious PCV2 mono vaccine resulted in recovery of performance (1). Porcine Circovirus type 2 (PCV2) and Mycoplasma hyopneumoniae (M.hyo) combination vaccines are relatively new to the Korean swine industry. The objective of this study was to compare efficacy like mortality between existing combo vaccine and ready-to-use vaccine with field swine practitioners in Korea.

Material & Method

Table 1. Trial Design

Swine vet	Farm	No. of Sow	No. of vaccinated pigs
Dr. Choi	MJJ	520	3407
Dr. Hwang	SUW	300	1240
Dr. Kim	CHG	600	4682
Dr. Ryu	URI	200	1716

The large comparative field efficacy observation was conducted on four different swine farms overseen by different swine consultants (see table 1).

10,145 piglets were included in this study and vaccinated with FLEXcombo® or FosteraTM PCV MH at 3 weeks of age according to the manufacture's recommendations. The mortality rate (including culling rate) before and after vaccination in each farm was reported for the whole post-weaning (wean-to-finish) period except CHG farm (grow-out period). Chi-square analysis was used to make comparisons between two groups for mortality.

Results

A statically significant difference between two-treatment groups showed in mortality ($P<0.001$). FLExcombo® vaccinated groups had significantly less mortality rate compared to FosteraTM PCV MH vaccinated group in all four farms.

Table 2. Total wean-to-finish mortality between FLEXcombo® and Fostera™ PCV MH vaccinations on the four farms.

	Mortality rate (%)		
Farm	FLEXcombo®	Fostera™ PCV MH	Diff.
CHG*	3.2	7.3	4.1
MJJ	4.8	7.7	2.9
SUW	5.7	9.3	3.6
URI	9.7	17.3	7.6

*CHG farm data comes from grow-finisher period.

Conclusions and discussion

The large-scale field observations confirmed the superior efficacy of FLEXcombo®. FLEXcombo® also proved consistent reduction of mortality comparing FosteraTM PCV MH regardless of farm performance. These are reflected in the fact that Ingelvac CircoFLEX® and Ingelvac MycoFLEX® are the preferred vaccines of Korean pork producers (2).

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IMM-035

Mincle and STING-Stimulating Adjuvants Elicit Robust Cellular Immunity and Drive Long-Lasting Memory Responses in a Foot-and-Mouth Disease Vaccine

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Introduction: Conventional foot-and-mouth disease (FMD) vaccines have several limitations, such as slow induction of the antibody, short persistence of antibody titers, and low vaccine efficacy and safety in pigs. Despite the importance of the cellular immune response in the host defense at the early stages of foot-and-mouth disease virus (FMDV) infection, most FMD vaccines focus on humoral immune responses. Antibody responses alone are not sufficient to drive complete protection against FMDV infection, and cellular immunity is also required. From these perspectives, it is necessary to develop a new strategy for a novel FMD vaccine to induce a more potent cellular immune response and a long-lasting immune response as well as to address safety. Previously, we demonstrated the potential of various pattern recognition receptor (PRR) ligands and cytokines as adjuvants for the FMD vaccine. Based on these results, this study aimed to verify the potential use of PRR ligands and recombinant cytokines as FMD vaccine adjuvants and thereby to develop FMD vaccine adjuvants optimized for both livestock species (cattle and pigs) and new strategy of vaccine compositions containing these adjuvants.

Materials and Methods: To address PRR ligands- and cytokines-mediated memory response and protective effect against FMDV infection, mice experiments were performed. For the analysis of peritoneal exudate cells (PECs) subpopulations from memorized mice, we used flow cytometric analysis. PRR ligands- and cytokines-induced LDH release and cell proliferation by BrdU incorporation assay were assessed in bovine and porcine peripheral blood mononuclear cells (PBMCs). To evaluate the potential use of PRR ligands and cytokines as vaccine adjuvants and to investigate their effect to induce cellular and humoral immune responses and long-term immunity, experiments were carried out using cattle and pigs in the fields. Vaccination was performed twice at a 28-day interval, and 1 mL vaccine (1 dose) was administered via the deep intramuscular route on the necks of the animals. Blood samples were from the cattle and the pigs for the serological assays including SP ELISA and virus neutralization test.

Results: Here, we investigated PRR ligands and a cytokines adjuvant-mediated memory response in mice and the cellular immune response in PBMCs isolated from cattle and pigs. The induction of robust memory responses and expansion of memory cells by PRR ligands and cytokines resulted in a complete protective effect against FMDV infection in all experimental groups. No cytotoxicity was observed in any of the adjuvant concentrations used in this study and these adjuvants significantly increased cell proliferation compared to the control group. We further evaluated target-specific adjuvants, including Mincle, STING, TLR-7/8, and Dectin-1/2 ligand, for their role in generating ligand-mediated, long-lasting memory responses in cattle and pigs. The combination of the Mincle and STING-stimulating ligands, induced a high level of antigen-specific and virus-neutralizing antibody titers at the early stage of vaccination and maintained the long-lasting memory immune response in pigs.

Conclusions: These findings will provide important clues for the development of a robust FMD vaccine that will stimulate both cellular and humoral immune responses to elicit a long-lasting, effective immune response and address the limitations of the current FMD vaccine.

Acknowledgement: This research was supported by the grant from the Animal and Plant Quarantine Agency, Republic of Korea.

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IMM-036

MYPRAVAC® SUIS vaccination at early stages improves piglet growth performance

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Introduction: *Mycoplasma hyopneumonia (M.hyo)* is one of the most common respiratory pathogens in swine and a main component of the Porcine Respiratory Disease Complex (PRDC). It usually causes lung damage, reduction in daily weight gain, a worse feed conversion rate and enhanced time to market weight[1]. Thus, *M.hyo* dramatically decreases the economic benefits of the farm. The aim of this study was to assess the efficacy of a *M.hyo* vaccine on a commercial pig farm in China.

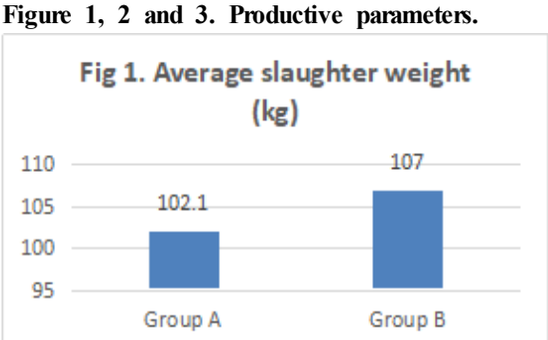
Materials and methods : A total of 634 piglets were randomly divided into two treatment groups: group A (n=413) was not immunized and group B (n=238) was immunized with an inactivated *M.hyo* vaccine (MYPRAVAC® SUIS, HIPRA) at 1 and 3 weeks of age. Both groups were monitored during the nursery and fattening stages. Mortality rate, average body weight at slaughter and average daily weight gain (ADWG) were analyzed at the end of the fattening stage.

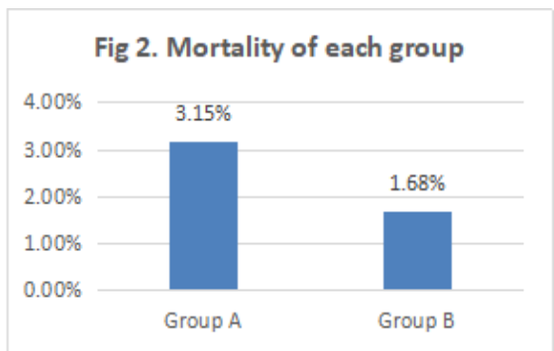
Table 1. Productive parameters

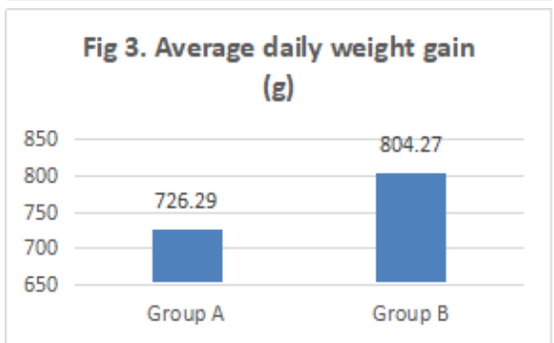
Items	Group A	Group B
Average weaning weight (kg)	31.65	29.79
Fattening period (days)	97	96
Average weight at slaughter (kg)	102.1	107.0
Mortality rate	13/413	4/238

Results: Immunized pigs showed an improvement in their productive parameters. Slaughter weight and ADWG were higher than in the controls (Fig 1 and Fig 3), whilst mortality was reduced by half in comparison to the control group (Fig 2).

Figure 1, 2 and 3. Productive parameters.







Conclusion: MYPRAVAC® SUIS vaccination improved growth performance and decreased the mortality rate in comparison to the non-vaccinated pigs. Therefore, two shots of MYPRAVAC® SUIS at an early stage can effectively solve the impairment of productive performance caused by *M.hyo* and offer protection throughout their lifecycle.

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IMM-037

Needle-free intradermal vaccination of pigs induced a good level of protection against Porcine Circovirus type 2 and *Mycoplasma hyopneumoniae*

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Introduction: Globally, porcine circovirus type 2 (PCV2) and *Mycoplasma hyoneumoniae (Mhp)* cause porcine respiratory disease complex (PRDC) and result in huge economic losses to pig industry [1]. Vaccines against PCV2 and *Mhp* are routinely used in the pig industry; however, intramuscular vaccination causes a high level of stress and increases the risk of cross-infection among pigs. In this study, noninvasive, needle-free intradermal vaccination was evaluated for safety and efficacy against two major pathogens of PRDC.

Materials and Methods: A total of 15, 6 week-old PCV2 and *Mhp* seronegative pigs were purchased and divided into 3 groups (Group A, B and C) of 5 pigs each. Group A and C were intradermally vaccinated with 0.2 ml of PCV2 *Mhp* vaccine using an intradermal applicator while group B pigs were kept as a non-vaccinated control (Table 1). Twenty- one days post-vaccination (dpv), both group A and B pigs were intranasally challenged with the 2.0 ml of PCV2b and *Mhp* while the other vaccinated group was kept unchallenged. Blood and nasal swabs were collected each week post-challenge for measuring pathogen loads and serological conversions using qPCR and ELISA, respectively. Lung and bronchial lymph node samples were collected from euthanized pigs at 28 days post-challenge (dpc) for measuring pathogen loads and histopathological evaluation. PCV2 neutralizing antibody assay was conducted at 0 dpv, 0 dpc, 28 dpc. Moreover, body weight of pigs was measured every-week after challenge to calculate average daily weight gain (ADWG).

Table 1.

Treatment	Group		
	A	B	C
Vaccinated	O	X	O
Challenged	O	O	X

Results: Compared to the vaccinated pigs, the non-vaccinated pigs displayed significantly escalated clinical symptoms and lower ADWG after challenge with PCV2 and *Mhp*. Moreover, PCV2 loads in sera, lungs, and nasal swabs of the non-vaccinated pigs were higher than the vaccinated pigs. Humoral response in the form of IgG's specific for PCV2 and *Mhp* started to appear 3 and 6 weeks post vaccination, respectively. The group A pigs displayed significantly higher IgG response as compared to the other two groups. In addition, vaccinated pigs presented significantly higher levels of PCV2 neutralizing antibodies. Further, based on pathological evaluation, higher pneumonia scores and severe lung lesions were perceived in non-vaccinated pigs as compared with the vaccinated pigs.

Conclusions: This study demonstrated that comparatively small volume of PCV2 and *Mhp* vaccines applied through intradermal vaccination against PCV2 and *Mhp* provides a good level of protection against these pathogens, which is probably due to efficient antigen delivery to abundant antigen presenting cells present in dermis.

Acknowledgement: This work was supported by the Technology Development Program for Bioindustry (Project No. 318038-2) of the Ministry for Food, Agriculture, Forestry and Fisheries of the Republic of Korea.

References:

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IMM-038

Parvoruvax protects against reproductive failure caused by porcine parvovirus-27a genotype strain

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Introduction: Concerns arouse about the protection capabilities of licensed vaccines against the highly virulent porcine parvovirus strain 27-a (PPV-27a), first reported in Germany [1]. The aim of this study was to evaluate the efficacy of Parvoruvax®, containing the K-22 strain of PPV and lysed bacterial cells of Erysipelothrix rhusiopathiae, in preventing reproductive failure caused by PPV-27a infection.

Materials and Methods: Eight gilts (8 in each treatment group) were vaccinated twice with Parvoruvax at 6 and 2 weeks prior to mating. Similar number of gilts was injected with PBS to serve as controls. At day 40 of pregnancy all gilts were challenged intranasally and i.m. (2-2 mL) with a PPV-27a genotype virus strain (PPV1-HUN) having a titer of 6.97 log TCID50/1mL. At day 90 of pregnancy all gilts in both groups were euthanized and all fetuses were aseptically delivered and euthanized. General condition of fetuses, their size and weight were recorded. Blood and tissue samples (lung and kidney) were collected from all fetuses for serology and qPCR, respectively. Serology was performed by an in-house sandwich blocking ELISA. qPCR was done according to Streck et al. (2015) and Foerster et al. (2016) [2, 3].

Results: Humoral immune response to vaccination was detected by ELISA in serum samples collected at the time of mating (38 days after the first vaccination), which declined by the time of challenge. Challenge triggered significant increase of antibody titers in both the vaccinated and control animals. No dead or mummified piglets were found in the vaccinated group, while all sows were affected in the control group (34.2% live, 5.4% dead and 60.4% mummified fetuses). The average litter size was 13.7 and 6.3 for the vaccinated and control group, respectively. The qPCR positive fetuses were 6% and 73% for the vaccinated and the control groups, having a mean of 1.5 log and 5.4 log TCID50/mL viruses in the tested organs, respectively. Regarding the weight and length of the live fetuses no significant difference was measured between the groups.

Figure 1. ELISA titers of gilts over time

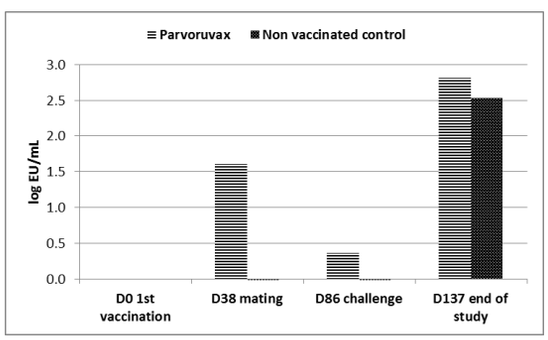
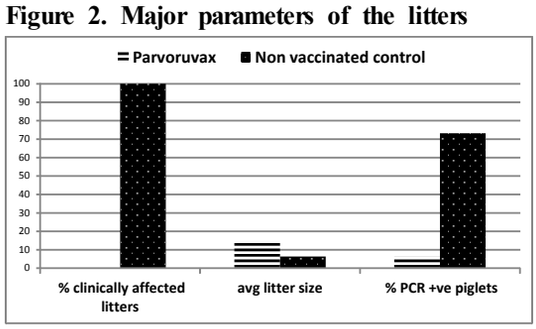


Figure 2. Major parameters of the litters



Conclusions: Parvoruvax® provided excellent protection against the effect of PPV 27a challenge by (i) completely preventing reproductive failure of vaccinated sows; (ii) reducing the ratio of sows with PPV +ve fetuses and the percent of PPV +ve fetuses/litters, which in turn resulted in significantly higher number of live piglets/litter in the vaccinated sow group.

Acknowledgement: The expert contribution of the staff of Prophly Ltd., Bár, Hungary, and Attila Cságola, Ceva-Phylaxia R&D is acknowledged.

References:

[1] Zeeuw E.J.L et al., 2007. J. Gen. Virol. 88, 420-427.
[2] Streck A.F., et al., 2015. J. Virol Methods, 218, 46-50
[3] Foester T., et al., 2016. J. Gen. Virol. 97, 1408-1413

IMM-039

Protective Erysipelothrix rhusiopathiae antibody response following vaccination with an Erysipelas-Parvo combo vaccine in a multicenter study

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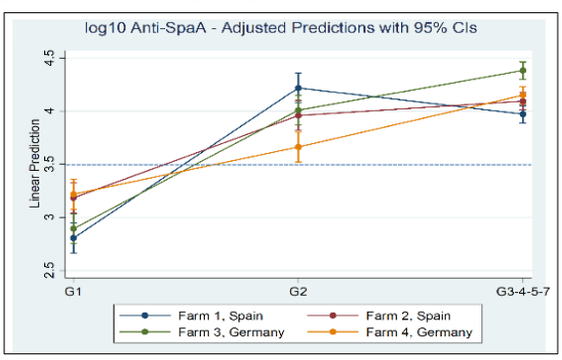
Introduction: *Erysipelothrix rhusiopathiae* (Ery), the cause of Swine erysipelas is ubiquitous and present in global swine production. When uncontrolled vaccination it is economically significant [1]. Spa are the surface located protective antigens of Ery. They are classified into three antigen types, including SpaA [1], the only Spa protein found in swine Ery strains, till now. Anti-SpaA antibodies are shown to be the central elements in swine Ery-protective immunity, via neutralization and opsonization they elicit a highly protective immunity [2]. An anti-SpaA ELISA is the only kind of test able to evaluate Ery protective immunity [3]. This is not to be confused with the titers of commercial tests for evaluating Ery field infection, not protective immunity [3,4]. Parvoruvax® (Ceva) has already shown to elicit a fast and potent Ery-protective immune response in an experimental setup [5]. The aim of this study was to assess the Ery-protective immunity by vaccination with Parvoruvax® in a multi-center field study on the basis of an anti-SpaA ELISA.

Materials and Methods: Two Spanish and two German swine farms, with no apparent Ery circulation and already using Parvoruvax® for over one year, were sampled. Sera were collected cross-sectional on female breeding stock in 5 groups of 10 animals each. In all farms 10 non-vaccinated gilts of approximately 6 months of age (G1) and 10 gilts 4 weeks following the second of the two primo-vaccinations (G2) were sampled. As well as 3 different sow parity groups >8 weeks following booster vaccination (G3-G7). The Ery protective immunity was assessed by anti-SpaA antibody titration using a rSpaA415 ELISA[4] with a positive minimal-value at 3000 (3.5log10) was performed at the Roslin Institute, University of Edinburgh, Scotland, UK. All titers were log10 transformed to conform normally distributed data within groups for accurate statistical calculations. The difference in mean log anti-SpaA values between the groups at farm level was modelled by fixed-effect ANOVA and significance evaluated by Holm adjusted p-value.

Results: The difference between mean Anti-SpaA values of Group 3, 4, 5 and 7 within farms was not found significant thus the data of those groups within each farm were pooled, G3,4,5,7 and compared to G1, as was G2. Data presented in fig.1.

All in-farms comparisons of G2-G1 and G3,4,5,7-G1 had same significant p-values at farm level: Farm1-Spain, p=0.0015; Farm2-Spain, p=0.0015; Farm3-Germany, p=0.001, and Farm4-Germany, p=0.0015.

Fig 1. ***Group-average log10*** **anti-SpaA titers**



Conclusions: Highly significant, highly Ery-protective immunity is demonstrated in all vaccinated groups in all farms. Already following primo-vaccination with Parvoruvax® high Ery-protective immunity values that plateaus out in all subsequent parities are achieved. Proving the immediate, high and durable protective immunity induced by Parvoruvax®.

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[1] Opriessnig and Coutinho. (2019) Erysipelas, Diseases of Swine 11th Ed.

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[4] Crussard et al. (2016) Proc. IPVS/ESPHM: P021

IMM-040

Quantitative detection of the foot-and-mouth-disease virus serotype O antigen by using virus neutralization test

Mun-Hyeon Kim[1], *Min-Goo Seo*[1], *Hyang-Sim Lee*[1], *Ji-Yeon Kim*[1], *Ji-ye Kim*[1], *Young-sang Kim*[1], *Seon-Jong Yun*[1*]

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Introduction: Foot-and-mouth disease (FMD) is infectious and contagious viral disease affecting biungulate species. To prevent and control of FMD, South Korea adopted vaccination policy throughout all over the country for FMD-susceptible animals. In Korea, many FMD vaccines of several companies were used. Quality control of commercial vaccines, formulated with inactivated FMD virus (FMDV), becomes more and more important with the strict requirement for the purity, potency, safety and effectiveness in vaccine production. In this study, we assessed serotype O FMD vaccine efficacy against antibody titer using virus neutralization test.

Materials and Methods: We used imported FMD vaccines from vaccine manufacturer. We tested growing pigs (6 weeks old) from a low-antibody level of FMD pig groups. Pigs were vaccinated intramuscularly in the neck region above the left shoulder with: 2 ml (full dose), 0.5 ml (1/4 dose) and 0.25 (1/8 dose). To investigate for vaccine's antibody titer, serum samples were collected on three weeks after vaccination and were analyzed by virus neutralization test in biosafety level 3 facilities. The antibody titers were calculated according to the Spearman-Kärber method and expressed as log10 of the reciprocal of the final serum dilution that neutralized 100 $TCID_{50}$ of virus in 50% of the well.

Results: Three concentration of FMD vaccine from each company were vaccinated against pigs. On three weeks after vaccination, all serum sample performed VNT. In serotype O FMD vaccine, serum sample of 2 ml vaccinated pigs showed the highest antibody value and 0.25 ml vaccinated pigs showed the lowest antibody value. The average VNT *r*-values for antibody titer were well evaluated.

Conclusions: In serotype O FMD vaccine, vaccine dose-dependently decreased antibody titer. Therefore, we confirmed that the higher the vaccination concentration, the higher the antibody titer. Previously, we performed measurement of antigen content in FMD vaccine by using HPLC. Results of antigen contents using HPLC will be correlated with VNT. If correlation is achieved measurement of antibody by VNT can be replace new methods of HPLC.

Acknowledgement: This study was supported by grants from the Animal and Plant Quarantine Agency (I-1543073-2018-20-02).

References:

[1] Spitteler MA et al., 2011. Vaccine 29: 7182-7187.
[2] Yang Y et al., 2017. Vaccine 35: 2413-2419.

IMM-041

Recombinant FMDV OVM can protect against diverse FMDV O serotype viruses

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Introduction: In this study, we evaluated a recombinant protein-based vaccine for foot-and-mouth disease viruses (FMDV).

Materials and Methods: To construct multi-epitope-based protein for FMDV vaccine, we selected epitope sites containing G-H loop and C-terminus known as major immunogenic sites in VP1 region from O serotypes FMDVs which had ever occurred or threatened in Korea and we conjugated each epitope sites. Through the E. coli expression system, the multi-VP1e protein was expressed in soluble form and purified by FPLC and IMAC system. By emulsifying with Montanide ISA 201, adjuvant known as efficacious in FMDV protection, we finally prepared recombinant FMDV vaccine candidate and used to immunize animals.

Results: Multi-VP1e protein was expressed in soluble form and purified by FPLC and IMAC system. Mice were inoculated with multi-VP1e protein emulsified with ISA 201 every two weeks by I.M injection and we demonstrated that multi-VP1e protein vaccine can induce humoral and cellular immunity and completely protect from lethal infection of FMDV O serotype. Additionally, multi-VP1e protein vaccine induced the production of sufficient neutralizing antibodies against each three serotypes of FMDV in pig and effectively protected pigs from FMDV O serotype challenge.

Conclusions: Thus, multi-VP1e protein vaccine which derived from E. coli expression system may be a safe and effective vaccine candidate against diverse FMDV.

Acknowledgement: This work was supported by Ministry for Food, Agriculture, Forestry and Fisheries (Grant no. 315044-3, 318039-3)

References:

[1] Lee HB et al., 2017. Microb Cell Frac. 16, 33.
[2] Cao P., 2014. Vet. Microb 168: 294-301.

IMM-042

Sensitivity of two serological Erysipelothrix rhusiopathiae tests on vaccine induced immune stimulation and protective immunity

Preben Mortensen[1], *Krisztian Kertesz*[2], *David Espigares*[3], *Christoph Waehner*[4], *Andreas Palzer*[5], *Han Smits*[2]

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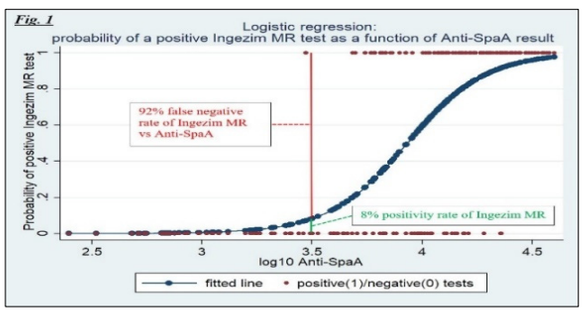
Introduction: No commercial test is available for indicating Erysipelothrix rhusiopathiae (Ery) protective immunity. The tests available are designed to reveal Ery field infection. This lack of readily available tools to evaluate vaccine induced protective immunity and vaccine take with a sufficient reliable sensitivity following Ery vaccination has already been addressed in experimental setup [1,4]. Anti-SpaA antibodies are identified as the central protective components in swine-Ery immunity[2,3]. A rSpaA415-microbead based serological fluorescent immunoassay (*SpaA-FMIA*) available for project-based testing has proven to have 100% sensitivity and specificity; far higher than commercial tests when compared[1], and following Parvoruvax® (Ceva) Ery-immunisation [4], under experimental settings.The aim of this study was to compare sensitivity in a commercial Ery-ELISA to the sensitivity of the *SpaA-FMIA*[1], in serum sampled in farms vaccinating with Parvoruvax® for Ery-protection.

Materials and Methods: In 2018, two Spanish and two German swine farms, with no apparent Ery circulation and already using Parvoruvax® for over one year, were sampled.On each farm, sera were collected cross-sectional in female breeding stock in 5 groups of 10 animals each. In all farms 10 non-vaccinated gilts of approximately 6 months of age and 10 gilts 4 weeks following the second of the two primo-vaccinations were sampled. As well as 3 different groups of sow parity one to five, >8 weeks following booster vaccination. The Ery protective immunity was assessed in a Luminex® based *SpaA-FMIA* [1], and the commercial test for Ery field infection, INgezim Mal Rojo (*INgezim MR*), Ingenasa, were both performed at the Roslin Institute, University of Edinburgh, Scotland, UK. Logistic regression was used to model the probability of positive *INgezim MR* test as a function of log10 *SpaA-FMIA* titre. The predicted positivity rate at 3.5 log10 of *SpaA-FMIA* with its 95% CI was obtained by the delta method.

Results: At low, protective *SpaA-FMIA* titres the *INgezim MR* provided a very high rate of negative results; At the lower positive titre (3000/3.5 log10) of *SpaA-FMIA*, *INgezim MR* has a predicted positive rate of only 8% (95% CI: 1% to 15%); hence 92% negatives (Fig.1). Increasing titres increases agreement of *INgezim MR* to *SpaA-FMIA*; close to the model predicted full agreement at 50,000/4.7log10 (Fig.1).

Fig 1. *Regression probability of a positive Ingezim MR test as a function of anti=spa result*



Conclusions: It takes a SpaA-based ELISA or FMIA to evaluate Ery vaccine efficacy and protective immunity [1]. Commercial Ery-ELISAs are not reliable tools for evaluating vaccine-take and protective efficacy, but designed for detecting previous Ery infection. Compared to the *SpaA-FMIA* test, the *INgezim MR*, is far less sensitive. At the low level of positive anti-SpaA antibodies the probability of a false negative is found to be 92% (Fig.1). The observation of low sensitivity on vaccine induced immune response in commercial Ery-ELISAs is in accordance with previous studies [1,4], showing the sensitivity of Ery-ELISAs, following vaccination, tested against the proven the 100% sensitive *SpaA-FMIA* revealing: only *22%* sensitivity of the *INgezim MR*, and only *44 %* sensitivity of the *Civtest Suis SE/MR*, Hipra [1].

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[1] Opriessnig and Coutinho., 2019 Erysipelas, Diseases of Swine 11th Ed.
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[4] Crussard et al., 2016. Proc. IPVS/ESPHM: P021

IMM-043

Suitability assessment of foot-and-mouth disease vaccine strains for FMD viruses recently isolated in Republic of Korea

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Introduction: Vaccination has been one of the most powerful tools in disease prevention and control including foot-and-mouth disease (FMD). However, FMD virus is a highly variable RNA virus, and in general, vaccination against one serotype of FMDV does not cross-protect between serotypes and even among other strains of the same serotype. Thus a reasonable antigenic match between the FMDV vaccine and the outbreak virus strains is considered important for the application of vaccination programs in FMD-affected regions as well as for the establishment and maintenance of vaccine antigen banks to be used in the event of new FMD threats. To select a suitable vaccine strain, serological tests are used for antigenic matching on the basis of the hypothesis that the level of protection in vivo is highly correlated with the antigenic match from the serological tests. In this study we investigated the protective capacity of conventional FMD vaccines against newly isolated FMDVs in Korea using two-dimensional virus neutralization test (vaccine matching test).

Materials and Methods: FMD viruses were isolated by Animal and Plant Quarantine Agency in the Republic of Korea (APQA). Bovine calf kidney (LF-BK) cells, received kindly from the Plum Island Animal Disease Center (USA), were used for all virus related work. Vaccine matching test was undertaken according to the protocol outlined within the OIE Manual [1]. The relationship between the field isolate and the vaccine strain is then expressed as an r1 value as :

$$r1 = \frac{\text{reciprocal arithmetic titer of reference serum against field virus}}{\text{reciprocal arithmetic titer of reference serum against vaccine virus}}$$

r1 values equal to or greater than 0.3 indicate that the antigenicity of the field isolate in question is sufficiently similar to that of the vaccine strain.

Table 1. FMDV genotyping

Vrius name	Host	Serotype	Topotype	Lineage
O/Boen/SKR/2017	bovine	O	ME-SA	Ind-2001e
O/Anseong/SKR/2019	bovine	O	ME-SA	Ind-2001e
A/Yeoncheon/SKR/2017	bovine	A	Asia	Sea-97
A/Gimpo/SKR/2018	porcine	A	Asia	Sea-97

Results: The serotype O field viruses isolated in 2017 and 2019 from Korea belonged to O/ME-SA/ Ind-2001e genotype by VP1 sequencing (Table 1.). Both viruses exhibited good cross-reactivity with the antisera of conventional vaccines used in Korea (r1 values $\geq$ 0.3). The serotype A field viruses isolated in 2017 and 2018 from Korea classified to A/Asia/SEA-97 genotype by VP1 sequencing (Table 1.). Both viruses exhibited similar antigenic reaction with the antisera of conventional vaccines applied nation widely in Korea.

Conclusions: New variant FMD viruses are emerging periodically and antigenic mismatch is one of the main reasons of vaccine failure. Thus FMD vaccine strain selection can be considered through antigenic and genetic characterization of recently circulating FMD viruses through vaccine matching.

Acknowledgement: This study was supported by a grant (B-1543386-2018-20-02) from the Animal and Plant Quarantine Agency's National Animal Disease Research Project.

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IMM-044

The protective efficacy of the recombinant B-subunit proteins of Stx2e against edema disease in post weaning piglets

Ja Young Moon[1], *Won Kyong Kim*[1], *Enkhsaikhan Ochirkhuyag*[1], *Jun woo Lee*[1], *Young Gyu Jo*[1], *Seon Min Kim*[1], *Je Myoung Lee*[1], *Geon Il Moon*[1], *So Hyun Park*[1], *Hyun Soo Lee*[1], *Sung Cheol Moon*[2], *Ho Kyeong Jung*[2] *and Jin Hur*[1]

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Introduction: Porcine edema disease (ED) caused by *Escherichia coli* producing F18 fimbriae and Shiga toxin 2e ($F18^{+}$STEC) is an enterotoxemia of post-weaned piglets that causes significant threats and economic losses to pig husbandry worldwide due to growth retardation and mortality. Stx is considered the key virulence factor involved in the pathogenesis of porcine ED. The typical Stx produced by *E. coli* isolated from pigs with ED is Stx2e whose gene has been found located in the chromosome since no Stx converting phages could be isolated opportunely. This study was carried out to examine whether intramuscular (im) immunization of piglets with the novel vaccine candidate could effectively protect the piglets against porcine edema disease.

Materials & Methods: The recombinant B-subunit proteins of Stx2e were over-expressed from *Escherichia coli* BL21 (DE3) transferred by pET28a containing the gene for Stx2eB subunit fragment among Stx2e and were purified. The purified recombinant proteins were used as one vaccine candidate to protect piglets from porcine edema disease. All the piglets were primed at 2 weeks of age and were boosted at 4 weeks of age. Group A piglets were im inoculated with PBS. Group B-D piglets were im immunized with 5 ELISA units/pig, 10 ELISA units/pig and 20 ELISA units/pig of the recombinant proteins, respectively. Seral IgG titers from all piglets were evaluated using ELISA kit. All piglets were challenged with wild-type F18+ Stx2e+ *E. coli* strain at 2 weeks post booster. All piglets were monitored daily for mortality for 14 days after challenge.

Results: Seral IgG titers from group B-D piglets were significantly higher than those of group A piglets. After challenge with the wild-type *E. coli*, mortality in group D piglets was not observed. However, mortality was observed in 80% of group A and B piglets, and in 20% of group C piglets.

Conclusion & Discussion : These findings indicate that im immunization of piglets with 20 ELISA units of the recombinant Stx2eB-subunit vaccine candidate can effectively protect the piglets from porcine edema disease.

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IMM-045

Tryptophanyl-tRNA-Synthetase on Virus Infection and Application for Adjuvant

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Introduction: Tryptophanyl-tRNA synthetase (WRS) is one of the aminoacyl-tRNA synthetases (ARSs) that possesses non-canonical functions. Full-length WRS is released during bacterial infection and primes the Toll-like receptor 4 (TLR4)-myeloid differentiation factor 2 (MD2) complex to elicit innate immune responses. However, the role of WRS in viral infection remains unknown.

Materials and Methods: To examine whether virus infection triggers WRS secretion, we infected human immune cell lines with VSV-gfp in a time-dependent manner. As virus infection proceeds, WRS level in the supernatant were increased from early time of infection like other kinds of antiviral cytokines. To assess the function of WRS, we purified recombinant human WRS (rWRS) produced in Escherichia coli and conformed it by immunoblot and coomasie blue staining. And after treatment of rWRS, cells were washed and followed by VSV-gfp or PR8-gfp infection for 2 h. At 24 h post infection, rWRS-treated cells showed markedly reduced level of GFP expression. To identify the antiviral function of WRS, we tested immune responses that is represented as antiviral cytokine secretion with rWRS treatment. We next addressed the effect of WRS in vivo. To assess whether rWRS bring about antiviral cytokine secretion of mice, rWRS was intravenously injected into mice tail vein. Mice stimulated by rWRS showed elevated IFN-β and IL-6 level in serum, which has peak at 3 h and 6 h post injection, respectively (Fig 6A). We further tested intravenous VSV-gfp infection after stimulating mice with rWRS for 6, 12 h. Consistent with serum cytokine level, infected VSV-gfp showed lesser replication in the serum of rWRS treated mice at 12 h post infection.

Results: Here, we show that full-length WRS is secreted by immune cells in the early phase of viral infection and functions as an antiviral cytokine. Treatment of cells with recombinant WRS protein promotes the production of inflammatory cytokines and type 1 interferons (IFNs) and curtails virus replication in THP-1 and Raw264.7 cells, but not in TLR4-/- or MD2-/- bone marrow-derived macrophages (BMDMs). Intravenous and intranasal administration of recombinant WRS protein induces an innate immune response and blocks viral replication in vivo.

Conclusions: These findings suggest that secreted full-length WRS has a non-canonical role in inducing innate immune responses to viral infection as well as to bacterial infection.

Acknowledgement: This work was supported by The Ministry for Food, Agriculture, Forestry and Fisheries (Grant No. 315044-3, 318039-3) and National Research Foundation (Grant No. 2018M3A9H 4078703)].

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Bact(Ent)-001

Antimicrobial Resistance of *Escherichia coli* from pig farms in Central China

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Introduction: Antimicrobials are widely used in livestock production for disease prevention and productivity promotion. However, these practices contribute to the emergence of antimicrobial-resistance bacteria and the spread of drug-resistant pathogens in both livestock and humans, posing a significant public health threat: drug-resistant pathogens can be transmitted from farms to humans through the environment and/or food products [1]. As a world's leading cause of food-borne and water-borne infections, *Escherichia coli* is widely isolated from food-producing animals as well as the environment in farms. In this study, we tested the antimicrobial susceptibility of *E. coli* isolates from pig farms in Central China, one of largest swine and pork producing regions in China.

Materials and Methods: *E. coli* was isolated from anus swabs of diarrheal pigs, health pigs, and also environmental swabs including floors, food, water, troughs, waterer, and pig wastes in scale farms (including breeding farms, weaner and finisher farms) and yard farms. Resistance profile of these isolates were determined by testing the minimal inhibitory concentration (MIC) values of 28 types of antibiotics on the bacteria using the broth microdilution method, according to CLSI guidelines (CLSI-VET06). Results were in interpreted using the CLSI breakpoints (CLSI-VET06, CLSI M100, 28th Edition). If a CLSI breakpoint is not available, a European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint is used for the interpretation. Each antibiotic was tested with three duplicates. *E. coli* ATCCR 25922 was used as quality control.

Results: Our results showed that *E. coli* isolates from pig farms in Central China were commonly resistant to trimethoprim/sulfamethoxazole (SXT, 81.44%), chloramphenicol (CHL, 92.24%), tetracycline (TET, 97.51%). 11.36% of the isolates were resistant to ertapenem (ETP), and imipenem (IPM); while 10.80% of the isolates were resistant to meropenem (MRP). 8.59% of the isolates were resistant to colistin (CL). In particular, 25.81% of the colistin-resistant *E. coli* were also resistant to the three carbapenems tested (ETP, IPM, MRP). Only a few (percentage < 10.00%) of isolates were resistant to fosfomycin (FOS, 9.70%), nitrofurantoin (NIT, 7.76%), and tigecycline (TGC, 0.28%) (Figure 1). Multi-drug resistance was also a common characteristic for *E. coli* isolates from farms. Approximately 96.95% of the isolates displayed resistance to more than three types of antibiotics. Isolates from different types of farms showed different levels of antibiotic resistance. Overall, isolates from scale farms had more serious resistance profile than those from yard farms; while isolates from weaner farms showed more serious resistance profile than those from breeding farms and finisher farms.

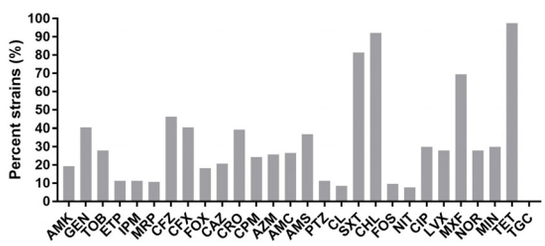

Figure 1 Percent of *E. coli* isolates from pig farms in Central China to the tested antibiotics.

Conclusions: Antibiotic resistance of *E. coli* from pigs remains a worrisome problem and should receive more attentions. More active actions should be taken to decrease the dependence on antibiotics in pig farms.

Acknowledgement: This work was supported in part by the National Key R&D Program of China (Grant numbers: 2017YFC1600101 and 2017YFC1600103).

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Bact(Ent)-002

Effects of phytogenic feed additive on post-weaned piglets naturally infected with *brachyspira hyodysenteriae*

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Introduction: *Brachyspira hyodysenteriae* is a causative agent of swine dysentery (SD), a severe mucohemorrhagic enteric pig disease. SD is associated with huge financial losses in pig production due to increased mortality, lower weight gain, poor feed conversion and increased costs for treatment. The increased bacterial resistance to antibiotics available for treating swine dysentery and a restricted use of antibiotics in the finishing period of pigs' fattening present great challenges for veterinarians to treat SD, nowadays. Thus, there is increased urge for finding novel solutions to control SD disease (1). Phytogenic feed additives (PFA) are considered as an alternative to antibiotic growth promotors based on wide spectrum of bioactivities such as, antimicrobial, antioxidant, and anti-inflammatory properties of plant bioactive compounds (2). Our previous research have demonstrated the beneficail effects of PFAs on post-weaned piglets which were naturally infected with *B. hyodysenteriae* (3) and *Lawsonia intracellularis* (4). The aim of current research was to asses *in vivo* efficacy of PFA to control of SD in weaned piglets naturally infected with *B. hyodysenteriae* by means of real-time PCR (RT-PCR) and fecal scoring.

Materials and Methods: The trial lasted 21 days and included 48 seven-week old weaned pigs divided into 3 groups: Trial group was fed with basic diet in which 2 kg/ton of commercial PFA (PATENTE HERBA® PLUS, PATENT CO. DOO, Serbia), Positive control group, where to basic diet 2 kg/ton of tiamulin was added, and Negative control group, where to basic diet no antibiotic or PFA were added. During the trial, fecal consistency was recorded daily while weight gain and feed conversion ration were monitored weekly. *B. hyodysenteriae* presence in fecal samples was monitored weekly in individual fecal samples by RT-PCR tests (3).

Results: The presence of *B. hyodysenteriae* in fecal samples was confirmed in all samples by both, the conventional PCR and RT-PCR techniques. The results of the RT-PCR quantification showed highest quantities of *B. hyodysenteriae* DNA copies in the Negative control group, and the least in Trial group on days 0 and 7, and the Positive control group days 14 and 21. However, statistical difference between groups in cycle-threshold (Ct) values measured by RT-PCR was not determined on any of the measuring days. On the other hand, differences of fecal consistency between pigs treated with the commercial PFA and the Negative control were statistically significant ($p<0.001$). The commercial PFA was as effective as tiamulin in preventing and controlling the outbreak of swine dysentery considering that differences in feces consistency were insignificant between those groups. For the whole trial period of 3 weeks, weight gain did not statistically differ between Positive and Trial groups, while significant statistical difference ($p \leq 0.05$) was detected between negative and commercial PFA group in Turkey test.

In third week of the trial, feed conversion ratio was highest in Negative control group 2.38, while in commercial PFA group was 1.96 and in Positive control group 1.95 (3).

Conclusions: The results of this study suggest that PFA could be applied in pig diet not just to improve production parameters in pigs naturally infected with *B. hyodysenteriae* but also to prevent and control swine dysentry outbreaks.

Acknowledgement: This study was supported by the Ministry of Education, Science and Technological Development, Republic Of Serbia, III46002, and PATENT CO. DOO partly funded this study.

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Bact(Ent)-003

Increase farm biosecurity by using virusnip™ to improve hygiene on four malaysian commercial piggeries

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Introduction: Biosecurity is a cornerstone of herd health maintenance (FAO, 2010). There are three biosecurity pillars: segregation, cleaning and disinfection. Performing all three consistently and correctly is mandatory. Segregation involves keeping potentially infected animals and materials away from uninfected animals. Most pathogen contamination on physical objects is contained in fecal material, urine or secretions that adhere to the surface; cleaning removes most of the pathogens. Disinfection is the final "polishing" step (FAO, 2010) in biosecurity, used after cleaning. Virusnip™ is an advanced soluble powder disinfectant with oxidizing and chlorine-releasing actions, widely used in livestock farming. The objective of the present study was to assess the efficacy of Virusnip™ in farrowing pens in four commercial Malaysian farms by determining the decrease in Total Aerobic Bacteria (TAB) populations.

Materials and Methods: In each of the four farms, fifteen empty farrowing pens (five/treatment group) were randomly selected and assigned to three treatment groups (Table 1). Virusnip™ and three commercial oxidizing disinfectant brands were used at 1% dilution.

Table 1. Treatment groups.

Farm	Pos. control	Treatment	Neg. control
#1	Brand A	Virusnip™	Distilled water
#2	Brand A	Virusnip™	
#3	Brand B	Virusnip™	
#4	Brand C	Virusnip™	

The general procedures of cleaning, disinfection and bacterial sampling of the pens occurred as follows, which differs from the ideal program proposed by Luyckx (2016):

1) Dry and/or wet cleaning with or without detergent
2) Drying period after cleaning (1 to 50 hours)
3) Sampling 1-2 hours before disinfection
4) Spraying disinfectant until run-off (>0.4 L/m^2)
5) Drying period after disinfection (22-25 hours)
6) Sampling 22-25 after disinfection

Sampling occurred using pre-moistened cotton swabs containing disinfectant neutralizer. Six locations (3 walls and 3 floors) in each pen were pre-determined using a 10x10 cm plastic frame. Swabs were kept on ice and sent to the laboratory within 24 hours. Swabs content was first diluted into 1 mL of broth dilution. Serial tenfold dilutions of the initial dilution were conducted. 1 mL of every dilution was pipetted and poured either onto Plate Count Agar (farm 1) or 3M™ Petrifilm™ Aerobic Count plate (farms 2, 3, 4). After a 24 hours incubation at 37℃, colonies were visually counted. Because many differences between farms existed regarding biosecurity and laboratory procedures, statistical analysis was only performed within farms (ANOVA, Tukey HSD test, JMP software).

Results: In each farm, Virusnip™ decreased numerically the TAB when compared to distilled water and the disinfectants with significant differences when compared to distilled water (Table 2).

Table 2. Effect of disinfection on bacterial population

Farm	Disinfectant	Mean TAB decrease after disinfection (log_{10} CFU/cm^2) ± Standard Deviation		
#1	Water	0.93^b	±	0.49
	VIRUSNIP™	2.96^a	±	1.31
	Brand A	2.28^a	±	1.59
#2	Water	0.28^b	±	0.36
	VIRUSNIP™	1.11^a	±	0.74
	Brand A	0.99^a	±	0.50
#3	Water	1.96^b	±	1.15
	VIRUSNIP™	3.99^a	±	1.79
	Brand B	3.11^a	±	1.60
#4	Water	0.89^b	±	0.96
	VIRUSNIP™	2.27^a	±	1.00
	Brand C	2.20^a	±	1.03

(Per farm, different superscript letters means $p<0.05$)

Conclusions: The disinfecting power of Virusnip™ can improve farm disinfection under commercial conditions, one of the three pillars of farm biosecurity.

Acknowledgements: The team thanks the commercial laboratories (Jubilee Sky, T&T, AmCen) for their work.

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Bact(Ent)-004

Swine dysentery: quantification of risk factors

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Introduction: Swine dysentery (SD), caused by *Brachyspira hyodysenteriae*, is an important disease in pig production worldwide due to severe intestinal lesions but also subclinical infections resulting in decreased growth rate and feed conversion [1]. However, quantitative data on risk factors are rare.

This case-control study aimed at identifying major risk factors related to presence of *B. hyodysenteriae* in pig herds.

Materials and Methods: Twenty pig herds with SD (cases) and 60 randomly selected herds without SD (controls) with a minimum herd size of '10 sows/ 80 fattening pigs' were analysed by means of a questionnaire-based interview and a herd examination. Cases and control herds were matched according to the production type. Herds with previous eradication of SD were excluded. All herds were located in Switzerland. Participation in the study was voluntary. The data from the questionnaire were transferred in a data management programme (Microsoft InfoPath 2010) and statistically analysed using the free software R (Ri386. v3.3.1). Risk factors were assessed by means of univariable and multivariable logistic regression models.

Results: The case herds comprised 17 grower-finisher herds (85%), two wean-to-finish herds (10%), and one farrow-to-finish herd (5%). Within the control herds, 54 (90%) were grower-finisher herds and six farrow-to-finish herds (10%). Due to the low number of herds with sows, piglets and/or weaned pigs, parameters describing these age categories are not analysed further.

Eight variables were identified as risk factors and three as protective factors (Table 1). The final multivariable logistic regression model identified 'more than 4 batches/ year purchased' (odds ratio (OR) = 7.5, 95% confidence interval (CI): 1.8-54.3) and 'contact to foxes' (OR = 5.9; CI: 1.2-34.6) as the strongest risk factors in our sample.

Conclusions: 'More than 4 batches/ year' implies continuous herd management supporting persistence of *B. hyodysenteriae* in an infected herd, but also increased the number of purchases each enhancing the risk of *B. hyodysenteriae* introduction. Foxes might be infected with *B. hyodysenteriae* by feeding on positive piglets or rodents. Besides, contact to foxes might represent a lack in biosecurity. In conclusion, the risk factors detected underline the importance of biosecurity in SD prevention and control and identify the need for further research.

Table 1. Risk and protective factors for Swine Dysentery in pig herds in Switzerland evaluated by logistic regression models. The first category presented is the reference category.

Variable	Categories	Odds ratio	95% CI (p-value)
BH-status of source herds	Neg Pos/susp	11.2	3.0-49.1 (0.001)
Regular treatment	No Yes	8.4	2.7-29.8 (< 0.001)
No batches/year	0-4 >4	6.7	1.7-44.8 (0.017)
Contact to foxes	No Yes	4.9	1.4-17.6 (0.013)
Diagnostics last 12 months	No Yes	4.4	1.5-13.3 (0.007)
Feeding system in fattener unit	Dry Wet	3.5	1.1-13.3 (0.042)
Rats on farm	No Yes	3.1	1.1-9.3 (0.034)
No fattening places	80-250 >250	3.1	1.1-9.3 (0.044)
Mixed batches of grower pigs	No Yes	0.3	0.08-0.8 (0.018)
Raptor birds in region	No Yes	0.1	0.03-0.5 (0.003)
Martens in region	No Yes	0.1	0.01-0.5 (0.024)

CI = confidence interval. Neg = negative. Pos = positive. Susp = suspected case.

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Bact(Ent)-005

The synergistic efficacy of chlortetracycline and neomycin to porcine enteric bacteria in Thailand

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Introduction: The diarrheal diseases caused by bacteria, are the major problems in pig industry. The major enteric bacteria caused diseases are *Escherichia coli* containing *mcr-1* gene (*EC+mcr-1*), *Salmonella* Choleraesuis (SC) and *Clostridium perfringens* (*CP*). Chlortetracycline (CTC) is the first tetracycline that uses for treatment of wound and respiratory infection in animals. Neomycin is one of aminoglycoside with excellent activity against Gram-negative bacteria, but it is partially effective against Gram-positive bacteria. To use of both antimicrobials to enteric pathogens, it may not be recommended as drug of choice [1]. However, it is interesting that the combination of these two antibiotics may improve the efficacy to enteric pathogens. The aims of this study were to determine the minimal inhibitory concentration (MIC) of enteric pathogens consisting *EC+mcr-1, SC*, and *CP* to CTC and Neomycin and to investigate their combination efficacies.

Materials and Methods: A total of 15 enteric bacterial strains consisted of five of each EC+*mcr-1*, *SC*, and *CP*. The colistin resistant EC+*mcr-1* gene were identified (MIC > 2 μ g/ml) [2]. The pure *EC* and *SC* were grown on TSA mixed 5% sterile sheep blood and confirmed using the routine biochemical test and an automated machine (VITEK® 2, bioMérieux). Only *CP* was anaerobically prepared by blood agar containing Neomycin. Agar dilution method was determined for following CLSI standard [3]. For *CP*, Brucella broth added 0.1% hemin solutions and 0.1% vitamin K solutions was used as the medium and interpreted the results at 48 hours of incubation [4]. Microdilution checkerboard susceptibility test were used. The concentrations of each antimicrobial were filled in the pits vary by row direction and another drug was diluted vary by column direction. Synergistic effects were determined by fractional inhibitory concentration (FIC) values [5].

Results: The MIC_{50} and MIC ranges of enteric pathogens are shown in Table. 1 and the combination efficacy is shown in Table 2. The results showed that all tested bacteria were in the high resistant level. Interestingly, MIC_{50} of *CP* to CTC and neomycin was apparently reduced under the combination and the results was consistent to all strains of *CP*. Nevertheless, the mechanism related to the synergistic efficacy is needed to investigate in the further study.

Table 1. MIC_{50} values and MIC ranges (μg/ml) of antimicrobials in single and combined reactions to the enteric bacteria.

Organisms	MIC_{50}	CTC		Neomycin	
	(μg/ml)	single	combined	Single	combined
EC+mcr-1	MIC_{50}	256	256	512	512
	range	128-256	64-256	512	128-512
SC	MIC_{50}	256	256	256	256
	range	256-512	64-512	256	128-256
CP	MIC_{50}	>512	64	>512	64
	range	>512	64	>512	64

Table 2. Combination efficacies between CTC and neomycin to porcine enteric bacteria (S: synergistic, I: indifferent, A: antagonistic).

Bacteria	No of isolates	Combination Efficacies		
		S	I	A
EC+mcr-1	5	1		4
SC	5	1		4
CP	5	5		

Conclusions: Our study demonstrated the synergistic combination efficacy between CTC and neomycin in *CP* and one strain of colistin resistant *E. coli* and *SC*.

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Bact(Res)-001

Case report : Effect of Introduction of Mycoplasma hyopneumoniae negative gilt on seropositive rate in growing pigs and Pig lung lesions at slaughter in Mycoplasma hyopneumoniae positive farm.

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Introduction: Swine enzootic pneumonia (EP) is one of the most important respiratory disease in swine industry. Mycoplasma hyopneumoniae (M. hyopneumoniae, MH) is the etiological agent of EP that swine mainly affects growing and finishing pigs. After the outbreak of foot-and-mouth disease in 2011 in Korea, MH seronegative pigs began to be imported from foreign countries and provided to the commercial pig farms. Since the distribution of MH free-gilt, many farms have experienced difficulty in acclimation of gilt against MH. Nowadays, limited information was researched on adequate MH acclimation protocol. While natural infection or vaccination is commonly used for acclimation, the effect of these methods on acclimation is not clear. Therefore, this case study was conducted to investigate the effect of introduction of M H-negative gilt on Antibody positive rate and pig lung lesions at slaughter in the MH -positive farm.

Materials and Methods: In this study, a 550 sows, farrow-to finish farm with MH in sow and growing pig was selected. Thirty MH-negative gilts were received monthly from January 2017, and for the M. hyopneumoniae acclimation, vaccination was conducted once during acclimation period. From September of 2017, gilts moved to the farrowing house and started to be delivered. Most sows were replaced in January 2019. The lung lesion was checked in slaughter house every three months. Blood samples were collected and tested for antibodies at 30, 60, 90, 120, and 150 days of age. Five Blood samples was taken from each age group. Correlation analysis between the percentage of MH-like lung lesions in the slaughter house and the seropositive rate at each stage of pigs was carried out through Excel.

Results: The percentage of MH-like lung lesion and the seropositive rate was highly correlated (r=0.89)(Table 1.). The seropositive rate of M. hyopnemoniae in farms is considered to reflect the positive rate of MH-like lung lesions at the slaughter house.(Figure 1.) The seropositive rate, as well as the respiratory symptoms, was increased by increasing the number of delivery of the Mycoplasma negative gilts which was acclimated by vaccination. The introduction of negative replacement stock into positive farms may contribute to the development of subpopulations of non-infected pigs, increasing the risk of pathogen re-circulation and its persistence in the farm. While the vertical transmission could not be identified in this study, it was assumed that the positive rate of each stage of pigs against Mycoplasma increased as the sows were replaced.

Table 1. Correlation between positive rate of antibody of stage of growing pigs and positive rate of MH like lung lesion at market stage.

	Seropositive Rate of MH	Rate of Mh like lung lesion
Seropositive against Mh	1	
Rate of Mh like lung lesion	0.89	1

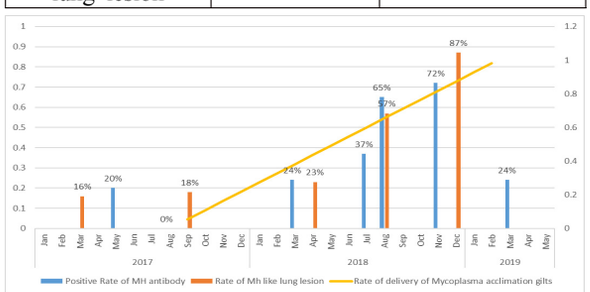

Figure 1. Comparison of seropositive rate of stage of growing pigs and positive rate of MH like lung lesion at market stage on quarterly

Conclusion: There is a very high correlation between the seropositive rate to M. hyopnemoniae and the positive rate of M. hyopneumoniae lesion at the slaughter house. On the farm, the MH-negative gilt for acclimation by using vaccination one time was not enough. Inadequate acclimation protocol using vaccination may increase bacterial shedding, causing activation of M. hyopnemoniae on farm. Therefore, an effective acclimating method for M. hyopnemoniae negative pigs is needed.

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Bact(Res)-002

Combination efficacy between chlortetracycline and tylosin against porcine pathogenic respiratory bacteria in Thailand.

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Introduction: Respiratory disease in pigs so-called "PRDC" causing by bacterial and viral infection, is the most important health concern for swine producers. To diminish severity of clinical sign, a proper management with antimicrobial administration become a routine execution in conventional farms, while the situation of antimicrobial resistance has been dramatically increased. Use of antimicrobial combination regimen is an alternative tool to enhance of the bactericide efficacy [1]. The objectives were to determine the minimal inhibitory concentration (MIC) to chlortetracycline (CTC) and tylosin and to investigate their combination efficacies against the respiratory bacteria; *Streptococcus suis* (SS) type 2, *Haemophilus parasuis* (HP), *Actinobacillus pleuropnuemoniae* (APP) and *Pasteuralla multocida* (PM) isolated from diseased pigs.

Materials and Methods: A total of 25 pathogenic bacteria comprising 5 PM, 10 SS type II, 5 HP, and 5 APP isolated from pigs (2017-2018) suffered from respiratory pneumonia were used in this study. PM and SS were grown on TSA mixed 5% sterile sheep blood and confirmed using the routine biochemical test and an automated machine (VITEK® 2, bioMérieux). APP and HP were grown on haemophilus test medium with supplement SR0158E, chocolate medium supplement with 1% NAD, and blood agar with staphylococcal streak in 5% CO_2 environment and were identified by the approved PCR [2]. The individual susceptibility procedure for CTC and tylosin against all bacterial strains were performed by agar microdilution method [3]. The combination of both antimicrobials was performed by checkerboard microdilution technique. The cut-off interpretation was detected by being transparent within well representing inhibition by antimicrobial combination. The fractional inhibitory concentration (FIC) index for combination was calculated follows the recommendation [4].

Results: The MIC50 and MIC ranges of all 25 pathogens are shown in Table 1 and the combination efficacies are shown in Table 2. The MIC of SS type II, PM and APP to CTC was higher and even higher than the breakpoint of CLSI guideline ($\geq 2\mu g$/ml) for PM [5]. Surprisingly, MIC of HP to CTC alone was in low level. MIC of all tested bacteria to tylosin was in high level but no available breakpoint. The CTC/ tylosin combination could decrease the MIC_{50} of HP and APP lesser than those of CTC or tylosin alone. The synergistic outcome was found in HP.

Table 1. MIC_{50} values and MIC ranges (μg/ml) of antimicrobials in single and combined reactions to the respiratory bacterial strains.

Organisms	MIC_{50}	CTC		Tylosin	
	(μg/ml)	single	combined	Single	combined
SS type II	MIC_{50}	256	256	256	256
	range	64-256	256	256-512	256-512
PM	MIC_{50}	4	4	16	16
	range	2-4	4	16	16
HP	MIC_{50}	2	1	128	64
	range	0.5-2	0.25-1	128	64
APP	MIC_{50}	128	32	32	4
	range	128	32	32	4-32

Table 2. Combination efficacies between CTC and tylosin to the 4 pathogenic species (S: synergistic, I: indifferent, A: antagonistic).

Bacteria	No of isolates	Combination Efficacies		
		S	I	A
PM	5		5/5	
SS	10		10/10	
HP	5	5/5		
APP	5		5/5	

Conclusions: In conclusion, this study confirmed the CTC/tylosin strong synergistic affect to HP and no antagonistic effect was observed from all samples.

References:

[1] Rybak and McGrath, 1996. Drug. 52(3):390-405.
[2] Angen et al., 2001. Vet Microbiol. 119(2-4):266-276.
[3] CLSI, 2013: 4th Edition: Approved Standard VET01-A4.
[4] Antibiotics in laboratory medicine 3rd edn. (1991)., pp. 432-492.
[5] CLSI, 2012: M11-A8, CLSI standard

Bact(Res)-003

Detection of Mycoplasma hyopneumoniae by nPCR in laryngeal swab samples of suckling pigs in Korea farms

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Introduction

Mycoplasma hyopneumoniae (M. hyopneumoniae) is a representative pathogen of enzootic pneumonia in pigs. Vertical transmission is reported to be the initial pathway of the disease, but the sensitivity of antigen detection is limited and varies with test methods and sample types (1). The present study aimed to investigate the sow herd stability with regard to *M. hyopneumoniae*. Laryngeal swabs of suckling piglets were investigated by nested PCR, which is reportedly the most sensitive method for detection.

Materials & Method

Twenty-two pig farms in Korea were selected for testing. These farms had a higher number of marketed pigs per sow per year (MSY) than the average MSY of Korean pig farms and reported signs of porcine respiratory disease complex (PRDC) due to M. hyopneumoniae based on previous serum ELISA and PCR results. Each farm had individual protocols for acclimation, antibiotic treatments, and gilt replacement (Table 1). Sampling was conducted as previously described (1). Briefly, a BD CultureSwab™ Liquid Stuart Single Swab (BD Diagnostics, USA) was inserted by a veterinarian behind the epiglottis of a piglet using a laryngoscope. Two suckling pigs of 2 ~ 3 weeks age were selected at random for the sampling from 11-15 sows of one farrowing batch depending on the farrowing batch size. The sampling was performed in three consecutive farrowing batches in each of the 22 farms. Chi-square and Fisher's exact test was used to compare the prevalence of infected piglets with the following parameters: herd size, gilts replacement rate, acclimation procedures, and antibiotic usage. A value of $P < 0.05$ was considered significant.

Results

In total, 1952 pre-weaning piglets were used for laryngeal swab sampling. *M. hyopneumoniae* DNA was detected in 14 farms (63.6%) and 127 piglets (6.5%). The prevalence of sows likely to transmit *M. hyopneumoniae* in herds was 11.1% and was calculated as the ratio of total sows to sows with at least one piglets positive for *M. hyopneumoniae*.

Table 1. Overview of farm management and *M. hyopneumoniae* detection in piglets.

Parameter	Group	Farms	piglets (+) rate
All		22	6.5%
Herd size (No. of sow)	≤ 550	11	5%
	> 550	11	8%
Gilt replace ment rate	≤ 40%	11	4.4%
	> 40%	11	8.6%
Serological status [a]	Positive	13	6.7%
	Negative	9	6.3%
Gilt source	Self-replacement	7	6.7%
	External (GP)	15	6.4%
Acclimation of gilts	Exposure	3	16.7%
	Vaccination	14	5.4%
	No treatment	5	3.3%
Antibiotics usage	For sow	11	8.2%
	For piglet	14	8.3%

Conclusions and Discussion

In summary, this study investigated the prevalenceof *M. hyopneumoniae* in piglets around weaning among farms. There was a significant difference of *M. hyopneumoniae* detection rate among farms with regard to herd size, gilts replacement rate and acclimation method. The results demonstrated that laryngeal swabs of suckling pigs provided useful information with regard to sow herd stability in farms with enzootic pneumonia.

Acknowledgement and references

[1] M. Pieters et al., Vet. Microbiol., 203 (2017), pp. 103-109

Bact(Res)-004

Efficacy of different PCV2+Mhyo or M. hyo single vaccination protocols to reduce lung lesions caused by Mycoplasma hyopneumoniae infection

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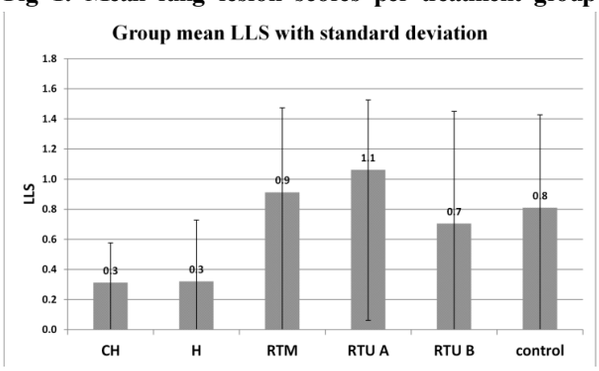
Introduction: PCVD and Enzootic pneumonia remain two of the economically most important diseases in pig farms. Vaccination against PCV2 and *Mycoplasma hyopneumoniae* (M.hyo) helps to reduce clinical manifestation of those infections and corresponding losses. Several commercial mono- or bi-valent vaccines are available. The aim of this study was to evaluate the efficacy of various PCV2 and M. hyo vaccination protocols against experimental M. hyo infection in a standardized challenge model.

Material and Methods: Three week-old piglets were vaccinated either with Circovac® plus Hyogen® (CH), administered simultaneously, or Hyogen® (H) only, or PCV2+M.hyo RTM vaccine or one of the two RTU (RTU A, RTU B) vaccines. At 7 WOA the animals were inoculated intratracheally with two different M. hyo strains on consecutive days. Five weeks later the pigs were slaughtered and the lung lesions scored, samples from affected lungs were collected for histopathology. Blood samples were collected for serology before vaccination, before challenge and before slaughter and tested by two M. hyo antibody ELISA kits (BioChek and IDEXX).

Results: Group mean lung lesion scores (LLS) in groups CH, H, RTM, RTU A, RTU B and positive control were as follows: 0.3; 0.3; 0.9; 1.1; 0.7 and 0.8. Circovac® plus Hyogen® and Hyogen® groups had significantly lower ($p<0,05$) LLS than any other vaccine groups or the positive control. They were not different from the negative control significantly. The LLS in other vaccine groups didn't differ significantly from the positive control, with RTU A being also different from RTU B ($p<0,05$). Histopathology confirmed the macroscopic scores.

Fig 1. Mean lung lesion scores per treatment group



Conclusion: This study demonstrated that some of the combined PCV2 and M. hyo vaccines may provide sub-optimal protection against M. hyo infection. Hyogen® administered either alone or simultaneously with Circovac® protected lungs the best against the development of the characteristic lung lesions.

Reference:

[1] Seo HW et al, Vaccine. 2014 May 1;32(21):2480-6

Bact(Res)-005

In vitro Susceptibility Study of Porcine Respiratory Pathogens to Chlortetracycline

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Introduction: *In vitro* antimicrobial susceptibility such as Minimum Inhibitory Concentration (MIC) is one of the basic criteria used for antimicrobial selection. It is also used to measure antimicrobial resistant (AMR) level of target bacteria in a specific period and area. Since Chlortetracycline (CTC) has commonly been used for of respiratory disease control and treatment, it is necessary to test for the susceptibility of porcine respiratory pathogens such as *Streptococcus suis* (SS), *Haemophilus parasuis* (HP), *Pasteurella multocida* (PM), and *Actinobacillus pleuropneumoniae* (APP). This study aimed to conduct the MIC test of porcine respiratory bacteria; i.e., SS, PM, HP, and APP, in Thailand to CTC, a member of tetracyclines.

Materials and Methods: Each 10 isolates of SS, HP, PM and APP (total N=40) derived from Thai pig farms during 2017-2018 were re-isolated and multiplied to 10^5 cfu/ml. Ten isolates of each microorganisms (SS, HP, PM, APP) were kept in media containing CAMHD + LHB 5% for broth microdilution method, CLSI 2018 (M07 - A11) [2]. CTC HCl (Pucheng Chia tai Biochemistry, China) was serially diluted from 0.025-128 µg/ml and mixed with 100 µl of microorganisms. The SS and PM isolates were incubated at 37℃ for 18-20 hours while the HP and APP isolates were incubated at 37℃, 5% CO_2, for 18-20 hours. MIC of each isolates were recorded and calculated for MIC_{50}, MIC_{90}, and MIC distribution. Expected resistance % of each isolate will be cut off by estimated breakpoint of resistance was from BSAC 2012 (V11.1) for SS (>2µg/ml) and PM (>1µg/ml) and from NCCLS M2-A4, 1990, for HP and APP (≥8µg/ml) [1, 3].

Results: Distribution of MIC, MIC range, MIC_{50}, MIC_{90} of four respiratory pathogens to CTC are shown in Table 1.

Table 1. Distribution of MIC, MIC range, MIC_{50}, MIC_{90} and Expected Resistance (%) of CTC to four respiratory pathogens (APP, HP, PM, SS).

Respiratory Pathogens	Concentration of CTC (µg/ml)										MIC			Expected Resistance %
N=10*4	0.025	0.5	1	2	4	8	16	32	64	128	range	50	90	
APP			1	6	1	1				1	1-128	2	8	20%
HP			3	6			1				1-16	2	2	10%
PM			1	8	1						1-4	2	2	90%
SS			5	5							1-2	1	2	0%

Conclusions: MIC_{90} of CTC to APP, HP, PM, and SS are 8, 2, 2, and 2 µg/ml, respectively. The expected resistance% of APP, HP, and SS to CTC are 0-20%. From this *in vitro* study, some respiratory pathogens such as APP, HP, and SS, are still susceptible to CTC.

References:

[1] BSAC version 11.1, May 2012.
[2] CLSI 2018 (M07-A11).
[3] NCCLS M2-A4, 1990.

Bact(Res)-006

Mycoplasma hyopneumoniae - the underestimated disease in Asia

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Introduction

Mycoplasma hyopneumoniae (M.hyo) causes mild to moderate losses to the pig industry. Without co- or secondary infections it does not result in high mortality and can be controlled by antibiotics. However, pig producers may underestimate the economic impact from this disease, especially in Asian countries where feed medication for disease prophylaxis is still allowed. The objective of this study is to prove that M.hyo vaccination, even in addition to medication, results in more benefit to the farms.

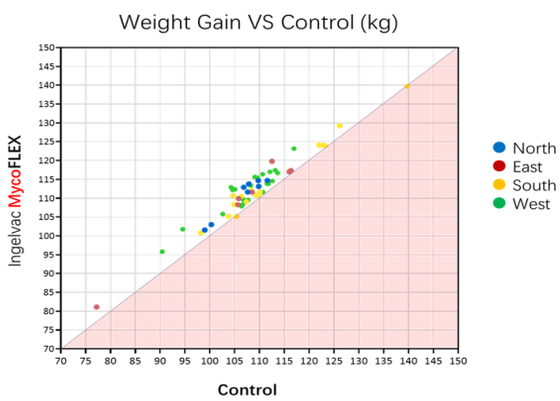
Material and Methods

Fifty two farms in North, East, South and West area of China that did not use M.hyo vaccine on the farm participated in side by side studies starting in July 2018. Piglets from each sow were divided into 2 groups based on piglet weight, in order to minimize the effect from different litters, and ear-tagged with different tag colors. Group 1 piglets were vaccinated with Ingelvac MycoFLEX® around 3 weeks of age; group 2 piglets served as non-vaccinated controls. Within each farm, groups were kept under the same environment and management; i.e., room, feed, medication and staff. Between farms there were differences in the number of pigs per group and basic medication program. Approximately 100-200 pigs per farm were included in each study depending on the individual farm size. The basic medication program was for example, amoxicillin 200 ppm + sulfa-trimethoprim 110 ppm in the feed for 28 days after weaning or Florfenicol 400 ppm after weaning etc. Slaughter weight and average daily gain (ADG) were the main study parameters. Group weights were collected at weaning (2-3 weeks of age) and at slaughter.

Results

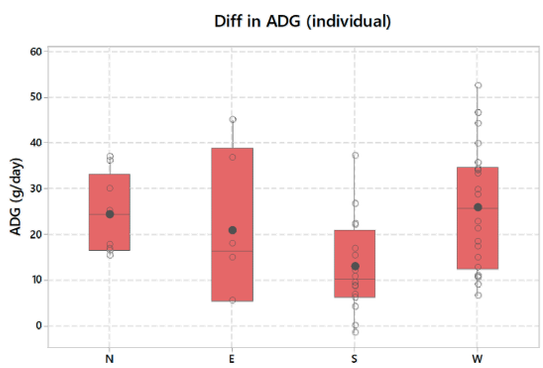
Ingelvac MycoFLEX® vaccinated groups gained more weight compared with non-vaccinated pigs. On average, the gain was around 3.5 kg (0.021 kg ADG) more between weaning and slaughter. The mean gain was also calculated for each area: in the North area the gain was around 4.1 kg (0.024 kg ADG), in the East area it was around 3.2 kg (0.028 kg ADG), in the South area around 2.7 kg (0.013 kg ADG) and in the West area around 4.4 kg (0.026 kg ADG). No differences were observed in mortality (data not shown).

Graph 1. Weight gain vs Control



In graph 1, each dot represents a farm and the color of the dot represent the area of the farm. If the dot is located in the white area, pigs of the Ingelvac MycoFLEX® vaccinated group gained more weight than pigs in the control group.

Graph 2. Diff in ADG (individual farm data) per region



Conclusion

Under the field conditions of these 52 farms in the North, East, South and West region of China, Ingelvac MycoFLEX® vaccination provided a clear benefit even in farms that used medication as a routine program.

Bact(Res)-007

Pathogenotypes of *Pasteurella multocida* isolates from pigs

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Introduction: *Pasteurella multocida* is a leading cause of porcine respiratory disorders worldwide. This Gram-negative pathogenic bacterium has been assigned in to five capsular genotypes (A, B, D, E, F) and/or eight lipopolysaccharide (LPS) genotypes (L1-L8), respectively. While it has been reported that both capsular types A and D are the main epidemic *P. multocida* capsular genotypes in pigs, the predominate LPS genotypes are still unknown. It is also lack of the knowledge about the multilocus sequence typing (MLST) genotypes as well as the virulence factors (VFs) associated genes of the epidemic *P. multocida* in pigs. Therefore, we determined the capsular genotypes, LPS genotypes, as well as the MLST genotypes of the *P. multocida* isolates from China between 2014-2018.

Materials and Methods: A total of 400 *P. multocida* isolates recovered from different samples (nasal swabs, lungs, tracheas) of pigs suffered/died from respiratory disorders in 29 provinces of mainland China between January 2014 and December 2018 were genotyped. Capsular genotypes, LPS genotypes, as well as MLST genotypes were determined by multiplex PCR methods according to Townsend et al. 2001 [1], Harper et al. 2005 [2], and Subaaharan et al. 2010 [3], respectively. Virulence genotyping was also determined by PCR detection of 23 main types of virulent genes, as described previously [4].

Results: PCR assays identified three categories of capsular genotypes: A (48.75%, 195/400), D (42.75%, 171/400), and F (2.75%, 11/400). Approximately 5.75% (23/400) of the isolates were non-typable. LPS genotyping determined three categories of LPS genotypes: L3 (25.00%, 100/400) and L6 (75.00%, 300/400). In combination with capsular genotypes and LPS genotypes, four categories of capsular: LPS genotypes were identified: A: L3 (16.50%, 66/400), A: L6 (32.25%, 129/400), D: L6 (42.75%, 171/400), F: L3 (2.75%, 11/400). MLST genotyping identified seven categories of MLST genotypes: ST3 (16.75%, 67/400), ST10 (34.25%, 137/400), ST11 (43.75%, 175/400); ST12 (2.75%, 11/400), ST16 (0.25%, 1/400), ST74 (0.75%, 3/400), ST75 (1.50%, 6/400). When combining the capsular genotype, LPS genotype with the MLST genotype, a total of eight categories of capsular genotypes were identified: A: L3: ST3 (15.75%, 63/400), A: L3: ST74 (0.75%, 3/400), A: L6: ST10 (32.25%, 129/400), D: L6: ST10 (0.50%, 2/400), D: L6: ST11 (41.25%, 165/400), D: L6: ST16 (0.25%, 1/400), D: L6: ST75 (0.75%, 3/400), F: L3: ST12 (2.75%, 11/400) (Figure 1). Virulence genotyping showed that several virulent genes such as *ptfA, fimA, exbB, tonB, Fur, ompA, ompH, oma87, sodA, sodC* were broadly characteristic of the porcine *P. multocida* isolates, as their detection rates were higher than 90.00%. Conversely, the detection rates of *toxA* and *tbpA* were lower than 10%. Other virulent genes including *hsf-1, pfhA, tadD, hgbA, hgbB, nanB, nanH, pmHAS* were moderately detected and their detection rates were located between 30.00%~80.00%. In addition, the distribution of some virulent genes displayed a certain level of "genotype -preference".

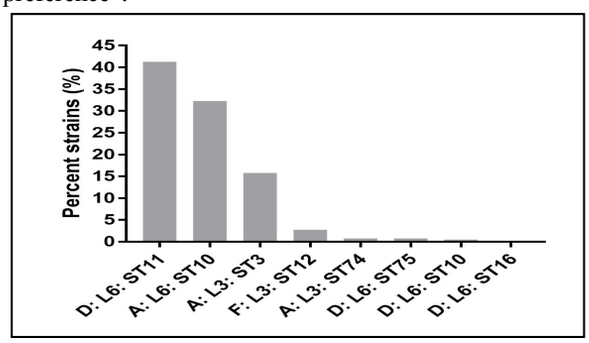

Figure 1 Distribution of capsular: LPS: MLST genotypes identified among porcine *P. multocida* isolates.

Conclusions: A capsule: LPS: MLST genotype D: L6: ST11 of *Pasteurella multocida* is likely to be strongly associated with swine respiratory disease in China.

Acknowledgement: This work was supported in part by the National Key R&D Program of China (Grant number: 2018YFD0500800).

References:

[1] Townsend KM et al., 2001. J. Clin. Microbiol. 39, 924-929
[2] Harper M et al., 2015. J. Clin. Microbiol. 53, 477-485.
[3] Subaaharan S et al., 2010. Vet. Microbiol. 141, 354-361.
[4] Khamesipour F et al., 2014. Front. Microbiol. 5, 536.

Bact(Res)-008

The efficacy of chlortetracycline/tiamulin combination against porcine pathogenic respiratory bacteria in Thailand.

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Introduction: PRDC is commonly found in fattening with high economic loss in pig industry. The diseases associate with both viral such as PRRS, PCV and bacterial infections such as *Streptococcus suis* (SS), *Haemophilus parasuis* (HP), *Actinobacillus pleuropnuemoniae* (APP) and *Pasteuralla multocida* (PM). Use of antibiotic is still a routine execution in conventional farms, however, the emerging of antimicrobial resistant bacteria is increasingly concerned in veterinary field and public health. The antimicrobial combination therapy becomes an alternative tool to increase the antimicrobial efficacy [1]. The objectives were to determine the minimal inhibitory concentration (MIC) to chlortetracycline (CTC) and tiamulin and to investigate their combination efficacies against the respiratory bacteria isolated from pigs in Thailand.

Materials and Methods: A total of 25 pathogenic bacteria comprising 5 PM, 10 SS type II, 5 HP, and 5 APP isolated from pigs (2017-2018) suffered from respiratory pneumonia were used. PM and SS were grown on TSA mixed 5% sterile sheep blood and confirmed using the routine biochemical test and an automated machine (VITEK® 2, bioMérieux). APP and HP were grown on haemophilus test medium with supplement SR0158E, chocolate medium supplement with 1% NAD, and blood agar with staphylococcal streak in 5% CO_2 environment and were identified by the approved PCR [2]. The individual susceptibility procedure for CTC and tiamulin against all bacterial strains were performed by agar microdilution method [3]. The antimicrobial combination was performed by checkerboard microdilution technique. The cut-off interpretation was detected by being transparent within well representing inhibition by antimicrobial combination. The fractional inhibitory concentration (FIC) index for combination was calculated follows the recommendation [4].

Results: The MIC_{50} and MIC ranges of all 25 pathogens are shown in Table 1 and the combination efficacy is shown in Table 2. The MIC_{50} of SS and APP were apparently high to both antimicrobials (over 32 μg/ml) demonstrating in high resistant group [5]. PM and HP show low MIC to CTC while MIC of tiamulin is high to all pathogens. On the other hand, tiamulin could enhance the efficacy of CTC and itself in combination testing. In combination, all MIC_{50} values were lower than that of single at 2-256 times, especially for SS. The synergistic effect was found against HP and SS.

Table 1. MIC_{50} values and MIC ranges (μg/ml) of antimicrobials in single and combined reactions to the respiratory bacterial strains.

Organisms	MIC_{50} (μg/ml)	CTC		Tiamulin	
		single	combined	Single	combined
SS type II	MIC_{50}	128	32	32	0.125
	range	64-256	32-256	0.25-128	0.125-2
PM	MIC_{50}	4	4	16	8
	range	2-4	2-4	16	8-16
HP	MIC_{50}	2	0.25	32	16
	range	0.5 -2	0.25	32-128	16
APP	MIC_{50}	128	64	128	64
	range	128	64	128	64

Table 2. Combination efficacies between CTC and tiamulin to the 4 pathogenic species (S: synergistic, I: indifferent, A: antagonistic).

Bacteria	No of isolates	Combination Efficacies		
		S	I	A
PM	5		5/5	
SS	10	4/10	6/10	
HP	5	3/5	2/5	
APP	5		5/5	

Conclusions: This study confirmed the synergistic effect of CTC/tiamulin to SS and HP and no antagonistic effect was observed from all samples.

References:

[1] Rybak and McGrath, 1996. Drug. 52(3):390-405.
[2] Angen et al., 2001. Vet Microbiol. 119(2-4):266- 276.
[3] CLSI, 2013: 4th Edition: Approved Standard VET01-A4.
[4] Antibiotics in laboratory medicine 3rd edn. (1991)., pp. 432-492.
[5] CLSI, 2012: M11-A8, CLSI standard

PCVAD-001

A Case Report: The resolution of a PCV2d outbreak by change in PCV2 vaccination program

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Introduction: Porcine Circovirus Type 2 (PCV2) is widespread in most pig populations throughout the world, as well as Japan. A clinical diagnosis of PCVAD is dependent on the following 3 conditions being present simultaneously-1) Clinical signs including growth retardation 2) Pathological findings such as lymphopenia and botryoid basophilic inclusion bodies 3) PCV2 virus isolated in tissues. Since 2008, PCV2 commercial vaccines have been licensed in Japan and PCVAD incidences have significantly decreased. Recently, there are cases where PCVAD has broken out in farms vaccinated with commercial PCV2 vaccines. One possibility is a global shift of PCV2 genotype has occurred[1]. It is currently reported that PCV2d is becoming more dominant than PCV2b worldwide[1].

Materials and Methods: The case farm is a conventional one-site system and has 460 sows. In August 2018, piglets (30 to 50 days old) showing neurological symptoms increased in the nursery barn. In September 2018, a 40-day-old piglet displaying neurological symptoms and ataxia was sent for necropsy and pathological examination. Interlobular edema was observed in the lungs. High levels of PCV2 copies were detected from lung by q-PCR (1.6×10^{11} copies/μ l) and PCV2d was detected by sequence analysis. PRRS, *Mycoplasma hyopneumoniae* and other bacteria were not detected in the lung. Histopathologically, botryoid inclusion bodies were observed (Figure1) and the sample was positive for PCV2 by immunostaining, thus confirming the diagnosis of PCVAD. The administration date of the vaccine (Porcilis PCV®) was advanced from one week after weaning (27 days old) to the time of weaning (20 days old). However, after weaning mortality was not improved. In November, blood samples were collected from piglets once every week after weaning in nursery. As a result, it was revealed early PCV2 challenge in the nursery had occurred. Therefore, the PCV2 vaccination program was changed again, to a two-shot program (0.5 dose administration at 4 to 7 days of age and 1 dose at 27 days of age)

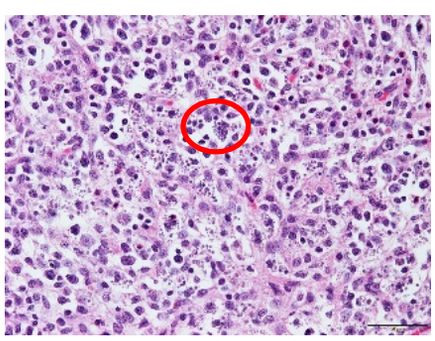


Figure.1 Botryoid inclusion bodies were observed.

Results:

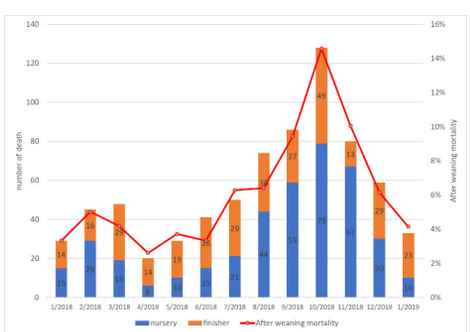
After the change of the vaccine program, the mortality at the nursery barn decreased and clinical observation also confirmed improvement of the health status of the piglets as of January 2019 (Table1).

Discussion

This case which is an early nursery infection of PCV2d was successfully resolved through changing to a two-shot vaccination program although it has not been possible to compare whether it was effective single early vaccination or two-shot-vaccination.

One of the possible reasons why the mortality dramatically increased is vaccination failure but there are no issues with the way of vaccination. Secondly, it has not been compared with past genotype and current genotype of PCV2 on this farm though, genotype shift to PCV2d might have occurred.

Table.1 After weaning mortality



References:

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PCVAD-002

A duplex real-time PCR assay for the differential detection of porcine circovirus 2(PCV2) and PCV3

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Introduction: Recently, a novel circovirus, designated as PCV3, was identified in pigs with PDNS, reproductive failure, and cardiac and multi-systemic inflammation in the US, China and South Korea [1, 2, 3, 4]. Based on prevalence studies, PCV3 was suggested to commonly circulate within pig populations in the countries. The clinical presentations of PCV3 are similar to those of PCV2 and to coinfection with PCV2 and PCV3 in pig populations. Therefore, a rapid and reliable diagnostic assay is needed for the differential detection of PCV2 and PCV3 in the field. In this study, we developed and evaluated a duplex quantitative real-time PCR (dqPCR) assay using primer sets capable of detecting and typing PCV2 and PCV3 in clinical samples.

Materials and Methods: For development of the dqPCR, primers and probe for PCV2 were used as described in a previous report [5], with some base modifications to reflect the genetic variation of the target gene sequences among the different genotypes of PCV2 strains in Korea. Primers and probe for PCV3 were newly designed using Primer Express software (version 3.0) (Applied Biosystems, USA) based on a total of 32 PCV3 genome sequences available in National Center for Biotechnology Information. To test the specificity of the dqPCR assay, the assay was performed with total nucleic acids extracted from seven viral samples (PCV2, PCV3 and other swine pathogens.) and two PCV3 non-infected porcine-origin cell cultures (ST cell and PK-15 cell) as negative controls. Limit of detection (LOD) of the assay was determined in triplicate using serial dilutions (from 10^6 to 10^0 copies/μ L) of each plasmid DNA containing the entire PCV2 or PCV3 capsid gene. In addition, evaluation of the dqPCR on field samples was performed on 46 tissue samples collected from PCV3-affected domestic pig farms in 2017.

Results: A dqPCR assay was successfully developed for the rapid and differential detection of PCV2 and were amplified using specific primers and probe sets, while no other porcine pathogen genes were detected. LOD of the assay was below 50 copies of the target genes of PCV2 and PCV3 and was comparable to that of previously described methods. The assay showed high repeatability and reproducibility, with coefficients of intra-assay and inter-assay variation of less than 4.0%. Clinical evaluation using tissue samples from a domestic pig farm showed that PCV2 and PCV3 co-circulated at the farm. Moreover, singular infection rates of PCV2 or PCV3 were 21.7% (10/46) or 6.5% (3/46), respectively, while the co-infection rate of PCV3 with PCV2 was 28.3% (13/46). PCV3 DNA was detected by the dqPCR in respiratory diseased piglets and aborted fetal tissue samples, suggesting that PCV3 infection is associated with porcine respiratory disease and reproductive failure in the pig farm.

Table 1. Comparison of diagnostic results for clinical samples by dqPCR and previously reported qPCR assays PCV2 and PCV3.

Type of PCV	Results of different methodsa (No. of positive/tested)				
	dqPCR (PCV2/3)	qPCR (PCV2)	qPCR (PCV3)	Olvera's qPCR (PCV2)	Palinski's qPCR (PCV3)
PCV2	10/46	10/46	-	10/46	-
PCV3	3/46	-	3/46	-	3/46
PCV2/3	13/46	13/46	13/46	13/46	13/46
Negative	20/46	23/46	30/46	23/46	30/46
Detection rate (%)	56.5	50.0	34.8	50.0	34.8

Conclusions: The dqPCR method is a rapid and reliable differential diagnostic tool for the monitoring and surveillance of PCV2 and PCV3 in the field.

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[1] Palinski et al., 2017. J. Virol. 91, 1-13.
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[4] Kwon et al., 2017. Vet. Microbiol. 207, 178-1.
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PCVAD-003

Comparative field study of porcilis® pcv m hyo versus other pcv2 and m hyo vaccines in two commercial farms in south korea

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Introduction: The aim was to compare and observe the field efficacy of Porcilis® PCV M Hyo against other frequently used combination or mixed PCV2 and Mycoplasma hyopneumoniae (M.hyo) vaccines in field conditions in South Korea.

Materials and Methods: This study was performed in two commercial farms in South Korea with 500 (Farm 1) and 700 (Farm 2) sows.

14 day old pigs were randomly allocated within litter to one of 3 treatments - Competitor 1) 50,100 piglets vaccinated at 3 weeks of age, Competitor 2) 50,100 piglets vaccinated at 3 weeks of age, Porcilis® PCV M Hyo) 50,100 piglets vaccinated at 3 weeks of age.

Efficacy parameters observed were lung lesion score at slaughter (LLS), rt-PCR for PCV2 and average daily weight gain from weaning to finishing(ADWG).

Safety parameters observed were local and systemic reactions up to 14 days post vaccination. Results were analyzed using Microsoft Excel Data Analysis Toolkit.

Results: Farm 1 PCV2 Area Under the Curve (AUC) was numerically different (12.15 vs 14.67 vs 1.16, P = 0.08). Farm 2 PCV2 AUC was numerically different (6.8 vs 8.31 vs 3.01, P = 0.54).

Farm 1 LLS was statistically different (AVG LLS 5.4 vs. 6.1 vs 2.2; P = 0.02).

Farm 2 LLS was numerically different (5.2 vs. 6.9 vs 3.7; P = 0.10).

ADWG from weaning to finish was numerically different in Farm 1 (694g vs 701g vs 703g, P = 0.57) and statistically different in Farm 2 (483g vs 504g vs 542g, P = 0.02) No local or systemic reactions were observed in the 14 day post-vaccination period, across all 3 different vaccines used in the study.

Table 1. Comparative data of Porcilis® PCV M Hyo versus other PCV2 and M Hyo vaccines by PCV2 Area Under Curve(AUC), Average lung lesion score(LLS) and average daily weight gain from weaning to finishing(ADWG)

		Comp.1	Comp.2	Porcilis® PCV M Hyo	P value
PCV2 AUC	Farm1	12.15	14.67	1.16	0.08
	Farm2	6.80	8.31	3.01	0.54
AVG LLS	Farm1	5.4	6.1	2.2	0.02
	Farm2	5.2	6.9	3.7	0.10
ADWG	Farm1	694g	701g	703g	0.57
	Farm2	483g	504g	542g	0.02

Conclusions: The post vaccination observations support the fact that all 3 vaccines observed had a high safety profile when used in commercial swine. In two commercial farms with PCV2 and M.hyo field challenge, pigs vaccinated with Porcilis® PCV M Hyo showed both numerical and significant reduction in 1) lung lesions associated with M.hyo, 2) average PCV2 viremia titres compared to pigs vaccinated with competitor vaccines. ADWG was also numerically or significantly improved by the use of Porcilis® PCV M Hyo.

PCVAD-004

Comparison of vaccine efficacy between PCV2 subunit vaccines

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Introduction

PCV2 is the cause of Porcine Circovirus Associated Disease (PCVD) one of the most important diseases for the swine industry, Vaccination with good management practices are the main tools to control the impact of PCVD on the farm. In China, most of the PCV2 vaccines in the market are whole virus killed vaccine, however since 2016 there are various PCV2 subunit vaccines registered in China. The objective of this study is to compare vaccine efficacy between Ingelvac CircoFLEX the first launched baculovirus expressed subunit vaccine in China with a local subunit vaccine

Material and Methods

This field observation was conducted in a 600 sow farm in Henan, China. The sow herd was stable for PRRSv. Piglets were weaned at 29-31 days of age. Age at slaughter is around 180-190 days old. PCV2 and Mycoplasma were vaccinated at 14-18 days old and the average wean-finish mortality was around 4-8 %.

In total 3,910 pigs of 12 weekly consecutive batches were evaluated. Farm was switching vaccine every 1-2 batches in order to reduce the environmental factor which might impact the study. 6 batches of 1,952 pigs, batch no. 1, 2, 4, 5, 8 and 10) were vaccinated with Ingelvac CircoFLEX while the other 6 batches of 1,958 pigs, batch no.3, 6, 7, 9, 11 and 12 were vaccinated with subunit vaccine A

Wean-finish performance such as average daily weight gain (ADG) and mortality rate are summarized in Table 1, Mann-Whitney was used for statistical analysis for ADG and Chi square test for mortality rate.

Results :

Average weaning weight was lower in CircoFLEX vaccinated group (group 1) compare with local subunit vaccine group (group 2) 7.16 vs 7.62 kg respectively, no statistic different was observed. Whereas selling weight in group 1 was 5.81 kg higher compare with group 2

The overall ADG in group 1, which was vaccinated with Ingelvac CircoFLEX was 53.4 g/day higher than in group 2 which vaccinated with local subunit vaccine (707.2 and 653.8 respectively, which is significant different $p<0.01$). Average wean-finish mortality and culling between both groups was also significant different (3.79 vs 7.76%, $p<0.01$)

Table 1 Summary parameter

Parameter	CircoFLEX	Vaccine A	Diff
Piglet in (n)	1,952	1,958	n/a
Average weaning weight (kg)	7.16	7.62	- 0.5
Wean age (day)	31	32	-1
Pig out (n)	1,878	1,806	n/a
% Loss	3.79%	7.76%	-3.97%
Average selling weight (kg)	117.33	111.52	+5.81
Weight gain (kg)	110.18	103.90	+6.28
Day on feed (day)	155.79	158.92	-3.13
ADG (g/day)	707.2	653.8	+53.4

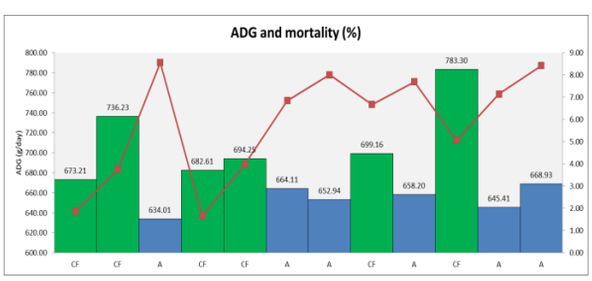

Figure 1: Chart of ADG (bar) and mortality (line)

In figure1.Batches which vaccinated with Ingelvac CircoFLEX had higher ADG (green bar) compare to batches vaccinated with local subunit vaccine (blue bar). Mortality trend line also lower in batches which vaccinated with Ingelvac CircoFLEX compared to local subunit

Conclusions :

In this field observation, pigs vaccinated with Ingelvac CircoFLEX had higher ADG and lower mortality compare to the animals which received the other subunit PCV2 vaccine. The results obtained reassure customers that the efficacy between PCV2 vaccines is different. Monitoring farm production performance is the best way to evaluate which vaccine can provide highest benefit to the farm.

PCVAD-005

Control of PCV2d infection with Circovac® vaccination of piglets in the experimental challenge trial

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[1]Ceva Santé Animale

Introduction: Circovac® (Ceva) is an inactivated vaccine, containing whole virus antigen of PCV2a genotype. The protection against virulent PCV2a as well as against experimental infections with PCV2b strains was already confirmed and published[1]. The obvious interest was to see if this vaccine provides efficient cross protection against the recent clinically most relevant PCV2d genotype and if the simultaneous administration with an inactivated M. hyopneumoniae vaccine (Hyogen®, Ceva) interferes with its efficacy.

Materials and methods: Circovac® 0,5ml used simultaneously with Hyogen® 2ml (Circovac+Hyogen), or a PCV2+Mhyo flex vaccine 2ml (Vaccine A) were administered to piglets at 3WOA. Control pigs (Positive control) were not vaccinated. All pigs were challenged at 12 WOA with a virulent PCV2d strain. Pigs were sampled weekly for 4 weeks post-challenge. VN test to measure antibody levels and qPCR to measure virus loads were used for efficacy evaluation of Circovac®.

Results: Viremia was reduced significantly in the vaccinated pigs compared to the controls. At the end of the observation period the Circovac® and Hyogen® vaccinated pigs had numerically lower amount of challenge virus in their serum than the ones receiving Vaccine A.

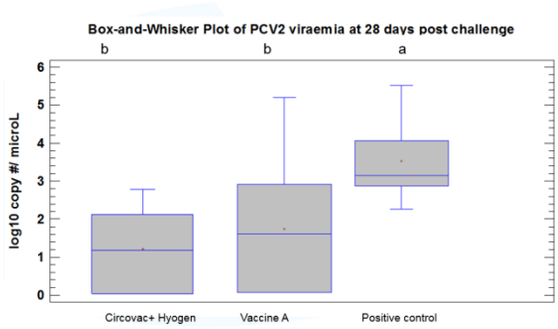

Fig 1.Virus amount in the serum 28 days post challenge

The amount of the virus in the lymphoid tissue of vaccinated groups was significantly lower than lower compared to the control at the end of the observation period. The viral load was numerically lower in the Circovac® & Hyogen® vaccinated pigs compared to Vaccine A.

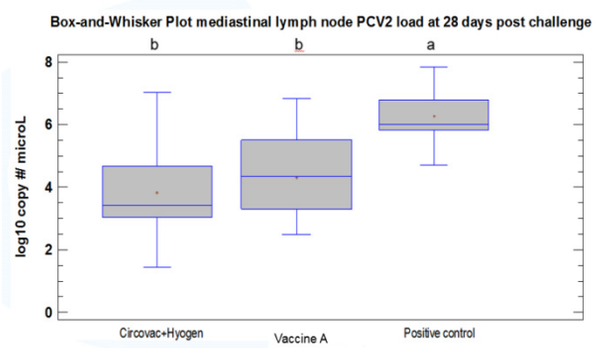

Fig 2. Virus load in the lymphoid tissue 28 days post challenge

Conclusion: PCVDs remain a common problem in many swine farms. Strains of different genotypes of PCV2 are circulating in the herds. The PCV2d seems to be dominant particularly in farms with clinical forms of PCVD. Vaccination of piglets with Circovac® & Hyogen® proved to be efficient in controlling the consequence of PCV2d infection. This efficiency as measured by some indicators such as reduction of viremia or of virus amount in the lymph nodes was numerically better, than the one obtained in Vaccine A (PCV2+Mhyo flex) vaccinated pigs. These results demonstrated that vaccination of piglets with Circovac® can be highly efficient tool in the control of PCVD.

Reference:

[1] Seo HW et al, Vaccine. 2014 May 1;32(21):2480-6

PCVAD-006

Development and Application of a Taqman-based Real-time Fluorescent PCR For Specific Detection of Porcine Circovirus 3

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Introduction: Porcine circovirus type (PCV) belongs to the Circoviridae family. PCV can be divided into PCV1, PCV2 and PCV3 [1]. Since PCV2 and PCV3 infections can cause similar clinical symptoms, it is urgent to develop a method to distinguish PCV3 from other viral infections. To detect the PCV3 sensitively, rapidly and specifically, we developed a TaqMan-based real-time fluorescent PCR (TaqMan qPCR) assay. The TaqMan qPCR assay is expected to be a more sensitive and specific compared to the conventional PCR. The method was used to evaluate the prevalence of PCV3 in Hubei province.

Materials and Methods: The primers (Forward: 5'-TATTCATTAGGAGGCCCACA-3', Reverse: 5'-GCAGTTTCCCATTCGTTTAG-3') and TaqMan probe (5'-FAM-ACTCCACCATGAACGTCATTTCC-TAMRA-3') for qPCR were designed on the basis of conservative ORF2 gene sequences of PCV3. Viral genome DNA were extracted using commercial kits according to the manufacture's protocol. Standard plasmids containing PCV3 ORF2 were used as templates for optimization and sensitivity detection of TaqMan qPCR assays. Tenfold serial dilution of plasmids were prepared at concentrations of 10^2 to 10^9 copies/μL and used to obtain standard curves. The reaction was carried out in a final volume of 10 μL containing 5 μL of 2 * iTaq™ Universal Probes Supermix (BioRad), 0.5 μL of each Forward and Reverse primers (10 μM), 0.5 μL of probe (10 μM), 2 μL DNA template and 1.5 μ L RNase-free water. The reaction went as follows: pre-denaturing at 50℃ for 2min and 95℃ for 10min, followed by 40 cycles of denaturation at 95℃ for 15s, annealing at 60℃ for 1min, and collecting the fluorescence signals at 60℃.

Results: The standard curves and linear regression equation of PCV3 were generated with a slope of -3.258 and a correlation coefficient of more than 0.99 (R2 > 0.99). The detection limit was 1:10^8 diluted plasmids containing 129 copies with lower than 28 cycles. The nucleotides of PCV2b, PCV2d, PEDV, PRRSV, PoRV, Hps, App, Bb and Pm could not be detected by TaqMan qPCR. As shown in Table 1 the standard deviation (SD) and the coefficient of variation (CV) ranged from 0.12 to 1.35 and from 0.69% to 1.47%, respectively. 112 clinical samples including lungs, spleens, lymph nodes and serums were collected from central provinces of China and detected by the TaqMan qPCR assay. Among 112 clinical samples, 10 samples were tested positive. Co-infection with PCV2 was demonstrated in all PCV3 positive samples. The results show that the method is successful and can be used for clinical testing.

Table 1. The intra- and inter-assay CVs for Ct values result of TaqMan qPCR

	Copies/μL	Ct (x ± SD)	CV (%)
Intra-assay	1.29×10^2	30.97 ± 0.21	0.69
	1.29×10^5	21.04 ± 0.23	1.08
	1.29×10^8	10.33 ± 0.12	1.16
Inter-assay	1.29×10^2	30.30 ± 0.35	1.16
	1.29×10^5	20.41 ± 0.30	1.47
	1.29×10^8	10.62 ± 0.16	1.47

Conclusions: The TaqMan qPCR assay can be applied for the rapid, specific, sensititive and reliable diagnosis of PCV3.

Acknowledgement: This work was supported by a grant from China Agriculture Research System (Grant/Award Number: No. CARS-35).

References:

[1] Palinski R et al., 2016. J. Virol. 91, 01879-16

PCVAD-007

Effect of intradermal inoculation of PCV2-MH intramuscular vaccine.

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Introduction: Post-weaning Multisystemic Wasting Syndrome(PMWS) is one of the most important diseases worldwide in swine industry. Porcine Circovirus type 2 (PCV2) is considered to be an important infectious agent as a major cause of PMWS, and Mycoplasma Hyopneumoniae (MH) is also a major factor. In order to PMWS control, it should be vaccinated against PCV2 and MH. It is more effective that 2-dose vaccination of PCV2 and MH for daily weight gain, mortality and Lung lesion index than once. Intradermal inoculation is known to induce a sufficient immune response by stimulating immune cells with a small amount of antigen. Therefore, the purpose of this study is to identify the possibility of application of intradermal inoculation of PCV2 and MH intramuscular vaccine in the actual farm conditions and to confirm the feasibility of the cost reduction of the vaccine, which is the largest portion of farm drug costs.

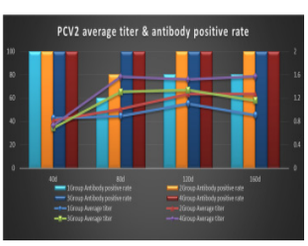
Materials and Methods: This study was conducted from July to November, 2018 in 350 sow farms in Hongseong, Chungnam, Korea. Total 60 piglets which were four piglets selected from each 15 sows were randomly divided into 4 groups of 15 piglets. Vaccination was performed as shown in Table 1 for each group. At the same time as the vaccination was carried out, ear notching was conducted and the weights were measured individually. Blood samples were taken at 40, 80, 120, and 160 days, and the survival rate and body weights were measure at 160 days.

Results: There was no statistically significant difference in body weight within the four groups. but a significant increase in body weight over the other groups (P-value 0.1) as shown in Table 1 for each group. In the PCV2 and MH serum test results, group 3 and 4 of the intradermal inoculation showed higher antibody positive rates and average antibody titers, especially in group 4, than in other groups (Figure 1, 2).

Conclusions: In this study, the best ADG was found in the 4 groups compared with the other groups. However, the results were not statistically significant (P-value 0.1). Blood serum tests showed the highest antibody positive rate and average antibody titer in group 4, This does not mean that the immune status is high. The experiment showed that the same or better effect could be expected on intradermal inoculation twice, while at the same time reducing the capacity of PCV2 and MH vaccine by one-half.

Table 1. Variables, route, dosage, Weight measurement results, ADG and survival rate by groups

	1Group	2Group	3Group	4Group
Variables	PCV2, MH vaccine mixing	PCV2-MH combination vaccine	PCV2-MH combination vaccine	PCV2-MH combination vaccine
Route	IM	IM	ID	ID
Dosage	2ml	2ml	0.5ml*2 (same time)	0.5ml*2 (interval 4weeks)
weight at starting	7.03	7.31	7.05	6.97
Std at start	0.88	0.76	0.71	0.93
weight at end	83.13	83.6	82.75	86.68
Std at end	7.69	8.24	8.2	5.76
ADG	0.56	0.56	0.56	0.59
head of survival/ head of starting	12/15	13/15	11/15	12/15



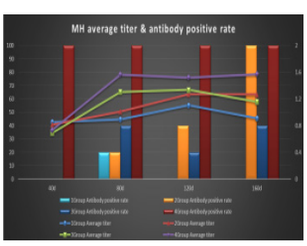


Fig 1, 2 PCV2 and MH serum test results.

References:

[1] D. Kim, et al., Vaccine. 2011 apr 12;29(17):3206-12.
[2] C. Chae, et al., Vet J. 2004 Jul;168(1):41-9

PCVAD-008

Efficacy comparison between two Porcine Circovirus type 2 vaccine under field conditions in Taiwan

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Introduction: Porcine Circovirus type 2 (PCV2) is an economically devastating disease, causing mortalities, decreased ADG and poor FCR[1]. This study compares the efficacy of Ceva's PCV2 vaccine Circovac® with competitor vaccine CircoFLEX® under field conditions in Taiwan, by comparing the serological response and performance parameters.

Materials and Methods: 1183 3-week-old piglets from 3 consecutive batches were included in this study. At 3 weeks of age (WoA), all the pigs were vaccinated with the same *Mycoplasma hyopneumoniae* vaccine, but different PCV2 vaccines, as shown in Table 1. It is worth noting that Group 3 is the routine practice in this farm.

Table 1. Experiment and control groups design

Group Number	Number of pigs	*Mycoplasma vaccine*	PCV2 Vaccine
Group 1	394	*MycoFLEX*®	CircoFLEX® (on the other side of the neck)
Group 2	381	*MycoFLEX*®	Circovac® (on the other side of the neck)
Group 3	408	*MycoFLEX*®	CircoFLEX® (mixing together)

In Group 1 and Group 2, blood samples were collected at 4, 8, 12, 16 and 20 WoA, and sent to National Pingtung University of Science and Technology for PCV2 antibody ELISA (BioChek) analysis. For each age group, 5 blood samples were collected.

Mortalities and feed intake were recorded both in the nurseries and growing-fattening units. Pigs were weighed as a group at 4 WoA, 12 WoA and before selling to the slaughterhouses at 28 WoA.

Results: Better FCR and a lower mortality are seen for Group 2 pigs, as shown in Table 2.

PCV2 serological response, Group 1 had a greater difference of average ELISA titer between 8 to 12 WoA than Group 2, shown in Fig. 1.

Table 2. Comparisons of mortalities and FCR

Group Number	Nursery pigs		Grower-Finishers	
	Mortality	FCR	Mortality	FCR
Group 1	1.27%	1.63	7.20%	2.94
Group 2	0.81%	1.60	3.79%	2.67
Group 3	2.95%	1.68	5.05%	2.83

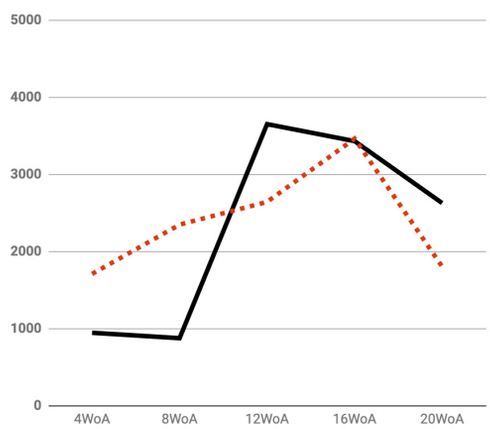

Figure 1. Average ELISA antibody curve: Group 1- solid line; Group 2- dotted line

Conclusions: Circovac® group (group2) showed better performance compared with the other groups. The difference is more pronounced in the growing-finishing period. Epidemiology studies and field observations indicate that PCV2 infection can often happen in the grower-finisher stage, thus finding the efficacious vaccine in combatting late infection is crucial.

Acknowledgment: The author is grateful for the owners and staffs of Chang-Xing farm, who were responsible for the animal phase of this study.

References:

[1] Vidigal et al, 2012. Virus Research 163 : 320-327.

PCVAD-009

Efficacy of the Porcilis® PCV M Hyo vaccine in pigs against heterologous challenge with a PCV2d strain

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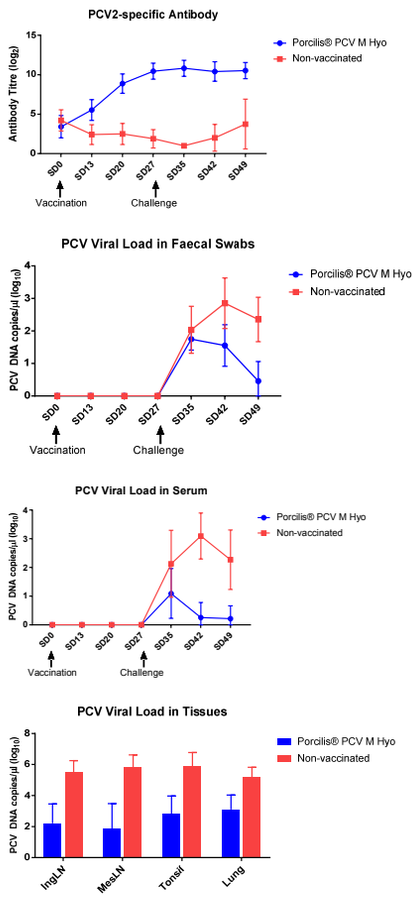
Introduction: Porcine circovirus type 2 (PCV2) is an economically important pathogen of pigs associated with a range of clinical manifestations, including post-weaning multi-systemic wasting syndrome. It has an extensive distribution and most pigs are infected during their lifetime. Effective vaccination is key to the management of PCV2-associated disease. There are currently five known genotypes of PCV2 (a - e) and in recent years PCV2d has been gaining prevalence in North America, South America, Europe and Asia [1]. Porcilis® PCV M Hyo is an effective ready-to-use, single-injection combination vaccine that protects against PCV2 and *Mycoplasma hyopneumoniae*. The PCV2 component of the vaccine is non-infectious capsid protein of PCV2a. In this study, the capacity of Porcilis® PCV M Hyo to protect against challenge with a PCV2d strain was evaluated.

Materials and Methods: Piglets (3 weeks-of-age, with low to moderate PCV2 antibody titres) were vaccinated intramuscularly with Porcilis® PCV M Hyo (n=10) and subsequently challenged intranasally with PCV2d (isolate DE-222-13), 28 days post vaccination. A non-vaccinated control group (n=10) was also challenged. The study concluded 21 days post challenge. Serum samples were tested for PCV2 antibody levels by ELISA. Viral loads in serum (viraemia), faecal swabs (faecal shedding) and tissues (inguinal and mesenteric lymph nodes, lung, and tonsil) were determined by qPCR of PCV2 genomic DNA.

Results: At the time of challenge, the average antibody titre in the vaccine group was significantly higher than in the control group. Following challenge, the viral load in serum, faecal swabs and all tissues tested was significantly reduced in the vaccinated group compared to the control group.



Conclusions: Vaccination with the PCV2a-based Porcilis® PCV M Hyo vaccine affords protection against heterologous challenge with PCV2d, reducing viraemia, faecal shedding and viral load in tissues in PCV2d challenged piglets.

References:

[1] Xiao CT, Halbur PG, Opriessnig T. *J Gen Virol* 2015; 96(Pt 7): 1830-41.

PCVAD-010

Evaluation of a porcine circovirus type 2a (PCV2a) vaccine efficacy against experimental PCV2a, PCV2b, and PCV2d challenge

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Introduction: Porcine circovirus type 2 (PCV2) is a small single-stranded circular DNA virus that has been linked to a numbers of diseases. PCV2 is currently classified in five different genotypes designated PCV2a, PCV2b, PCV2c, PCV2d, and PCV2e [1]. PCV2d is also currently the most prevalent genotype in the US and Korea. Cross-protection against PCV2d infection by currently commercially available PCV2a-based vaccines is very important because PCV2d is the most prevalent genotype causing PCVAD resulting in enormous economic losses in Asian pork industry. The objective of this study was to evaluate and compare the protection efficacy of a PCV2a-based vaccine against experimental challenge of PCV2a, PCV2b, and PCV2d strains isolated in Korea.

Materials and Methods: At -28 days post challenge (dpc, 3 weeks of age), pigs in the Vac/Ch2a, Vac/Ch2b, and Vac/Ch2d groups were injected intramuscularly with a 1.0 mL dose of CircoFLEX (Boehringer Ingelheim Vetmedica, St. Joseph, Missouri, USA). Pigs in the UnVac/Ch2a, UnVac/Ch2b, UnVac/Ch2d, and UnVac/UnCh groups were similarly administered 1.0 mL of PBS. At 0 dpc (7 weeks of age), pigs in the Vac/Ch2a and UnVac/Ch2a groups were inoculated intranasally with 2 mL (1 mL/nostril) of tissue culture supernatant containing 10^5 $TCID_{50}$/mL of PCV2a (SNUVR000032, GenBank no. KF871067, 5th passage in PCV-free PK-15 cell lines). Pigs in the Vac/Ch2b and UnVac/Ch2b groups were inoculated intranasally with 2 mL (1 mL/nostril) of tissue culture supernatant containing 10^5 $TCID_{50}$/mL of PCV2b (SNUVR000463, GenBank no. KF871068, 5th passage in PCV-free PK-15 cell lines). Pigs in the Vac/Ch2d and UnVac/Ch2d groups were inoculated intranasally with 2 mL (1 mL/nostril) of tissue culture supernatant containing 10^5 $TCID_{50}$/mL of PCV2d (SNUVR140004, GenBank no. KJ437 506, 5th passage in PCV-free PK-15 cell lines).

Results: Real-time PCR was used to quantify genomic copies in blood samples collected from each group. At 14, 21, and 28 dpc, pigs from the Vac/Ch2a, Vac/Ch2b, and Vac/Ch2d groups had significantly ($P < 0.05$) less genomic copies of PCV2a, PCV2b and PCV2d DNA respectively compared to the corresponding control groups. In addition, Vac/Ch2a, Vac/Ch2b, and Vac/Ch2d groups had similar number of genomic copies of PCV2a, PCV2b, and PCV2d DNA respectively. There was also no significant difference on genomic copies of PCV2a, PCV2b, and PCV2d DNA respectively between UnVac/Ch2a, UnVac/Ch2b, and UnVac/Ch2d groups. Pigs in the Vac/Ch2a group were negative for PCV2b and PCV2d DNA, pigs in the Vac/Ch2b group were negative for PCV2a and PCV2d DNA and pigs in the Vac/Ch2d group were negative for PCV2a and PCV2b DNA throughout the experiment. No PCV2a, PCV2b, or PCV2d genomes were detected in the sera of pigs from the UnVac/UnCh group throughout the experiment. At the time of vaccination, serum samples from pigs in all seven groups were negative for PCV2 against neutralizing antibody (NA). Pigs from the Vac/Ch2a group had significantly higher ($P < 0.05$) NA titers against PCV2a compared to the UnVac/Ch2a group between 0 and 28 dpc. Similarly, pigs from the Vac/Ch2b and Vac/Ch2d groups had significantly higher ($P < 0.05$) NA titers against PCV2b and PCV2d respectively, compared to their respective control group between 0 and 28 dpc. No NA titers against either PCV2a, PCV2b, or PCV2d were detected in serum samples collected from pigs in the UnVac/UnCh control group throughout the study

Conclusions: The results presented here demonstrate that PCV2a-based PCV2 vaccine is efficacious against experimental challenge with Korean PCV2a, PCV2b, and PCV2d strains. The same PCV2a-based PCV2 vaccine has been previously shown to provide good protection against experimental challenge with a PCV2b and a PCV2d strain isolated in the US.

Acknowledgement: This study was supported by contract research funds (Grant no. 550-20180107).

References:

[1] Gou et al., 2012. PLos One 7, e41463.

PCVAD-011

Evaluation of cross-protection with porcine circovirus type 2 recently circulated in the Republic of Korea

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Introduction: Porcine circovirus type 2 (PCV2) is associated with a variety of disease manifestations referred to as porcine circovirus-associated diseases (PCVAD). PCV2 is a small, non-enveloped virus with a circular, single-stranded DNA genome and comprised of two major proteins, ORF1 and ORF2. The ORF2, namely capsid protein on the complementary strand, binds to the host receptor and induces immune responses. Recently, rapid change of PCV2 genotypes has been proceeding in the world. Nowadays, PCV2d is a major prevalent genotype. This genotype was isolated from cases of vaccine failure under the current vaccination [1]. Current PCV2a-based vaccine program might not completely protect PCV2d genotypes. Therefore, it needs to evaluate cross-protective ability of different genotypes and identify the cause of viral escape.

Materials and Methods: Three different genotypes of PCV2 were used in this study; PCV2a (DS-PCV2a), PCV2b (QIA418) and PCV2d (QIA169 and QIA244). Anti-sera of specific pathogen free (SPF) pigs vaccinated with Ingelvac CircoFLEX (Boehringer Ingelheim Vetmedica) and guinea-pigs immunized by DS-PCV2a, QIA418, QIA169, QIA244 and Ingelvac CircoFLEX, respectively, were used for viral neutralization. A serum neutralization (SN) assay was adapted from the method by Meerts et al., [2]. The serum neutralizing activity was expressed as the percentage of viral neutralization (% VN) estimated the number of infected cells per $1x10^4$ cells by counting nuclei.

Results: To evaluate serum neutralization between PCV2 genotypes, anti-sera of pigs vaccinated by Ingelvac CircoFLEX as PCV2a-based vaccine were used for viral neutralization. Serial diluents of 12-week pig serum of post-vaccination were neutralized with 200 $TCID_{50}$ of DS-PCV2a as a homologous genotype. The antigen was completely neutralized by 64-fold dilution of the anti-sera (95.4%). To evaluate cross-protection ability of the PCV2a-based vaccine, PCV2b (QIA418) and two PCV2d isolates (QIA169 and QIA244) were neutralized by the SN titer (1:64) of 12-week serum. % VN of QIA418, QIA169 and QIA244 was 97.8%, 85.1% and 29.3%, respectively. To compare the cross-protective ability between different genotypes of PCV2, guinea-pigs were immunized with DS-PCV2a, QIA418, QIA169, QIA244 and Ingelvac CircoFLEX, respectively. Anti-sera of guinea-pigs neutralized with each homologous genotype of PCV2. SN titer was decided by VNT90 (up to 90% neutralization rate). Based on SN titer decided with homologous PCV2, cross-protection among different genotypes of PCV2 was estimated in 2-, 4-, and 8-fold titer. QIA244 isolate represented relatively low neutralization rate against anti-sera of heterologous PCV2 virus (Ingelvac CircoFLEX, DS-PCV2a, QIA418 and QIA169). Interestingly, anti-sera of QIA244 effectively neutralized QIA244 as well as heterologous PCV2 used in this study.

Conclusions: QIA244 as one of PCV2d isolates was not protected by neutralizing antibody of PCV2a vaccine in pigs as well as PCV2a, 2b and even 2d in guinea-pigs. However, all PCV2 used in this study was effectively neutralized by anti-sera of QIA244.

Acknowledgement: This work was supported by a grant (grant No. QIA M-1543083-2019-21-02) from Animal and Plant Quarantine Agency under the Ministry of Agriculture, Food, and Rural Affairs, Republic of Korea.

References:

[1] Seo HW et al., 2014. Can. J. Vet. Res. 78, 233-236.
[2] Meerts P et al., 2005. J. Interferon Cytokine Res. 25, 684-693.

PCVAD-012

First Report in Malaysia of Reproductive Failure associated with PCV-2 and Improved Production Results after Sow Herd Mass Vaccination

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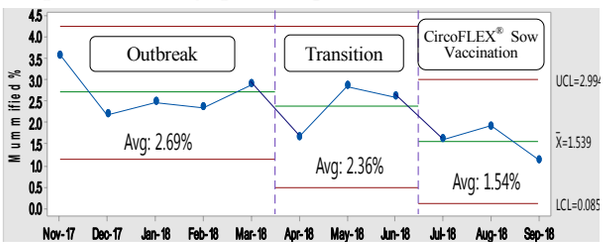
Introduction: PCV2 infection has traditionally been associated with weaners since the initial recognition of post-weaning multi-systemic wasting syndrome (PMWS) in 1991. However, there have been reports of reproductive disease caused by PCV2 infection in breeding herds such as early termination of pregnancy, increased numbers of mummified fetus, still born and weak-born pigs. Reproductive performance has been shown to improve after use of mass vaccination of the breeding herd against PCV2. This study reports a case of PCV2 infection in sow herd for the first time in Malaysia, and the impact of sow herd PCV2 mass vaccination on the reproductive performance.

Materials and Methods: The farm of this case report is a single site, farrow to finish farm with 800 sows in Malaysia; where it experienced an increase in the percentage of mummified fetuses (Mean: 2.69%) from sows of multiple parities from November 2017 to March 2018. Breeder herd in the farm was vaccinated against CSF, PRRS, Aujeszky and FMD; while the porkers were vaccinated for PCV2 & CSF. The farm was positive for PRRS (stable), negative but vaccinated for Aujeszky's disease. In March 2018 samples of lungs, hearts and lymphoid organs of the mummified fetuses were tested positive for PCV2 antigen by Real-Time PCR. Following these findings, the sows were mass vaccinated with Ingelvac CircoFLEX® in April and May 2018 and, mass revaccinated every 6 months. Reproductive parameters were evaluated for the period before (November 2017-March 2018) and the period after the implementation of sow vaccination (April 2018-September 2018). The statistical analysis was generated using Minitab software version 17.

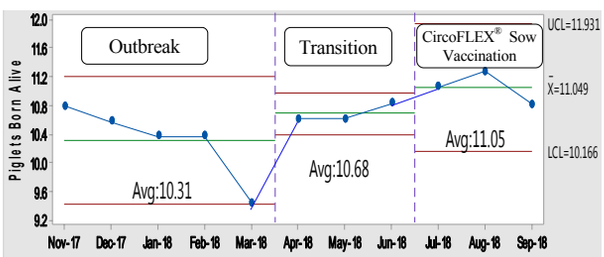
Results: As compared to before sow mass vaccination with Ingelvac CircoFLEX®, there has been a 57% reduction of percentage of mummified fetus from 2.69% during outbreak to 1.54% (Graph 1). As for the piglets born alive per litter, it has also been increased from 10.31 to 11.06 piglets born per litter after application of Ingelvac CircoFLEX® mass vaccination in sows (Graph 2).

The results are summarized in graph 1 & 2.

Graph 1: Monthly percentage of mummified fetus



Graph 2: Monthly piglets born alive per litter



Conclusions: PCV2 was first suspected to be the cause of reproductive failure as PCV2 antigen was detected by Real-Time PCR from the organs of mummified fetus. The diagnosis was then further supported by the improvement of reproductive performances after mass vaccination with Ingelvac CircoFLEX® in the sows. This study reported for the first time in Malaysia, the presence of PCV2 in mummified fetus with improvement in reproductive performances after sow vaccination with Ingelvac CircoFLEX®. In addition, mass vaccination of the sow herd was proven to be safe with this vaccine and it can be a useful tool to reduce/prevent reproductive failure associated with PCV2 infection.

Acknowledgement: We would like to acknowledge the farm owner, Mr Lee for the support and providing the farm performance data for this submission.

References:

[1] Madson, D. M., & Opriessnig, T. (2011).. Animal Health Research Reviews, 12(01), 47-65.

[2] E. D., Chevez, J. C., & Angulo, J. R. (2011). International Symposium on Emerging and Re-emerging Pig Diseases, 1

PCVAD-013

Genetic variations in open reading frame 2 gene of porcine circovirus type 2 isolated in Korea during 2016-2017

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Introduction: Porcine circovirus type 2 (PCV2) is a small, nonenveloped, circular single-stranded DNA virus belonging to the genus *Circovirus* of the family *Circoviridae* [1]. Open reading frame 2 (ORF2) of PCV2 encodes a major immunogenic capsid protein that induces protective immunity against PCV2 infection and plays an important role in binding to a receptor, heparan sulfate [2]. The ORF2 gene of PCV2a has a high level of nucleotide similarity with that of PCV2b, but is significantly different from that of PCV2d, which spread rapidly worldwide [3]. To allow molecular diagnosis and vaccine development, further studies are required to understand the genetic variation of the ORF2 gene associated with production of neutralizing antibodies against PCV2 genotypes. In this study, we sequenced on either full-length or ORF2 gene of PCV2 isolates in Korea from 2016 to 2017, and investigated the heterogeneity in the ORF2 gene of the PCV2 isolates.

Materials and Methods: A total of 244 blood and saliva samples were randomly selected from 23 pig farms in Korea from 2016 to 2017. For phylogenetic analysis, the ORF2 genes of Korean PCV2 isolates were compared with 27 reference PCV2 sequences deposited in GenBank. A multiple sequence alignment was generated using the ClustalW program. Phylogenetic tree was constructed by the neighbor-joining method and bootstrap analysis with 1,000 replicates using MEGA 6.0 software.

Results: The full-length genomes (n = 7) or ORF2 genes (n = 10) of PCV2-positive samples from 8 pig farms were successfully sequenced. The ORF2 nucleotide sequences from the PCV2 isolates consisted of 702 or 705 bp. The ORF2 genes of the PCV2d isolates were three nucleotides (705 bp) longer than that of the PCV2a and PCV2b isolates. The degree of identity of ORF2 from the 17 PCV2 isolates ranged from 88.3% to 100% (86.3% to 100%) at the nucleotide (deduced amino acid) level. Among the 17 isolates, 2 (11.8%), 2 (11.8%), and 13 (76.4%) were determined as PCV2a, PCV2b, and PCV2d, respectively, based upon the DNA sequencing of ORF2 (Fig. 1).

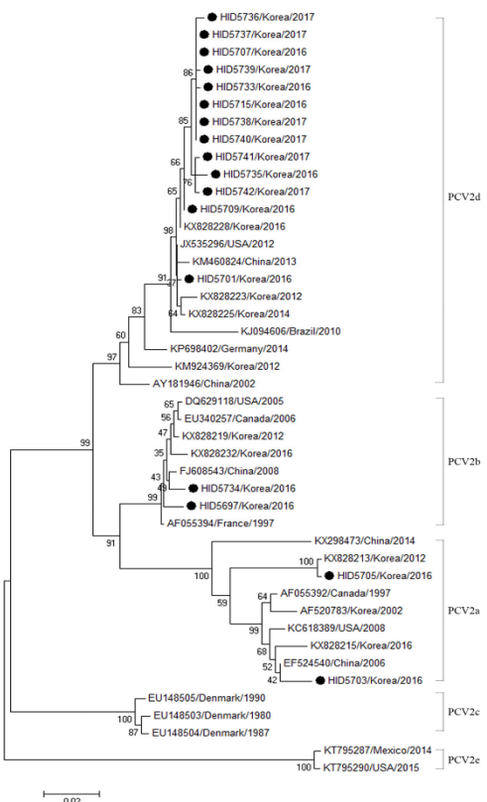

Figure 1. Phylogenetic analysis of the Korean PCV2 isolates based on the nucleotide sequences of the ORF2 gene.

Conclusions: This study revealed that major PCV2 genotypes (PCV2a, PCV2b, and PCV2d) co-circulate and that PCV2d is currently the predominant genotype in the Korean pig population.

References:

[1] Allan G et al., 2012. Virus Res. 164, 4-9.
[2] Misinzo G et al., 2006. J. Virol. 80, 3487-3494.
[3] Olvera A et al., 2007. Virology 357: 175-185.

PCVAD-014

Investigation of PCV3 situation at farrow-to-finish farms in Japan

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Introduction

Since PCV3 (Porcine Circovirus 3) was first identified in 2016, the same cases have been reported worldwide and it is suggested that it also causes PCV2-like diseases such as PDNS (Porcine dermatitis and nephropathy syndrome). In Japan, the occurrence showing PCVAD type symptoms with identified PCV3 have been also reported. In this survey, the situation of Japanese field PCV3 cases have been investigated.

Materials and methods

1. Pooled 594 serum samples collected based on the respective parity and age groups from 23 farms in 2018 were checked by qPCR for PCV3.
2. Blood of sows and pigs from 30 days to 150 days old in 8 farms were monthly collected from Apr. 2017 and Oct., 2018. Totally, 74 pooled samples from each farm were checked for PCV3.
3. On 3 PCV3 positive farms among 8 above, totally 67 blood samples were monthly collected from 30 until 150 days old of 3-6 pigs/herd and were checked for individual PCV3 situation.

Results

1. 21 farms out of 23 were confirmed PCV3 positive by means of PCR (91.3%). The positive rate was highest at 90 days (60.9%), and both gilts and sows were the second (47.8%). The lowest was at 30 days (8.7%)(Fig 1).
2. In 3 sow positive farms, no PCV3 were detected from their piglets. In the farms with PCV3 positive piglets, it tended to continuously show PCV3 positive. The situations were, however, varied from herd to herd (Table 1).
3. Pigs in 3 farms, only one animal of each was positive but other pigs were negative throughout the investigation. The positive individuals were positive continuously had PCV3 until 150 days of age (Table 2).

Discussion and conclusion

This survey demonstrated that PCV3 was widely spread in Japan. Since 30 days old pigs showed low positive rate while 90 days old and sows were high, the maternal antibody might have influenced. Difference in PCV3 situations between pig groups and between individuals were obvious; although it turned out that the herds were highly positive, the individual morbidity was low. Furthermore, the survey on individual animals demonstrated that the viremia lasted more than 120 days. All the pigs in this survey had no clinical symptoms, therefore interaction with clinical diseases was unknown. PCV3 situation and its involvement in diseases such as PDNS should be continuously monitored and explored.

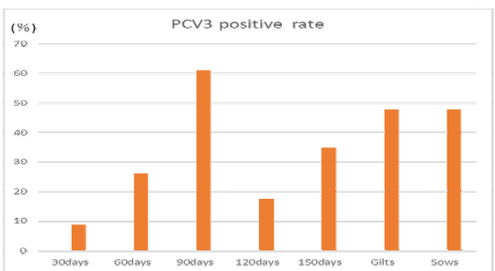

Fig 1 PCV3 detection in the serum of pigs with respective age group

Table 1 PCV3 detection in respective pig herds

Farm A	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017		−	−	−		
October 2018		−	−	−		
Farm B	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017	+	−	−			
October 2018	−	+	+			
Farm C	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017		−	−	−	−	−
October 2018	−	+	+	+	+	+
Farm D	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017		−	−	−	−	−
October 2018	+	−	−	−	−	
Farm E	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017	−	−	−	−	−	−
October 2018		−	−	−	−	
Farm F	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017		−	+	+	+	+
October 2018	−	−	−	−	+	
Farm G	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017	−	−	−	−	−	−
October 2018	−	−	−	−	−	−
Farm H	sow	30-day old	60-day old	90-day old	120-day old	150-day old
April 2017		−	+	+	+	
October 2018	+	−	−	−	−	

Table 2 PCV3 detection in individual animals

	PigNo.	30-day old	60-day old	90-day old	120-day old	150-day old
HerdA	536	+	+	+	+	+
	537	−	−	−	−	−
	538	−	−	−	−	−
	539	−	−	−	−	−
	540	−	−	−	−	−
	541	−	−	−	−	−
HerdB	31	−	−	−	−	−
	32	+	+	+	+	+
	33	−	−	−	−	−
HerdC	46	−	−	−	−	−
	47	−	−	−	−	−
	48	−	−	−	−	−
	49	+	+	+	+	+
	50	−	−	−	−	−

PCVAD-015

Isolation and genetic diversity of porcine parvovirus from aborted pig fetus

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Introduction: Porcine parvovirus (PPV) is one of the important pathogens to porcine. PPV causes not only reproductive failure in susceptible sows, but also skin lesions, diarrhea and non-suppurative myocarditis in piglets. Moreover, co-infection of PPV and porcine circovirus type 2 (PCV2) leads to the post-weaning multisystemic wasting syndrome (PMWS). However, there is limited information regarding distribution and phylogenetic characterization of PPV in the Republic of Korea. Here, we provide the isolation and the phylogenetic characteristics of PPV from aborted pig fetus.

Materials and Methods: Porcine samples (lung tissues) were obtained from aborted pig fetus in 2019. The PPV positive samples were lysed with medium and centrifuged at 3,000 rpm for 10 min to obtain the supernatant. All supernatants were aliquoted and stored at -80℃ until further genetic analysis. The virus was isolated successfully in the ST cell line. The cytopathic effects on the cells were observed and an indirect immune fluorescence assay was performed to identify ST cells infected by PPV. PCR amplifications were conducted with a pair of primers (PPV1F and PPV1R) for VP2 gene detection and genome sequencing. The phylogenetic tree of PPV VP2 genes were constructed using the Mega 7 software with reference sequences in GenBank database, neighbor-joining method and 1,000 replicates of the bootstrap values.

Results: We detected nine PPV strains, and sequenced each VP2 genes. The lengths of all of amplified VP2 genes were 1,740bp. The PPV VP2 sequences in this study were deposited in GenBank under accession numbers KY - KY. The similarities of sequenced VP2 genes to NADL-2 strain (vaccine strain) were 99.1 - 99.4% for nucleotides and 97.8 - 98.6% for deduced amino acids. These strains were divided in three different groups in the VP2 gene-based phylogenetic tree analysis (Fig. 1).

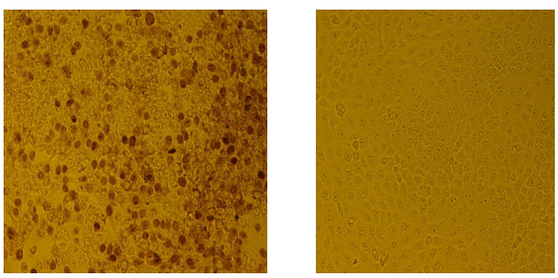

Fig. Indirect immunofluorescence of assay of ST cells infected with PPV.

Conclusions: In conclusion, our study provides insight into the evolution of PPV. Further studies on the antigenic variability and genetic analysis of PPV isolated from different geographical regions are needed for understanding the pattern of evolution of PPV. This study will provide up-to-date genetic diversity information and understanding into the development of an effective vaccine.

Acknowledgement: This study was supported by the Research of Animal and Plant Quarantine Agency.

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[1] Park et al., 2017. Viruses. 9, 196.
[2] Park et al., 2018. J Vet Sci. 2018. 19(6).

PCVAD-016

Loop-mediated isothermal amplification for rapid and sensitive detection of porcine circovirus 3

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Introduction: Porcine circovirus 3 (PCV3) has been identified in pigs from China, Korea, and Poland with various clinical conditions [1, 2]. Given that the clinical presentation of PCV3 infection is similar to that of PCV2 infection, and that the virus may be highly prevalent globally, the development of a rapid and simple diagnostic assay for the specific detection of PCV3 is necessary. Although several of loop-mediated isothermal amplification (LAMP) assays have been successfully developed for the rapid and highly sensitive and specific detection of PCV2, and other porcine viruses, no LAMP assay is currently available for PCV3. In this study, therefore, a LAMP assay for the rapid and visual detection of PCV3 DNA was developed and evaluated using clinical samples collected from PCV3-infected pig farms.

Materials and Methods: The web-based primer design software Primer Explorer version 4 (Eiken Chemical, Japan; http://www.primerexplorer.jp/e/) was used to design primers targeting the conserved regions of the capsid gene of PCV3. The reaction conditions were optimized by testing the amplification in a temperature range from 53℃ to 66℃ using reaction times ranging from 30 to 60 min. The reactions were terminated by heating at 80℃ for 5 min. Positive LAMP results were visually detected as a color change of the reaction mixture owing to the presence of the metal ion indicator HNB (hydroxy naphthol blue). The limit of detection (LOD) of the LAMP assay for PCV3 was evaluated and compared with previously described conventional polymerase chain reaction (PCR) and real time PCR (qPCR) assays using ten-fold serial dilutions (10^6-10^0copies/μL) of the cloned PCV3 capsid gene as a template. The clinical usefulness of the assay was evaluated using 78 clinical samples from a PCV3-infected Korean pig farm.

Results: The amplification of LAMP assay could be completed in 40 min at 62℃, and the results could be visually detected by the naked eye. The assay specifically amplified PCV3 DNA and not amplified other porcine viral nucleic acids. LOD of the assay was 50 PCV3 DNA copies, which was comparable to that of qPCR and lower than that of conventional PCR. In the clinical evaluation, the PCV3 detection rate of the LAMP assay was higher than that of PCR and agreed 100% with that of qPCR. These results indicate that the LAMP assay will be a valuable tool for the rapid, sensitive, and specific detection of PCV3 in clinical samples.

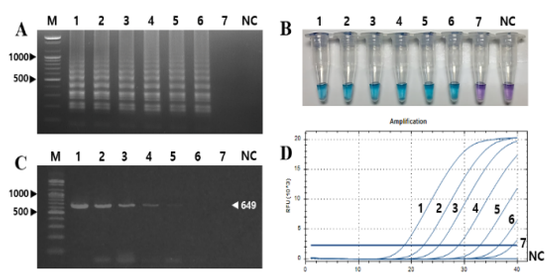

Fig. 1. Comparative sensitivity of the LAMP, PCR, and real-time PCR assay for PCV3.

(A) Electrophoretic analysis of the LAMP amplicons. (B) Visualization of the LAMP amplicons. (C) Electrophoretic analysis of the PCR amplicons. Lane M, 100-bp DNA ladder. (D) Amplification curves of the qPCR assay. The numbers in (A), (B), (C) and (D) represent serial 10-fold of cloned PCV3 DNAs (from 5×10^6 to 5×10^0 copies, lines/tubes 1-7). NC, negative control.

Conclusions: The LAMP assay is simple, rapid, highly specific, and highly sensitive, and the assay results can be observed directly by the naked eye without the need for any analysis equipment. Therefore, this assay will be a valuable tool for the rapid diagnosis of PCV3 in clinical samples, even in under-equipped laboratories.

Reference

[1] Ku et al., 2017. J. Virol. 91, 1-13.
[2] Kwon et al., 2017. Vet. Microbiol. 207, 178-181.

PCVAD-017

Performance follow up after vaccination with Circovac® 0.5 ml in piglets in a commercial farm in Thailand

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Introduction: Porcine circovirus type2 (PCV2) is an important swine pathogen which causes significant economic losses in swine production worldwide [1]. This virus is one of the factors involved in the disease entity known as porcine respiratory disease complex (PRDC) [2]. Circovac® is registered as PCV2 vaccine in piglet once for 0.5 ml [3]. The purpose of this study was to compare efficacy between Circovac® 0.5 ml once in piglet with a PCV2 vaccine previously used on the same farm

Materials and Methods: This study was carried out in 4,000 sow contract operation. Around 2,200 piglets were weaned and divided into 3 groups. All groups were sent to different farms but in the same area. Pigs were vaccinated with PCV2 vaccine at 6 weeks of age. Group A and B were vaccinated with vaccine which farm had used previously. Group C received Circovac 0.5ml. Pig performance and PCV2 serology (PCV2 Biocheck®) were compared among 3 groups. Performance was measured as mortality rate (%Loss), average daily weight gain (ADG), and feed conversion ratio (FCR). Serum samples were collected 10 of each group at 6, 10, 14, 18 and 22 week of age. Return on investment (ROI) from pig performance of vaccination in group C was compared with group A and B calculated by the Respinomics™ application.

Results: The results show that there are no significantly difference between the serological responses($p>0.05$). All groups seroconverted between 10-14 weeks of age (indicating all group had a natural infection during that time) then decreased until 22 weeks of age.

Pig performances results differed among each other in the 3 groups. Group C had higher performance in term of survival rate, average daily weight gain and feed conversion ratio. All performances were of each inline. The group which has best ADG will have best FCR and survival rate too. ROI of Group C compared with group A is 7 times and with group B is 11 times. Due to less of % loss, feed to gain one kg and day in house.

Fig 1. Serological response

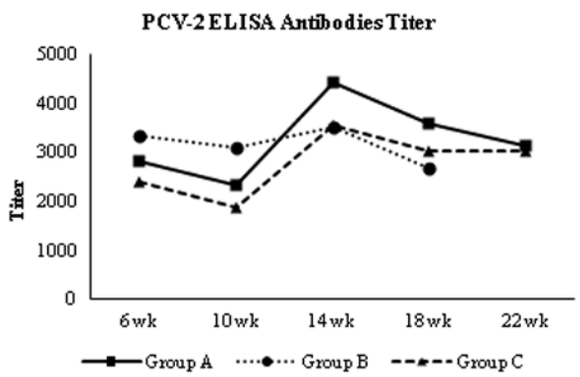

Table 1. Performance comparison data

	Group A	Group B	Group C
Pigs in	724	745	719
Weight in (kg)	4.77	4.95	4.88
Pigs out	647	604	693
Weight out (kg)	90.8	85.1	96.6
%Loss	10.60%	18.90%	3.60%
ADG (g/day)	626	558	695

Conclusions: The results observed indicate that under the conditions of this study Circovac (group C) outperformed groups A and B, for mortality, ADG and FCR.

Reference:

[1] Vidigal et al, 2012. Virus Research 163 : 320-327.
[2] Opriessnig et al, 2007. J VetDiagn Invest,19:591-615.
[3] Circovac® Thai leaflet.

PCVAD-018

Prevalence of porcine circovirus 3 in Korea

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Introduction: Porcine circovirus 3 (PCV3) was recently reported in pigs with cardiac and multi-organ inflammation and in sows that died acutely with porcine dermatitis and nephropathy syndrome (PDNS)-like clinical signs and reproductive failure in USA. PCV3 also has been reported in China, South Korea and European countries. However, clinical presentations (symptomatic or asymptomatic) and pathogenesis of PCV3 was not well established. Despite the growing concerns about PCV3, the distribution of PCV3 in Korea is limited. Therefore, this study investigated the prevalence of PCV3 in Korea.

Materials and Methods: To examine the infection of PCV3 in Korean pig farms, we collected blood samples from 112 commercial farrow-to-finisher farms in 2017. Five sera per growing stage (sow, sucking piglet, weaned pig, growing pig, finisher pig) were mixed and screened by dual real-time PCR for PCV2 and PCV3. We also tested diverse clinical samples (lung, tonsil, lymph node, intestine, kidney, spleen and brain) of pigs submitted to diagnostic division of APQA from 2012 to 2018.

Results: In a total of 112 commercial farms (487 pooling sera), PCV3 was detected in 63 farms (56.3%) and 101 sera (20.7%). PCV3 was most frequently detected in sow (33.3%) and low in suckling piglet (13.5%). In pigs of other stage, the positive rates of PCV3 were about 20-25%. Although the average positive rate of PCV2 of same farms was similar to PCV3, PCV2 were more detected as pigs are growing. On the other hand, of 884 pig farms (1,782 pigs) submitted to APQA from 2012 to 2018, PCV3 was detected in 120 farms (13.6%) and 175 pigs (9.8%). Clinical symptoms of the PCV3 positive pigs were divided into systemic (47 pigs), respiratory (44 pigs) reproductive (29 pigs), digestive (25 pigs), and sudden death (5 pigs), respectively. PCV2, PRRSV, *Streptococcus suis*, E. coli and *Clostridium perfringens A* were more frequently detected in PCV3 positive pigs. The PCV3 positive rates in intestine, lymph node, tonsil, lung, kidney and spleen mixture and brain were 74.0% (57/77), 73.0% (65/89), 72.6% (53/73), 69.3% (79/114), 58.0% (47/81) and 56.5% (35/62), respectively.

Conclusions: Despite of relatively low detection of PCV3 in pig samples submitted to APQA, the prevalence of commercial farms in which many serum samples were tested was high, indicating that PCV3 was widely distributed throughout Korean pig populations. Because clinical understanding of PCV3 in the field was not enough, further studies are required to isolate PCV3 and explore pathogenic characteristics.

References:

[1] Palinski R et al., J Virol 2017, 91, e01879-16.
[2] Tischer I et al., ArchVirol 1986, 91, 271-276

PCVAD-019

Safety and efficacy trials in piglets of a PCV2 vaccine based on PCV2d genotype

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Introduction: PCV2d is now the predominant genotype in Korea [1] and has been previously isolated in field vaccination failure cases [2]. Three studies were performed in Korea to assess safety and efficacy of a recently registered PCV2 vaccine based on PCV2d genotype.

Materials and Methods: The challenge test was performed on 8 SPF piglets allocated to 2 groups of 4 each. Group T was vaccinated (Suigen/Porcigen® PCV2, Virbac) and group C received a saline serum. Injections were done at 3 weeks of age (1 ml/piglet by IM route). All piglets were intranasally challenged at 6 weeks of age (2 ml inoculum per piglet containing 10^5TCID$_{50}$/ml of a PCV2b strain). Blood samples were taken on all pigs at 3, 6, 8 and 9 weeks of age. Pigs were necropsied at 10 weeks of age. Clinical examinations were performed weekly between challenge and necropsy. Serum PCV2 neutralizing antibodies (NA) were determined by a virus neutralizing assay. Serum and tissue genomic copies of PCV2 were quantified by PRC. Immunohistochemistry (IHC) was done on inguinal lymph nodes to count the number of PCV2 infected cells per 0.25 mm^2 area.

The field trials were performed in 3 farrow to finish Korean farms where 70 piglets were allocated to 2 groups of 35 each per farm. Group T was vaccinated against PCV2 at 3 weeks of age (Suigen/Porcigen® PCV2, Virbac, 1 ml/piglet by IM route) while group C was not vaccinated against PCV2. Between weaning and finishing, pigs were weekly examined clinically and regularly weighed. At finishing (25 weeks of age), 5 pigs/group/farm were necropsied and PCV2 infection was determined by PCR in inguinal lymph nodes, lungs and tonsils.

The safety test was done on 36 piglets from the same 3 farms. Half of the piglets received twice the PCV2 vaccine dose recommended (2 ml/pig by IM route) and were compared to the same number of non vaccinated piglets for local and general side effects (including hyperthermia). Groups were compared in the 3 studies by appropriate parametric or non parametric analysis.

Results: No adverse effects were reported after vaccination in the 3 studies. In the challenge test, the vaccination induced significantly higher NA titers and lower viremia (peak values at 8 weeks of age). The tissue PCV2 burdens were also significantly lower in vaccinated pigs. In the field trials, performances were significantly higher and tissue PCV2 infection rates at finishing significantly lower in vaccine group.

Table 1. Mean ± SD of peak immunity, viremia and viral burdens in inguinal lymph nodes in challenge test

Group	T	C
Log_2 NA titers	6.8 ± 0.7^a	2.5 ± 0.5b
Serum Log_{10} copies/ml	3.3 ± 0.5^a	5.6 ± 0.7b
Lymph node Log_{10} copies/ml	0.5 ± 0.3^a	3.1 ± 0.5b
IHC scores	9.7 ± 1.6^a	52.5 ± 20.1b

$^{a, b}$: Different superscripts indicate significant differences

Table 2. Performances and PCV2 tissue infection rates at finishing in field trials

Group	T	C
Pig weight at 19 weeks (kg)	78 ± 10^a	73 ± 10^b
Mortality rate (wean to finish)	7.6%^{a}	12.4%^{b}
Lymph node PCR positive rate	10%^{a}	100%^{b}
Lung PCR positive rate	6.7%^{a}	100%^{b}
Tonsil PCR positive rate	20% ^{a}	100%^{b}

$^{a, b}$: Different superscripts indicate significant differences

Conclusions: These trials confirm the safety and the efficacy of the tested vaccine both in challenge and field conditions according to immunological, virological and clinical criteria.

References:

[1] Seung-Chai K et al., 2018. BMC Vet Res 14:294.
[2] Seo HW et al., 2014. Arch Virol 159, 3107-3111.

Keywords: PCV2d, Vaccine, Safety, Efficacy

PCVAD-020

SDS-page electrophoresis and Western blot result from different PCV2 vaccines in China

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Introduction: China is a biggest swine raising country with more than 50% of worldwide pig production. For PCV2 vaccination, more than 30 different products have been launch in the market i.e. whole virus killed vaccines, baculovirus subunit vaccines and *E.coli* subunit vaccines. The objective of this study is to monitor the quality of the main PCV2 vaccines in China by using SDS-page electrophoresis and Western blot analysis

Material and Methods: 13 products (A-L,N) from 19 samples were transfer to collecting tubes and sent to the laboratory for testing (blinded). Some brands had two different batch production numbers i.e. A1,A2,B1,B2 etc. The purpose of this testing is to check the consistency of vaccine batches.

The principle of SDS-page electrophoresis is to use electricity to separate different molecular weight substances in the sample i.e. protein in the vaccine.[1]

The target molecule in this study is a 28 kDa molecule which is the molecular weight of PCV2 capsid protein. Western blot method will be applied later to confirm whether detected molecules were PCV2 capsid protein or not by using specific monoclonal antibody.[2]

Table 1. ID, type and adjuvant of each vaccine

ID	Type of antigen	Adjuvant
A1,A2,B1, B2,C1,C2	Whole virus	Oil
D1,D2	Whole virus	Non-oil (water)
E1,E2	Subunit	Non-oil (polymer)
F1,F2	Whole virus	Oil
G	Whole virus	Non-oil (water)
H,I,J	Subunit	Non-oil (water)
K,L,N	Whole virus	Oil

For oil adjuvanted vaccines, an additional step for antigen extraction by chloroform was needed before running SDS page.

Results : SDS page results demonstrate that all samples except F2, G, H, J, K had a 28 kDa band which might be a PCV2 capsid protein, however after further analysis by Western blot, we found that only in product D1,D2,E1,E2 and I could the28 kDa band be identified. Moreover, Western blot result show that there was also bands at 56, 84 and 112 kDa which were assumed to be dimer, trimer and tetramer of PCV2 capsid protein.

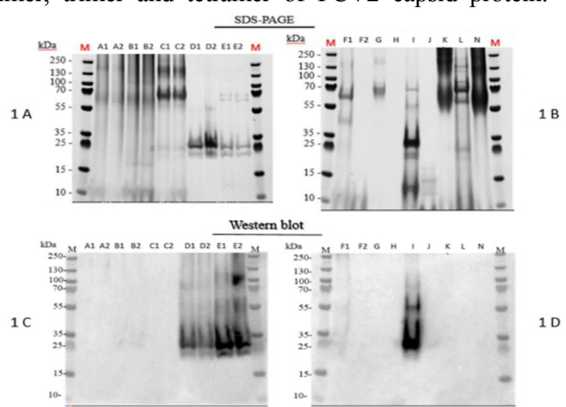

Fig 1. SDS page (1A,1B) and Western blot result (1C, 1D) / Target band is 28 kDa. M lane is a molecular weight marker

Conclusion: For samples that did not contain 28 kDa band from SDS page can imply that the samples did not contain PCV2 capsid protein (F2, G, H, J and K). Therefore this method can be used as a screening test, however in order to confirm whether vaccine contain capsid protein or not, Western blot method is needed in order to differentiate between capsid protein and other molecules that have similar molecular weight.

In summary, there are still unqualified PCV2 vaccines in the market and producers should avoid these kind of vaccines. Although these methods can help identify unqualified vaccines producers cannot just look at the SDS page or Western blot results because there are many factors that can influence vaccine efficacy. The best practical way to evaluate vaccine efficacy is still monitor the production parameter on the farm.

References :

[1] Virginie et al., 2017.Therapeutic Advances in Vaccines 2017, Vol. 5(2) 31-38
[2] Michiyo et al., Arch Virol (2008). 153:2291-2295

PCVAD-021

Superior efficacy of FLEXcombo™ compared with another PCV2 subunit vaccineand M.hyo vaccine combination

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Introduction: PCV2 vaccine is one of the key vaccines for the swine industry and several market surveys have found that efficacy is the most important parameter for selecting PCV2 vaccine followed by safety and convenience respectively. The easiest way to determine vaccine efficacy is to monitor the production performance in the farm which mainly focus on ADG and mortality rate. The objective of this study is to compare the production performance between FLEXcombo™ (freshly mixed Ingelvac CircoFLEX + Ingelvac MycoFLEX administered in one injection) and another PCV2 subunit vaccine and Ingelvac MycoFLEX (separate injection) in a single site farrow to finish farm

Material and Methods: The side by side study was conducted in a 500 sow farm in Hunan, China. The sow herd was stable for PRRSv. Piglets were weaned at 24-26 days of age. Age at slaughter is around 190-210 days old. PCV2 and Mycoplasma were vaccinated at 14-18 days old and average wean-finish mortality was between 4-8 %. In total 601 17-21 days old piglets from 60 sows were divided to 3 different groups, Group 1 FLEXcombo™ = 247 pigs, Group 2 PCV2 Subunit vaccine and Ingelvac MycoFLEX® = 244 pigs and Group 3 control = 110 pigs. Each sow contained all group of piglets and each groups were ear tagged with different color. In order to minimize the impact from different litter and batch. Individual vaccinated weights of each pig were recorded. Wean-finish performance such as average daily weight gain and weight gain were recorded

Results : Due to the ASF outbreak in China, it was difficult to visit the farm and weight pig one by one as planned, so group weighting were performed by farm staff when selling the pigs. Each cut of pigs were weighed as a group, number of pigs in each cut and day on feed are summarized in table 1

Table 1. No. of pigs, Day on feed in each cut

	No. of pig in each group		
	FLEXcombo	Subunit+MF	Control
1st cut weight (Day 190).	6	8	4
2nd cut weight (Day 199)	10	10	3
3rd cut weight (Day 201)	14	10	5
4th cut weight (Day 204)	9	13	3

Mean slaughter weight for each cut are summarized below (Figure 1). In short, group 1 had higher slaughter weight than group 2 and 3 in 1st, 3rd and 4th cut, while group 2 had higher slaughter weight than group 1 in 2nd cut. Both vaccinated groups had higher weight gain than control group in every single cut.

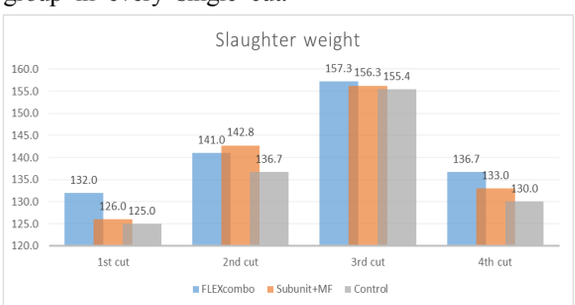

Figure 1. Summary of weight gain in each cut

When combining all data i.e. total number of pigs, total weaning weight & total slaughter weight and total day on feed from each group it was found that average ADG and average weight gain in group 1, which was vaccinated with FLEXcombo™ was 23 g/day higher than in group 2 which vaccinated with subunit vaccine + Ingelvac MycoFLEX (700.9 and 677.9 g/day) respectively, both were higher than ADG in control group 674 g/day. The average weight gain for group 1, 2 and 3 were 139.8 kg, 135.1 kg and 133.5 kg respectively

Conclusion: In this side by side study, pigs vaccinated with FLEXcombo™ had higher ADG compared to the pigs which received the other subunit PCV2 vaccine with Ingelvac MycoFLEX®. Not been able to collect individual slaughter weights did not allow for statistical analysis. The results obtained reassure that the efficacy between PCV2 are different, monitoring farm production is the best way to see which vaccine can provide highest benefit to the farm.

PCVAD-022

The leak of porcine circovirus type 2 (PCV2) vaccine efficacy and genotyping of prevalent PCV2 in conventional pigs

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Introduction: The subunit PCV2 vaccines have been widely utilized to against PCV2 infection for decades. Since PCV2 showed highly evolutionary rate under the stress of vaccine induced immune response, evaluation of the changes of PCV2 genotypes is crucial for disease control. In this study, PCV2 vaccine efficacy was monitored in a routinely vaccinated conventional pig farm and the genotypes of PCV2 in culled pigs collected from several pig farms before and after the onset of PCV2 vaccination was identified.

Materials and Methods: A conventional continuous flow pig farm located at middle Taiwan was selected. In this farm a conventional subunit PCV2 vaccine (based on PCV2a) was routinely used to immunize pigs at 3 to 4 weeks old since 2012. In 2016, total 30 piglets in this farm were selected and randomly assigned to two groups. Piglets in group A (n=15) were immunized with one dose of subunit PCV2 vaccine and the group B (n=15) pigs were immunized with placebo (PBS) at 4-week-old respectively. Serum samples were collected from 4 to 24 weeks old at 4 weeks interval to profile PCV2 infection in conventional pigs. The serum PCV2 viral load was detected with quantification PCR to profile infection status at different ages. Besides, average daily weight gain (ADWG) between 4 and 24 weeks old was calculated as an indicator to clarify the PCV2 vaccine efficacy. Furthermore, in order to identify the prevalent PCV2 genotypes, full length PCV2 open reading frame 2 (ORF2) nucleotides from culled pigs collected from several pig farms around this area were sequenced. The ORF2 sequences were aligned at codon level using the ClustalW method and a Maximum Likelihood tree was constructed using MEGA7 software.

Results: The results of serum profiling revealed the dynamic of PCV2 viral load between 4 and 24 weeks old. The serum PCV2 viral load steeply increased at 8 weeks old followed by decline at 20 weeks old, revealing the infection of PCV2 in conventional pigs (Figure 1). Pigs in group A had a slightly higher PCV2 viral load between 16 and 20 weeks old. However, there was no significant difference in serum PCV2 viral load and ADWG between group A and B during 4 to 24 weeks old. These results reveal the leak of PCV2 vaccine efficacy on virus eradication. Therefore, it is important to clarify the possible virus strain which escapes from vaccination. The PCV2 ORF2 sequences from culled pigs at middle Taiwan before onset of PCV2 vaccination (before 2012, n=3) and recent samples (2013~2018, n=24) were aligned on codon level with ORF2 sequences collected from GenBank (2001~2011 Taiwan isolates, n=19). The aligning result showed that the genotypes distribution of PCV2a (68.2%), PCV2b (27.3%), and PCV2d (4.5%) before 2012, but PCV2d (91.7%) is highly prevalent during 2013~2018 (Table 1).

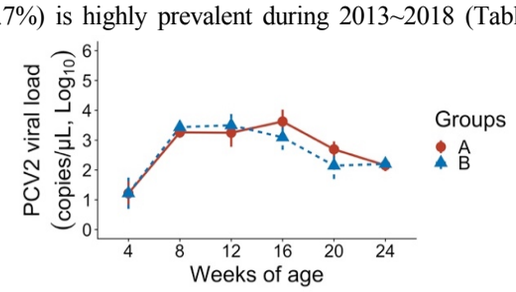

Figure 1. Profiling of serum PCV2 viral load in pigs

Table 1. The genotypes of PCV2 isolates before and after routine vaccination

Genotypes	Before 2012	2013~2018
PCV2a	15	2
PCV2b	6	0
PCV2d	1	22
Total	22	24

Conclusions: Vaccination induces highly specific immune response against PCV2; however, based on phylogenetic results, it reveals the gradually changing of prevalent PCV2 genotype in conventional pig farm after receiving routinely PCV2 vaccination. Therefore, the possible leak status of PCV2 vaccine efficacy and the variation of PCV2 genotypes may be reciprocal causation. These results may provide useful information for further PCV2 vaccine investigation.

Prac-001

Antimicrobial resistance and molecular characterization of *Campylobacter coli* isolated from pigs and carcasses

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Introduction: *Campylobacter* spp., such as *Campylobacter jejuni and Campylobacter coli*, are normal intestinal flora in animals. Contamination of food products during processing is the main source of food poisoning in humans. Macrolide antimicrobials are widely used in animal industry. The potential risk that macrolide-resistant *Campylobacter* spp. will be transmitted from animal products to humans has raised concerns about failure of treatment of human infections. The aims of the present study are to examine antimicrobial resistance of *C. coli* and to investigate the molecular mechanisms involved in macrolide resistance

Materials and Methods: *C. coli* strains were isolated from pig faeces and their carcass samples using a selective agar and identified by Maldi-Tof or PCR. Antimicrobial susceptibility testing was performed by broth dilution using a commercial MIC plate (Campy, Sensititre). Domain V of the 23S rRNA, L4 protein and L22 protein were amplified by the PCR and sequenced at Macrogen (Seoul, Korea). DNA sequences of resistant and susceptible strains were compared with the sequence of the *C. coli* JV20 genome

Results: A total of 643 *C. coli* were isolated from pigs and their carcass samples at slaughterhouses throughout Korea during 2010-2018. Resistance to ciprofloxacin and nalidixic acid (88%) was highest followed by tetracycline (78%), clindamycin (43%), telithromycin (42%), and erythromycin (39%). Resistance to macrolide and phenicol antimicrobials was gradually increased during study periods. Most *C. coli* isolates were resistant to one or more antimicrobials, and 60% of *C. coli* isolates exhibited multi-drug resistance. All macrolide-resistant *C. coli* isolates possessed a 23S rRNA mutation (A2075G). In addition, several amino acid substitutions in the L4 and L22 ribosomal proteins also observed.

Conclusions: We discovered a high rate of antimicrobial resistance in *C. coli,* with a mutation in the 23S rRNA gene mainly responsible for erythromycin resistance in *Campylobacter* isolates. The effect of the amino acid substitutions in the L4 and L22 proteins on macrolide resistance required further evaluations. To prevent the transmission to humans of resistant *Campylobacter* spp. via the food chain, we urge more prudent use of critically important antimicrobials such as fluoroquinolones and macrolides in swine industry, as well as constant monitoring of resistance among *Campylobacter* isolates in animals and animal products.

Acknowledgement: This work was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (B-1543084-2017-19-01).

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[1] Lim SK et al., 2017. J Vet Med Sci . 78, 1791-95
[2] Bolinger HK et al., Foodborne Pathog Dis. 15, 698-700.

Prac-002

Antimicrobial resistance and molecular characterization of *Staphylococcus aureus* isolated from pig carcasses

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as an important pathogen in human. Recent reports have documented MRSA infections in animals, and it is now considered as one of the most important zoonotic pathogens. The aims of study were to investigate the antimicrobial resistance and characterize the methicillin-resistant *S. aureus* isolated from pork carcasses.

Materials and Methods: *S. aureus* strains were isolated from carcass samples using a selective agar and identified by Maldi-Tof or PCR. Antimicrobial susceptibility testing was performed by broth dilution using commercial MIC plates (EUST). Methicillin resistance gene and SCC*mec* types were identified by PCR. In addition, multilocus sequence typing, and *spa*-typing were performed to determine the genetic relatedness of the MRSA isolates.

Results: A total of 1,198 **S. aureus** were isolated from pork carcasses at slaughterhouses throughout Korea during 2010-2018. Resistance to penicillin (82%) was highest followed by tetracycline (47%), chloramphenicol (40%), clindamycin (39%) and erythromycin (34%). Overall, resistance rates and multiple-drug resistance gradually increased during the study period with liner trends of increasing resistance. In total, 97 (8.1%) S aureus isolates were identified as MRSA. For all antimicrobial tested, except for fusidate, frequencies of resistance were much higher for MRSA than MSSA. Sixteen different clones were observed in MRSA isolates with human associated (27%) and livestock associated (73%): ST398-*spa* t571 (36%), ST72-*spa* t324 (17%), ST398-*spa* t034 (19%) and 13 other types (28%). Notably, LA-MRSA was emerged in pork carcasses from 2012 and predominant with various variant clones.

Conclusions: This study demonstrates that increase of antimicrobial resistance in *S. aureus* isolated from pig carcasses. High proportion of livestock associated-MRSA and the emergence of new ST398 and ST541 variant type identified in this study highlight the necessity and importance of MRSA surveillance in pig production industry.

Acknowledgement: This work was supported by a grant from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (B-1543084-2017-19-01).

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Prac-003

Asian multicenter study of a nutritional oral gel in the farrowing house

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Introduction: Pre-weaning piglets mortality is a remaining multifactorial issue where increased prolificacy plays a role due to possible insufficient sow milk supply for all the litter. Objective of this study was to test the effect of a nutritional palatable gel dedicated to the 10 first days of life, in the reduction of pre-weaning mortality and improvement of growth between birth and weaning.

Materials and Methods: Study was performed in 3 farrow to finish sites owning between 700 and 2000 sows, respectively located in Taiwan (site 1), Philippines (site 2) and Vietnam (site 3). In each site, farrowing sows from the same batch were randomly allocated between 2 groups according to parity and number of born alive. Cross fostering was allowed only within the same group. Litters from the T group received Porcistart® (Virbac) *ad libitum* in a specifically designed tray placed close to the sow head from birth (D1) to D10. Litters from the C group did not receive any nutritional complement to sow milk between D1 and D10. From D11 to weaning, both groups received the farm usual feeding procedure. Piglets were individually identified and weighed at birth and weaning in sites 2 and 3. In site 1, identification and weighing was at sow level. Pre-weaning mortality cases, attributed to low body weight and poor condition, were recorded. Average daily growth (ADG) was compared between groups from birth to weaning according to the t test. Pre-weaning mortality rate was compared between groups by the Cochran-Mantel-Haenzel test.

Results: A total of 678 piglets was included in the study, corresponding to 9 sows in site 1, 10 sows in site 2 and 44 sows in site 3. Parity ranged between 1 and 8 and number of born alive per litter between 9.0 and 13.4, without statistical difference between groups in each site. Pre-weaning mortality rates were numerically lower in tested than in control group for all sites, multisite difference being close to significance
($p = 0.0675$). Pre-weaning growth was numerically higher in T than in C group for all sites, difference being statistically significant in site 3 ($p < 0.05$).

Table 1. Pre-weaning mortality and growth

Site	Mortality (%)		ADG (g/d)	
	T	C	T	C
1	2.3	6.9	267 ± 26	253 ± 18
2	3.8	9.0	158 ± 5	150 ± 5
3	4.8	7.0	230 ± 6[a]	214 ± 6[b]

[a, b]: Different superscripts indicate significant differences

Conclusions: Effects of the tested product on pre-weaning reduction of mortality and increased growth have been previously reported in European conditions [1]. Interest was confirmed here in Asian conditions, though differences with control group were not always statistically significant, possibly due to limited numbers of included piglets (particularly in sites 1 and 2). In site 1, a side test was performed on 29 weak piglets (mean birth weight of 1 kg) pooled in 2 litters receiving the tested product. Pre-weaning mortality was equal to 13.8% on these piglets while it could reach 50% on this category of animals from farm history. Though this finding is outside the controlled study design, it would deserve further trials for confirmation.

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Prac-004

Comparative trial of the humoral immune response and reproductive parameters of two commercial reproductive vaccines in Korean pig farms

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INTRODUCTION

Swine erysipelas (SE) and porcine parvovirus (PPV) infections are major causes of abortions, mummified fetuses and low reproductive productivity, among others, in breeding herds [1]. To prevent these problems, most of pig farms are using complex vaccines composed with *Erysipelothirix rhusiopathiae* and PPV.

For evaluating potency (humoral immune response) and efficacy (reproductive parameters) of two commercialized vaccines, ERYSENG® PARVO (HIPRA, Spain) and Vaccine B (adjuvanted with *Amphigen*®) were tested in a Korean swine farm.

MATERIALS & METHODS

Twenty-four animals were selected as experimental sows, and those animals were divided into 2 different groups. Each group was composed by 8 multiparous sows and 4 gilts, and vaccinated and revaccinated with ERYSENG® PARVO and Vaccine B following manufacturers' instructions.

To evaluate antibody titres against *E. rhusiopathiae* and PPV, serum samples of experimental sows were tested by using CIVTEST® SUIS SE/MR (Hipra, Spain) and VDPro® PPV HI Reagent (Median dinostics, Korea), respectively [2]. Regarding the efficacy of reproductive vaccines, sow reproduction performance was recorded in each group.

RESULTS

In farrowing day (22.3 weeks after first vaccination), average IRPC titer (IRPC, Positive > 40) of ERYSENG® PARVO group was 52.2 and 49.5 in multiparous sows and gilts, respectively. After the vaccination with ERYSENG® PARVO, 88% of multiparous sows and 75% of gilts were sero-positive until farrowing day.

On the other hand, only 50% of sows vaccinated by Vaccine B were sero-positive at farrowing day (Figure 1). Besides, HI titer against PPV was slightly higher in ERYSENG® PARVO group until the farrowing day.

Regarding the sow performance, there was no significant difference between both vaccines in multiparous sow. However, in the gilts, ERYSENG® PARVO showed less abnormal newborn piglets. Hence, average number of weaned piglets in ERYSENG® PARVO group was significantly higher than Vaccine B group (Table 1).

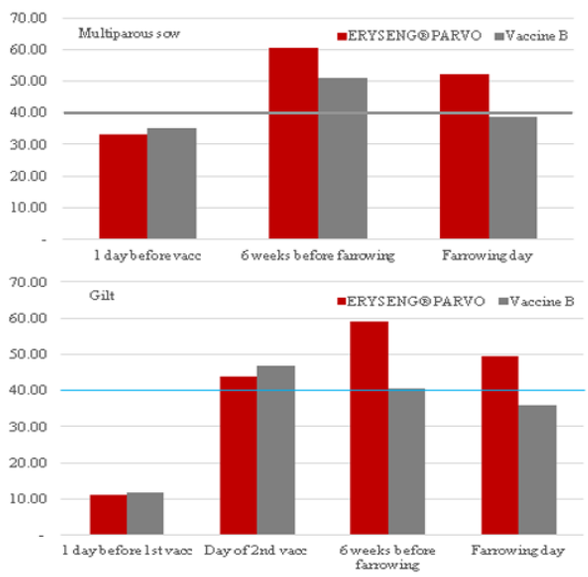

Figure 1. Antibody titers against E. rhusiopathiae (IRPC, Positive > 40).

Table 1: Productivity of experimental sows vaccinated with reproductive vaccines

Experimental sow	Group	Total born	Still born/ mummy	Weaned piglets
Multiparous sow	ERYSENG® PARVO	10.75	1.13	9.63
	Vaccine B	10.38	1.00	9.38
	p-value	0.696	0.841	0.766
Gilt	ERYSENG® PARVO	11.75	0.50	11.50*
	Vaccine B	10.50	2.25	8.25*
	p-value	0.302	0.277	0.025

DISCUSSION & CONCLUSION

ERYSENG® PARVO elicited higher antibody titers against *E. rhusiopathiae* and PPV compared to Vaccine B in both multiparous and gilts. Furthermore, ERYSENG® PARVO provided higher productivity on gilts, which were not immunized before with other vaccines. Based on all results, ERYSENG® PARVO could be a useful immunization method against SE and PPV.

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Prac-005

Comparison of efficacy of Hyogen® with another commercially available M.Hyo vaccine under Philippine field condition

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Introduction: Vaccination against M. hyo infection is a common practice in commercial swine farms, to achieve improvements in lung lesion scores as well as improvements in production parameters such as average daily weight gain (ADWG) and Feed Conversion Ratio (FCR)[1]. In this field study, the objective is to compare the performance of Hyogen® with a combo 6 in 1 vaccine (with M.hyo.) in terms of improving lung lesion scores and growth performance of growing pigs under Philippine field conditions

Materials and Methods: A four thousand sow farm located south of Manila, Philippines has been using a commercially available M. hyo vaccine in piglets (3 and 5 weeks) for years. Lung lesion scoring using Ceva Lung Program (CLP) App was conducted in October of 2017 (50 pigs) to assess the lung health status (figure 1). Since November of 2017, the farm used Hyogen® (M. hyopneumoniae vaccine - Ceva Animal Health) at 5 weeks of age. CLP was conducted 8 months (50 pigs) after the implementation of Hyogen vaccination (figure 2). The two CLP results, together with the individual weight of the slaughtered animals in the group were compared for analysis and evaluate possible correlation between lung lesion scores and production performance such as average daily gain (ADG).

Results: The result showed Hyogen® group had 38% lower of Bronchopneumonic lungs (Figure1), 0.4 Kgs heavier in weight at slaughter and 5 days less of the age at slaughter than vaccine A group (Table 1).

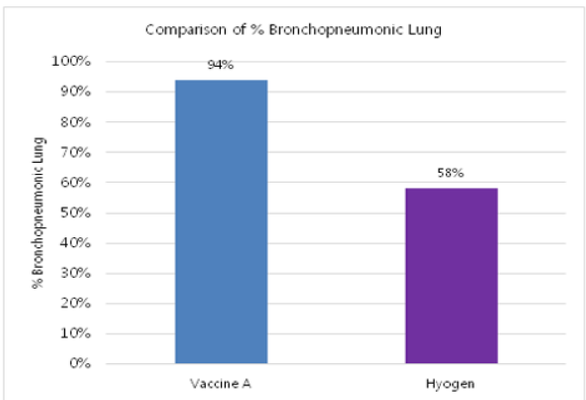

Figure 1. Comparison of % of Bronchopneumonic Lungs between the 2 groups

Table 1: Summary of Result

	Vaccine A	Hyogen®	Improved
%Bronchopneumonic Lungs	94	58	38%
Slaughter Weight(kgs)	94.7	95.1	0.4kgs
Slaughter Age (days)	160	155	-5 days

Conclusions: The results showed 38% improvement in the percentage of healthy lung in the Hyogen group translated into additional 0.4 kgs per pigs and a reduction of 5 days in the number of days to slaughter. .

References:

[1] Moreau, I. (2004) Vaccine 22 (17-18).

Prac-006

Detection and phylogenetic analysis of hemoplasma species in domestic pigs from the Korea

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Introduction: Two hemoplasma species, *Mycoplasma suis* and *M. parvum*, previously known as *Eperythrozoon suis* and *E. parvum*, respectively, have been identified in pigs. Swine hemoplasmosis has worldwide distribution, and *M. suis* infection presents an economic problem for the pig industry throughout the world. The objective of this study was to investigate the nationwide frequency and distribution of hemotropic mycoplasmas in pig farms of Korea.

Materials and Methods: We randomly selected 1,867 samples from 464 pig farms located in four regions of Korea. Hemoplasmas were first identified based on the amplification of the 16S rRNA gene with universal primers fHf1/rHf2 and *M. suis*-specific primers f2/r2; then, positive results were confirmed at the species level by PCR using cmsf2/cmsr2 and msf2/msf2 primer sets to amplify the 16S rRNA genes of *M. suis, M. parvum,* and a novel hemoplasma species.

Results: Among 1,867 pigs evaluated in the study, three (0.2%), 51 (2.7%), and one (0.1%) were infected with *M. suis, M. parvum*, and novel hemotropic *M. haemosuis*, respectively. The 16S rRNA sequences of *M. suis, M. parvum*, and *M. haemosuis* had high identity (99.3-100%, 99.6-100%, and 99.6-100%, respectively) with those of *Mycoplasma* spp. isolated from other countries. To the best of our knowledge, this is the first nationwide, large-scale study on the molecular detection of *Mycoplasma* spp. in domestic pigs in Korea.

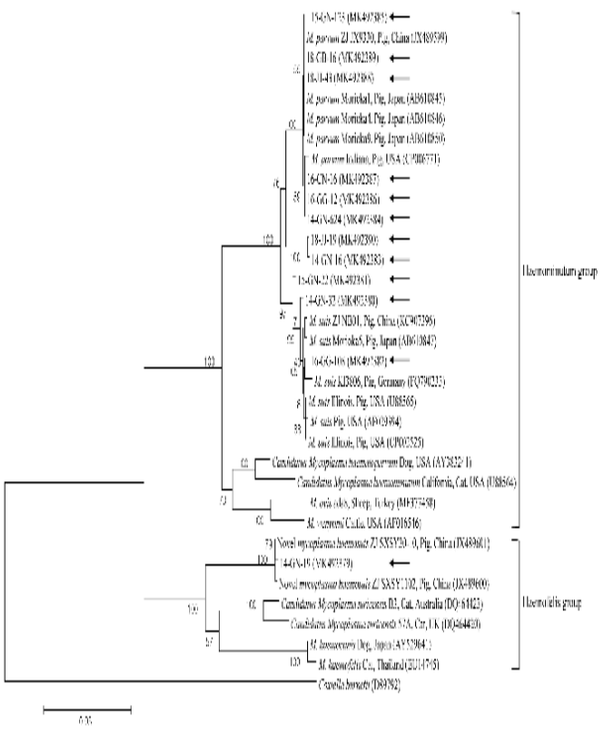

Fig. 1. Phylogenetic tree of *Mycoplasma* spp. based on the 16S rRNA gene. The tree was constructed using the maximum likelihood method.

Conclusions: Our results indicate that *Mycoplasma* infections are widespread among domestic pigs in Korea; therefore, continuous monitoring and control strategies are required to prevent the transmission of hemoplasmas, which cause economic losses in the pig industry and pose a potential threat to public health.

Acknowledgement: This work was supported by a grant from the Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education (grant number NRF-2016 R1D1A1B02015366).

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Prac-007

Detection of Porcine Enteric Viruses from Korean Wild Boar: 2016-2018

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Introduction: Viral enteritis is one of the important diseases in pig with high morbidity and/or mortality, causing significant economical loss worldwide. Many enteric RNA viruses were known to cause severe virus enteritis and diarrhea in pigs. Wild boars are also known to play important roles in the transmission of many viral diseases affecting domestic pigs. Many previous reports confirmed that wild boar as reservoir host of virus pathogens of domestic pig population. Porcine epidemic diarrhea virus (PEDV), Transmissible gastroenteritis virus (TGEV) and Rotavirus (PRV) were known to cause severe viral diarrhea and economically important pathogens in pigs. In addition, Porcine Astrovirus (PAstV), Kobuvirus (PkoV), Sapovirus (PSV) and Sapleovirus (PSapV) considered as emerging enteric viruses, but its pathogenicity in pigs were controversial. In present study, we examined the presence of seven porcine enteric viruses including PEDV, TGEV, PRV, PAstV, PkoV, PSV and SapV in Korean wild boar population and their relationships with other enteric viruses.

Materials and Methods: Fecal samples from wild boar were collected from different regions of Korea, 2016-2018. Total RNA was extracted from the sample using Qiagen RNeasy kit and cDNA were prepared by Nanohelix Easy cDNA Synthesis kit. Virus-specific PCR were performed using previous published primers (1-5). Briefly, Spike gene of PEDV/TGEV, VP6 gene of PRV, RNA-dependent RNA polymerase/ORF-2 gene of PAstV, 3D region of PkoV, RNA polymerase gene of PSV and 5'-UTR gene of PSapV were detected by PCR. The amplified PCR products were sequenced at the Cosmogentech Institute by using an ABI Prism 3730xi DNA sequencer. Multiple sequence alignments and phylogenetic tree analysis were carried out using BioEdit Sequence Alignment Editor, CLC Main workbench system and Mega 6 program.

Results: Based on the national wild boar surveillance program, total 752 fecal samples were collected from Korean wild boars in 2016-2018. The number of fecal samples collected per year as follows: 187 samples in 2016, 261 samples in 2017 and 304 samples in 2018. These fecal samples were collected from all 16 different regions of Korea. PEDV, TGEV and PRV were not detected in 2016-2017 samples. However, PSV and PSapV were determined PCR-positive in samples from 2018. PAstV and PkoV were detected 6% and 14% for 3 years respectively. No other enteric viruses such as PEDV, TGEV and PRV were detected in PAstV or PkoV positive samples.

Conclusions and discussion: In this study, we determined the presence of seven porcine enteric viruses in Korean wild boar population. PAstV, PkoV, PSV and PSapV were detected PCR-positive in fecal samples. Interestingly, there were no PEDV, TGEV and PRV in these positive samples. These results indicate that wild boar population may serve as potential reservoir of porcine enteric viruses. Further study is needed to characterize viral genome sequence of these viruses detected in Korean wild boars.

Acknowledgement: This study was supported by a grant from the Animal and Plant Quarantine Agency (Project Code No. B-1543083-2019-21), Ministry of Agriculture, Food, and Rural Affairs, South Korea.

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Prac-008

Development and Application of a *TaqMan* quantitative Real-time PCR for Specific Detection of Porcine Parvovirus 3

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Introduction: Porcine parvovirus 3 (PPV3) belongs to the parvoviridae subfamily, parvoviridae. Porcine parvovirus disease is characterized by stillbirth, mummified fetus, fetal death and infertility. In 2008, PPV3 was first detected in pig serum and other samples in Hong Kong, and later detected in Germany, Canada, Japan, South Africa and other countries[1-5].

Materials and Methods: Reference virus were keep in our lab. The primers and probe were designed on the basis of conservative VP1gene sequences. To construct the standard curve, qPCR amplifications were performed with serial recombinant plasmid. Sensitivity, specificity and repeatability of the method was evaluated by sensitivity test, specificity test and repeatability test. 53 aborted fetuses were evaluated by qPCR to confirm the feasibility of this technique in detection of PPV3.

Results: The results showed that the standard curve produced a good linear relationship between cycle threshold value and the concentration of standard plasmids. The assay could specifically detected PPV3 and no positive targets were detected from samples that were positive to porcine circovirus 2, porcine epidemic diarrhea virus, porcine reproductive and respiratory syndrome, porcine pseudorabies virus, porcine parvovirus 2, porcine parvovirus 5, porcine parvovirus 6, *Haemophilus parasuis, Bradetella bronchiseptica*. In addition, The limit of detection (LOD) determined as plasmid copies per reaction was 987,which 100-fold lower than that of conventional PCR. The method was highly reproducible and a coefficient of variation of intra-assay and inter-assay is 1.12%-1.64%% and 0.87%~2.03%, respectively. Clinical samples of 53 aborted piglets from hubei province were tested, with a positive rate of 19% (10/53). The coincidence rate of common PCR and qPCR is 100%.
Conclusions: This study established a *TaqMan* real-time PCR method with high sensitivity, good specificity and good reproducibility.

Acknowledgement: This work was supported by the national pig industry technology system, China(CARS-35).

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Prac-009

Early application of parenteral toltrazuril-iron combination (Forceris®) is comparable to later treatment in the control of experimental cystoisosporosis in suckling piglets

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Introduction:

Cystoisosporosis (coccidiosis) is a leading cause of diarrhea in suckling piglets and is controlled by metaphylactic toltrazuril application. Recently, a single dose combination product (Forceris®) has been developed for the prevention of piglet cystoisosporosis and iron deficiency anaemia. It is applied intramuscularly between the 2nd and 4th day of life (dol) (24h- 96h after birth). In previous experimental studies, it was shown that treatment with Forceris® on the 2nd day of life (dol) followed by experimental infection with *Cystoisospora suis* on the 3rd dol reduces significantly the oocyst shedding and diarrhoea and to consequently improves body weight gain and health of treated piglet compared to infected untreated control [1].

Materials and methods:

A subsequent study with experimental infection conducted on the 1st dol and treatment on the 2nd dol was conducted to determine the efficacy of Forceris® when applied after the onset of neonatal infections. Piglets were randomly assigned to the Forceris® group (n=13; 45 mg toltrazuril + 200 mg iron/piglet), and to the Control group (n=11; 200 mg iron/piglet). General animal health was recorded daily and body weight was determined weekly during the study (1st - 29th dol). Individual faecal samples were collected from the 5th - 18th dol and examined for faecal consistency and the presence of oocysts.

Results:

In the Control group all piglets shed countable oocysts, while the Forceris® group remained negative ($p<0.0001$). Diarrhoea was seen in all animals in the Control group and in one animal in the Forceris® group ($p<0.001$). The body weight gain was significantly depressed in the Control group compared to the Forceris® group during the first two weeks post-challenge ($p=<0.0001$).

Table 1: Comparison of groups: Oocyst excretion, Faecal consistency, Body weight

	Forceris®	Control
N piglets	13	11
N sampling days	179	154
Oocyst excretion		
N [%]piglets positive in AF/MM	0 [0.0]	11 [100]
Mean [min-max] excretion days/piglet AF	0	7.1 [3-12]
Mean [min-max] excretion days/piglet MM	0	6.1 [2-11]
N [%] excretion days AF	0 [0.0]	79 [51.3]
N [%] excretion days MM	0 [0.0]	67 [43.5]
Faecal consistency		
N [%] piglets with diarrhoea	1 [7.7]	11 [100]
Mean [min-max] diarrhoea days/piglet	0.4 [0-5]	3.6 [1-6]
N [%] diarrhoea days	5 [2.8]	40 [26.0]
Body weight development		
Mean BWG (g) 1st -29th dol [%]	5701.7* [501.8]	4894.5 [439.8]
Mean daily BWG (g) 1st - 29th dol	203.6	174.8
Mean daily BWG (g) 8th - 15th dol	212.3	63.9

(AF: autofluorescence, MM: McMaster, OpG: oocysts per gram of faeces), diarrhoea [faecal scores (FS) 3 and 4] and body weight gain (BWG). dol: day of life

Conclusions:

Forceris® was safe to use and effective in a single application against experimental infections with *C. suis* on the 1st dol and can be recommended for treatment of porcine coccidiosis in neonatal piglets

References:

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Prac-010

Effect of ascogen® supplementation on immunity response post vaccination on sows and sucking piglets

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Introduction: Recently, several antibiotic alternatives in the disease prevention of pigs, including vaccines, probiotics, prebiotics, oils extracted from plants, antibacterial peptides, passive immunoglubulin, digestive enzymes, organic acids,... with different direct or indirect impact mechanisms [1]. ASCOGEN® is a commercial products (CHEMOFORMA, Switzerland), which is a unique formulation of purified RNA is extracted from yeast and purified nucleotides, the organic acid, and fungal unactivated yeast. Nucleotide effects dislay the improvment of immune system, development of small intestine growth, take part in divided of the cell to increase animal resistance with the diseases [2]. In pig farms, vaccination against classical swine fever (CSFV) and porcine reproductive and respiratory syndrom virus (PRRSV) is applied routinely due to the important nature of their epidemiological properties. Therefore, The objective of this study was to evaluate the effect of ASCOGEN® on the performance and antibody response after vaccination on gestating sows and their piglets.

Materials and Methods: 60 pregnant sows in good health at a pig farm were divided into two trial groups: group A (control, no additional preparation of feed); group B (treament, supplemented in feed preparations ASCOGEN®, dosed recommended by the manufacturer). 15 piglets of sows in each experimental group (A, B) will be randomly selected for trial classified into 4 groups (Table 1) aimed at comparing the health and growth potential of the piglets; additional preparation time after weaning (starter feed) until weaning (28 days old). PRRS and CFS vaccines is especially about the time of injection and injection techniques to ensure uniformity between the two treatment groups.

Table 1: Additional layout ascogen experimental of piglets (x: no adding; o: adding)

Group	code	No. Sows	No. Piglets	Ascogen
A (Control)	A1	15	150	o
	A2	15	150	x
B (Treament)	B1	15	150	o
	B2	15	150	x

In batch experiments pigs, Ascogen be added to feeds prior to the PRRS and CFS vaccination two weeks; while sows in the control group no additional preparations. Analyzing IgG antibody levels (ELISA, IDEXX), PRRS and CFS resistance before and after the addition of the composition. Results: At the time of vaccination, there was a significant difference ($P<0.05$) of maternal derived antibody (MDA) in piglets coded B1, B2 compared with piglets coded A2; and MDA of piglets coded A1 was significantly higher ($P<0.05$) than the piglets coded A2. Postvaccinated, the S/P PRRS in piglets coded B1 was higher than piglets coded B2, ($P<0.01$) and piglets coded A2 ($P<0.01$). This difference were also expressed in piglets coded A1 compared with piglets coded A2 ($P<0.05$) (Figure 1). There was a significant difference of performance between ASCOGEN® supplemented versus control sows at their piglets' average birth weight, ($P<0,05$) and at average weaning weight ($P=0.060$).

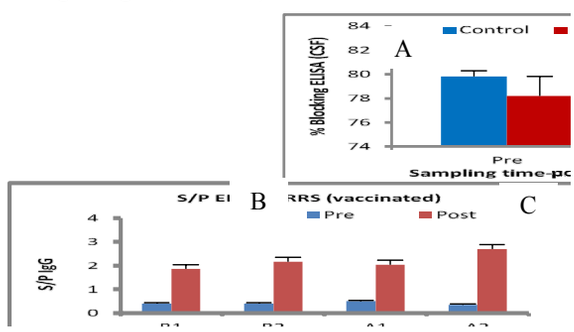

Figure 1: A - Comparison of antibodies against CFSV in sow (A), (MDA) in piglets (B) and PRRSV in piglets (C)

Conclusions: Results showed that ASCOGEN® supplementation was effective in improving the immune response after vaccination and improving pig performance.

Acknowledgement: This work was supported by CMS Ltd., Vietnam; CHEMOFORMA Ltd., Rheinstrasse 28-32, CH-4302 Augst, Switzerland

References:

[1] Thacker, PA., 2013, J. Ani. Sci. and Biotech. 4(35): 1-22.

[2] Hess, JR., & Norman, AG., 2012. Official Pub. of the American Society for Parenteral and Enteral Nutrition. 27: 281-94.

Prac-011

Efficacy of an injectable combination of toltrazuril and iron against experimental infection with Cystoisospora suis in suckling piglets

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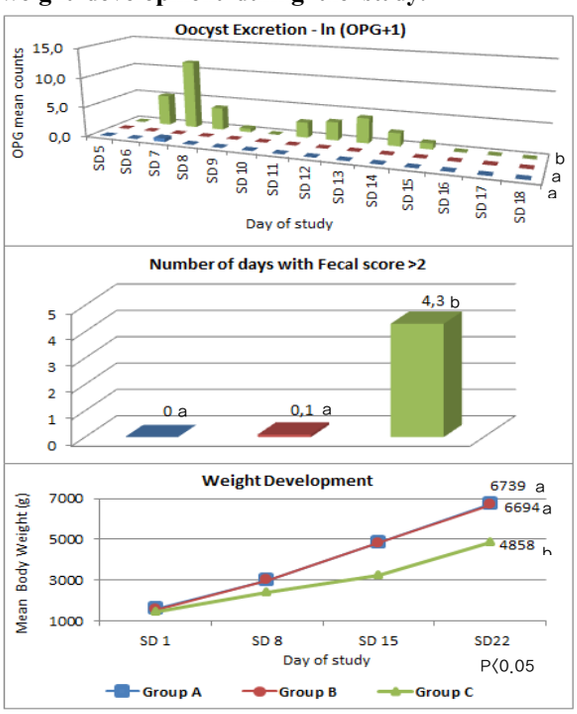
Introduction: Coccidiosis caused by *Cystoisospora (C.) suis* is a major cause of piglet diarrhea, resulting in reduced performance and economic losses. The current prevention method of choice is individual oral toltrazuril administration from day three to five of life, at 20mg/kg body weight (BW). This study evaluated the efficacy of a novel injectable combination of toltrazuril and iron against experimental *C. suis* infections in suckling piglets.

Materials and Methods: On the day of birth (SD1), piglets were allocated to the three groups (n=8-9 animals/group) and challenged with 1,000 oocysts of *C. suis*. Treatments were administered two days post challenge (SD3): Group A - injectable toltrazuril plus iron combination; Group B - oral toltrazuril suspension (Baycox® 5%) + commercial iron injection; Group C (control) - oral water + commercial iron injection. Both toltrazuril groups received 20 mg/kg of BW. Individual fecal samples were examined from SD5 to SD18. Efficacy of treatment was evaluated regarding oocyst excretion (qualitatively and quantitatively as oocysts per gram of feces; OPG), fecal consistency (fecal score FS1 to FS4, with FS3 and FS4 being diarrhea) and BW development (recorded weekly from SD1-22) in comparison to each other and the control group.

Results: Treatment resulted in suppression of countable oocyst excretion in groups A and B. Only one animal excreted a negligible amount of oocysts (OPG = 333) for a single day in group A. All piglets in group C excreted oocyst (max. OPG = 941,724) and had watery diarrhea (FS4) for at least one day. Diarrhea was not observed in groups A and B, except for one piglet for one day in group B (FS3). Both toltrazuril treated groups were superior to the control group in the parameters oocyst excretion and diarrhea ($P<0.05$). Body weights at SD22 and average daily weight gain from SD1 to SD22 were lower ($P<0.05$) in the control group (4,858g, 153.9g/day) and comparable in the treated groups (Group A: 6,739g, 233.3g/day, and Group B 6,694g, 233.5g/day).

Figure 1. Oocysts excretion, fecal score and average weight development during the study.



Conclusions: The injectable toltrazuril plus iron combination at 20 mg toltrazuril/kg BW was effective in reducing oocyst shedding, diarrhea incidence and the negative effect of coccidiosis on piglet weight gain.

Acknowledgement: This work was supported by Bayer Animal Health, GmbH. Monheim, Germany.

References:

[1] Mundt HC, Joachim A, Becka M, Daugschies A. *Isospora suis*: an experimental model for mammalian intestinal coccidiosis. Paras. Res. 2006; 98(2): 167-75.

[2] Scala A, et al. Toltrazuril and sulphonamide treatment against naturally *Isospora suis* infected suckling piglets: is there an actual profit? Vet Parasitol. 2009; 163(4): 362-365

Prac-012

Evaluation of Correlation between Purpura and Pathogens Infection of Nursery Pigs in Southern Taiwan

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Introduction: Skin disease causes a significant decrease in growth rate and feed efficiency in swine herd [1]. Purpura occur on limb, abdomen and in the perineal area is caused by an leakage of blood from a vasculature into the skin due to vasculitis [2]. The pathogens of purpura present in nursery pigs is still unknown. The purpose of this study is to evaluate the association among purpura, endotoxin and pathogens causing vasculitis which consist of porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), porcine circovirus type 3 (PCV3), *Mycoplasma hyorhinis* (Mhr) and systemic bacteria.

Materials and Methods: Thirty nursery pigs with purpura and thirty-two nursery pigs without pupura were selected from various herds of South Taiwan. Blood samples were collected from those pigs for pathogens detection, blood culture, platelet count, prothrombin time test (PT) and activated partial thromboplastin time test (aPTT). PRRSV, PCV2, PCV3 and Mhr were detected by real-time PCR. Endotoxin was detected by ELISA.

Results: Blood culture showed no growth of bacteria. Lesions group of PT was significantly higher than non-lesions group. Endotoxin, platelet count and aPTT had no statistical significance. The detection rate of PRRSV, PCV2, PCV3 and Mhr were 100%, 30%, 10% and 53% in lesions group, and 69%, 34%, 0% and 19% in non-lesions group, respectively. The results showed statistical significance at detection rate of PRRSV and Mhr. The odd ratios were detected to be 29.4 for PRRSV and 5.2 for Mhr.

Conclusions: PRRSV and Mhr may related to purpura. Nevertheless, septicemia could prolong PT and cause thrombocytopenia due to endotoxin [3]. Endotoxin activate NF-kB to cause endothelium cell apoptosis lead to hemorrhage [4]. However, endotoxin tolerance will inhibit proinflammatory cytokines production and delay NF-kB activation [5]. Non-lesions group may long-term exposure in endotoxemia result in different symptom.

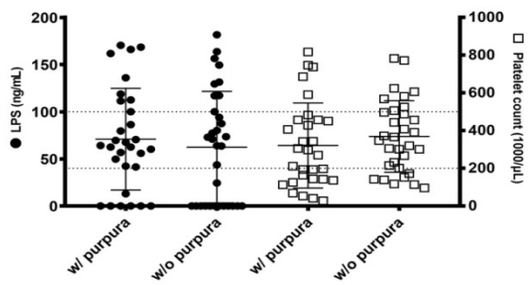

Figure 1. Concentration of endotoxin and platelet count. Dotted line represent normal range of platelet.

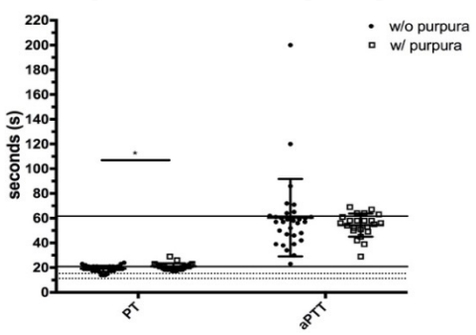

Figure 2. Coagulation parameters in two groups. Solid line represent normal range of aPTT, dotted line represent normal range of PT

References:

[1] Cargill CF et al., 1979. Vet. Rec. 104, 33-36
[2] Leung AK et al., 2001. Am. Fam. Physician. 64, 419-28
[3] Mavrommatis AC et al., 2000. Crit. Care. Med. 28, 451-457
[4] Dauphinee SM et al., 2006. Lab. Invest. 86, 9-2
[5] West MA et al., 2002. Crit Care Med. 30, S64-7

Prac-013

Evaluation of NeoPrime™ supplementation during pre- and post-weaned phases on selected fecal bacterial populations and growth performance

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Introduction: Supplementation of NeoPrime during the nursery phase has been shown to improve growth performance and reduce diarrhea in weaned pigs. The development of gut microflora is vitally important for pig health and performance during the transitions associated with weaning. This study was designed to test the effects of NeoPrime when supplemented both pre-and post-weaning (day 0-56 after birth) on selected microbial populations in feces.

Materials and Methods: On d 0, 16 litters of newborn piglets from a terminal line with similar body weight were randomized to 2 treatments. Eight litters received 2 oral doses of 300 mg of NeoPrime in water, one immediately after birth and the second 2 h after consuming colostrum. Piglets were offered NeoPrime-supplemented creep feed (0.15% w/w) from d 7 until weaning at d 21. The other 8 litters received isovolumic sham (water) doses with identical scheduling to the NeoPrime treatment. On d 21 when the pigs were weaned, 50 pigs from NeoPrime- or control-treated litters continued to be assigned to treatment with or without NeoPrime supplemented in the feed (0.15% w/w), respectively (10 pens/treatment and 5 pigs/pen). The effects of NeoPrime supplementation and growth phases as main factors were studied on selected fecal microbiota constituents. A qPCR-based method was used to determine concentrations of *Escherichia coli, Lactobacillus* spp., *Clostridium perfringens* and *Salmonella enterica* in fecal samples collected at three time points (i.e., d 21, d 35 and d 56). Suitable primers targeting the 16S rRNA gene for each targeted constituent were used. Standard curves were constructed from known concentrations of each reference bacterial strain genomic DNA and CFU plotted against the respective cycle threshold (Ct) value. Fecal sample DNA and CFU for each of the selected microbiota constituents were determined by interpolating the Ct values into the appropriate standard calibration curve. All microbial CFU data were transformed to respective $\log_{10}$ values before being analyzed. Repeated measure ANOVA was performed by using a mixed model approach (JMP 13).

Results: Pigs receiving NeoPrime treatment trended towards having greater weight gain at d 35 (10.57±0.62 vs. 8.99±0.54, P=0.07) and d 56 (57.16±2.17 vs. 50.51±3.01, P=0.09). The main factors of this analysis, treatment and growth phase, had demonstrable effects on selected fecal microbiota constituents. The main effects of the growth phase were evident on the abundance of *E. coli, Lactobacillus* spp. and *C. perfringens* (P<0.05). The abundance of *E. coli* and *Lactobacillus* spp. of pigs in the post-weaning phases (d 35 and d 56) were significantly higher than that of pigs in the pre-weaning phase (d 21, P<0.05), whereas the abundance of *C. perfringens* of post-weaning phases was significantly lower than that of the pre-weaning phase (P<0.05). NeoPrime treatment significantly reduced the abundance of *E. coli* compared to control treatment (P<0.05). Further there was an evident interactive effect between treatment and growth phase on *E. coli* abundance. NeoPrime resulted in a decreased *E. coli* population in the feces compared to control (P<0.05) on d 21 and significantly increased fecal *Lactobacillus* spp. population on d 56 (P<0.05).

Conclusions: Weaning can significantly affect selected fecal bacterial populations, suggesting a natural shift in the gut microbiota occurs during the phase. NeoPrime supplementation decreased the negative effects of weaning on performance, which may be partially attributed to a beneficial modulatory effect on the gut microbiota.

Prac-014

Evolution of lung lesion scores in The Philippine (2015-2018) using the Ceva Lung Program (CLP) application

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[1]*Ceva Animal Health (Philippines). Inc.*

Introduction: Lung scores vary between pigs, farms, and countries. For a meaningful assessment of respiratory health, it is important to correctly identify and quantify lung lesions during the slaughterhouse inspection [1]. Different from post-mortem investigation, it targets assessment of lung health in the whole batch of animals [2]. The objective of this study was to know the evolution of lung lesion scores in the Philippines since 2015, as scored using the Ceva Lung Program (CLP) App.

Materials and Methods: The data covers the period from April 2015 to December 2018. Two hundred fifty one (251) batches of pigs with a total of 16,115 lungs from different farms in the Philippines were scored using the Ceva Lung Program (CLP) application. Enzootic pneumonia (EP)-like lesions, cranial pleurisy, dorso-caudal pleurisy (*Actinobacillus pleuropneumoniae*-like lesions) and percentage of scarring were recorded.

Results: EP-like lesions: the affected lungs with bronchopneumonia ranged between 70-84%, 2016 being the lowest and 2018 the highest. The scars were found from 8, 3, 2, and 2% for the year 2015, 2016, 2017, and 2018 respectively. The % cranial pleurisy ranges between 13-23%, 2017 being the lowest and 2015 the highest. Dorso-caudal pleurisy was found from 40-28-32-36% for the year 2015, 2016, 2017, and 2018 respectively. The distribution of APP index (APPI) values ranges from 1.01 to 0.74.

Table 1. EP - like lesions

	2015	2016	2017	2018
%Bronchopneumonic Lungs	81	70	75	84
%Ave. Lungs with active pneumonia	20	11	11	13
%Scar score	8	3	2	2
%Cranial pleurisy	23	15	13	14

Table 2: App - like lesions

	2015	2016	2017	2018
%Dorsocaudal pleurisy	40	28	32	36
APP index	1.01	0.74	0.82	0.88

Conclusions: Based from the results, it is suggested that there is a high prevalence and extension of lung lesions in finishing pigs attributed to Enzootic pneumonia and *Actinobacillus pleuropmeumoniae* in the Philippines from 2015 to 2018. Though a high prevalence of lung lesions, there were some farms that achieved better lung lesion scores compared with the results, signifying that improvement is feasible.

References:

[1] Mazerolles P. (2015). Lung lesion survey using Ceva Lung Program in the Philippines and Vietnam compared to other countries, 7th APVS.

[2] Krejci R. (2013). Lung lesion survey in the Philippines, 6th APVS (OR6).

Prac-015

Field efficacy of an injectable combination containing toltrazuril and iron compared to Baycox® 5% oral in the control of coccidiosis in piglets

Octavio Orlovsky Eckhardt[1], *Luiz Felipe Lecznieski*[*1], *Kristina Streyl*[2], *Sofia Klein*[2], *Matthias Pollmeier*[2]

[1]*Global Veterinary Scientific Affairs Swine, Bayer Animal Health, Monheim, Germany;* [2]*Clinical Development, Bayer Animal Health, Monheim, Germany*

Introduction: Suckling piglets are often infected with coccidia, primarily *Cystoisospora suis*, resulting in non-hemorrhagic diarrhea. Toltrazuril is commercialized as an oral suspension (Baycox® 5%, Bayer Animal Health, Germany) which has been successfully used for prevention of *Cystoisospora suis* infections. An injectable combination of toltrazuril and iron simplifies the control of coccidiosis and anemia in piglets, but ensuring accurate toltrazuril dosing (20 mg/kg body weight) is essential to ensure short- and long-term efficacy of this molecule. The objective of this study was to compare the efficacy of toltrazuril plus iron combination administered intramuscularly at 20 mg toltrazuril per kilogram body weight to Baycox® 5% orally in the prevention of oocyst shedding and diarrhea in piglets in a farm with history of coccidial infections.

Materials and Methods: In total, 106 piglets from 12 litters naturally infected with *Cystoisospora suis* were included in this study. On the 2nd day of life (SD2) piglets were weighted and randomly allocated to the three Groups: A (control) - intramuscular injection of commercial iron (200 mg/animal, Ursoferran®); B - intramuscular injection of a combination of toltrazuril and iron (20 mg/kg and 91 mg/kg, respectively); or C - oral toltrazuril (20 mg/kg, Baycox® 5%) and intramuscular injection of commercial iron (200 mg/animal, Ursoferran®). Treatments were administered on SD3. From SD6 to SD14, individual fecal samples were collected and fecal consistency assessed on alternating days. Fecal samples were analyzed by fluorescence microscopy for the presence of oocysts and OPG calculated using a modified McMaster technique. Final weight was measured on SD20.

Results: Oocyst excretion was largely prevented in groups B and C while excretion was significantly higher in group A ($p<.0001$), Figure 1. In group A, median fecal scores at peak oocyst excretion were 2 (pasty). Fecal scores in groups B and C were normal during the whole trial. Total weight gain from SD2 to SD20 was higher in groups B (4,058g) and C (3,987g) when compared to group A (3,801g), Figure 2.

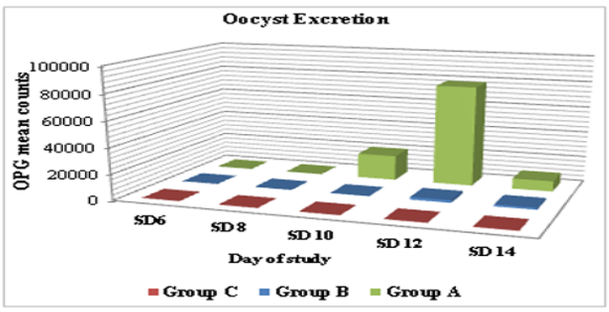

Figure 1. Average group oocyst excretion (oocyst per gram - OPG) according to the Day of Study.

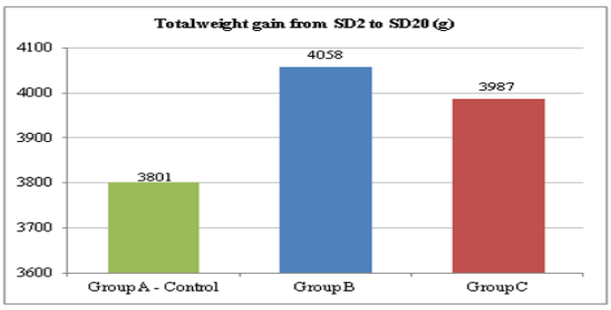

Figure 2. Body weight gain from SD 1 to SD 20.

Conclusions: The injectable combination of toltrazuril plus iron is efficacious in controlling coccidiosis in piglets when administered at 20 mg toltrazuril per kilogram body weight, ensuring equivalent results compared to commercial Baycox® 5% oral solution.

Acknowledgement: This work was supported by Bayer Animal Health, GmbH. Monheim, Germany.

References:

[1] Mundt HC, Joachim A, Becka M, Daugschies A. Isospora suis: an experimental model for mammalian intestinal coccidiosis. Paras. Res. 2006; 98(2): 167-75.

[2] Scala A, et al. Toltrazuril and sulphonamide treatment against naturally Isospora suis infected suckling piglets: is there an actual profit? Vet Parasitol. 2009; 163(4): 362-365

Prac-016

Genotypic analysis of *Enterocytozoon bieneusi* in pigs in Korea

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ntroduction: *Enterocytozoon bieneusi* is a type of microsporidia recognized as one of the most important pathogens that can infect a variety of hosts, including humans and pigs. *E. bieneusi* can be classified into several groups based on genotypes, including group 1, which contains zoonotic, and groups 2 to 9, which are known as the host adaptation group [1]. The purpose of this study was to investigate the *E. bieneusi* prevalence in domestic pigs in Korea and to investigate the relationship between breeding environment and parasite.

Materials and Methods: The fecal samples were collected from five provinces of Korea (Chungnam, Gyeongbuk, Gyeongnam, Jeju, Jeonnam) between May 2017 to April 2019. We obtained 949 DNA samples extracted using a commercial kit. To detect *E. bieneusi*, the ITS region was amplified by nested PCR [2]. Nucleotide sequencing and phylogenetic analysis was done for genotyping.

Results: Among 949 samples tested, 334 were positive to *E. bieneusi*. The overall prevalence rate was 35.2%. The infection rate was highest in the grower pigs (53.7%, between 2 - 3 months), followed by weaner (41.6%, between 1 - 2 months), fattener (36.1%, between 3 - 6 months), piglet (26.3%, less than 1 month) and sow (4.9%, older than 6 months) (P<0.01). The *E. bieneusi* infection rate in diarrheal feces was 39.5% which was higher than normal (30.9%) (P = 0.03). Regionally, the infection rate was highest in Jeonnam province (43.9%) (P = 0.01). By sequencing analysis, all the sequenced samples were classified into group 1.

Conclusions: The findings suggest that *E. bieneusi* in pig samples implicate potential zoonotic transmission. Considering these results, close monitoring and increased surveillance on *E. bieneusi* are needed for proper prevention and treatment.

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

Table 1. Prevalence of *Enterocytozoon bieneusi* according to age, fecal type, and region

Group	No. tested	No. positive (%)
Piglet (< 1 month)	114	30 (26.3)
Weaner (1 - 2 months)	380	158 (41.6)
Grower (2 - 3 months)	147	79 (53.7)
Fattener (3 - 6 months)	166	60 (36.1)
Sow (> 6 months)	142	7 (4.9)
Normal	476	147 (30.9)
Diarrheal	473	187 (39.5)
Jeju	37	5 (18.5)
Gyeongnam	736	277 (37.6)
Jeonnam	41	18 (43.9)
Chungnam	36	12 (33.3)
Gyeongbuk	109	22 (20.2)
Total	949	334 (35.2)

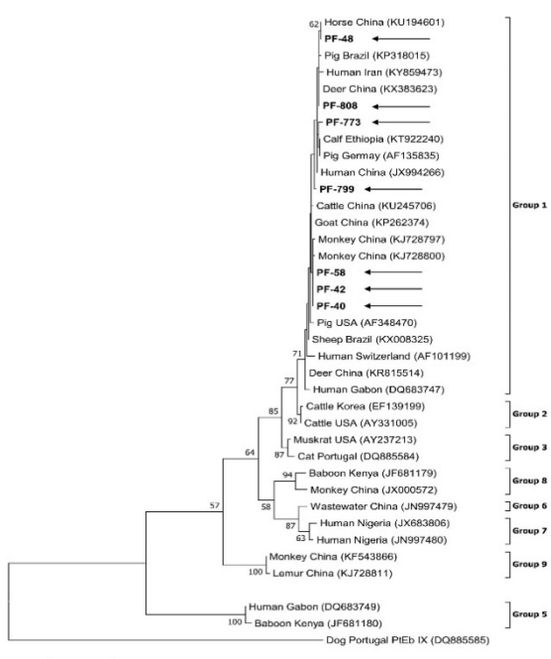

Figure 1. Phylogenetic tree of *Enterocytozoon bieneusi*

References:

[1] Li W, et al. 2016. Parasitol Vectors. 9: 142
[2] Lee JH. 2007. Parasitol Res. 101: 391 - 396

Prac-017

Gut microbiota profiles in sows when supplemented feed with multi-strains probiotic BACTOSAC-P during late gestation

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Introduction: Colonized gut microbiota are important for health and development of animal via gut fermentation, stimulating gut mucosal immune system, preventing pathogens [1]. In the present study, we evaluated the gut microbiota profile in late gestation and lactating sows after supplemented feed with multi-strains probiotic (BACTOSAC-P^{TM}) during late gestation of sows.

Materials and Methods: Altogether 40 pregnant-sows (at week 12th) were selected and equally divided into 2 groups (control and treatment). Control-group was fed with normal feed while treatment-group was daily fed with normal feed plus 5 grams of BACTOSAC-P^{TM} (powder form) until farrowing. The pool samples of feces were collected from both group sows at week 12th of pregnancy and at 1 week after farrowing. Total DNA was extract from fecal sample using QIAamp Stool DNA Extraction kit (QIAGEN, Germany). 16s rRNA was amplified from pool DNA sample and then sequenced by Illumina MiSeq platform. Overlapping paired end reads were assembled using PEAR. The reads were processed using Quantitative Insights into Microbial Ecology (QIIME) v1.9.0 for OTU picking and taxonomy.

Results: The results of genome (DNA) sequencing at the level of phylum are presented in Figure 1. A total of 16 phyla were found in the feces samples of sows in this experiment. Among which, *Firmicutes* and *Bacteroidetes* were the largest shares comparing more than 87.5% of the total sequences, *Firmicutes* for more than 78.9% and *Bacteroidetes* for approximately 8.6%. At the genus level, the most abundant genera containing *Clostridium, Lactobacillus, Ruminococcaceae* and *Turicibacter*. A higher proportion of *Firmicutes/Bacteroidetes* was observed in control group than that in treatment group. From the fecal sample microbiota profile, a lower rate of fecal shedding of *Lactobacillus* was found in treatment group than control group, especially at 1 weeks after farrow (1.84 vs 2.06%, respectively). In contrast, the proportion of *Clostridium* shedding in feces was higher in control and in the treatment group at the same period of study (15.91 vs 14.40%, respectively).

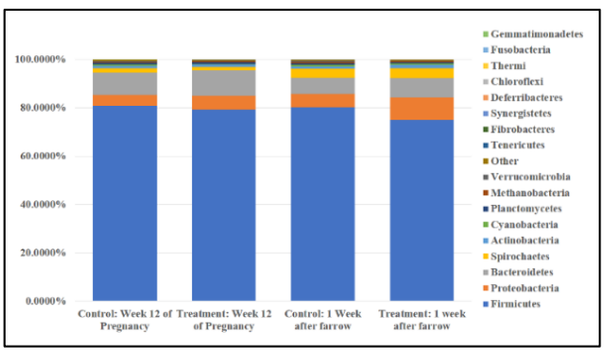

Figure 1: Distribution of the phylum as a percentage of the total number of identified 16s rRNA sequences from fecal samples of the sows.

Discussion and Conclusion: The gastrointestinal tract ecosystem is complex, and play role in both promote health and prevent disease. From present study, we found was *Firmicutes* and *Bacteroidetes* are the dominant phyla in pregnant and lactating sow's gut. Changes in microbial proportions were observed due to the change of production stage. More important, sow fed with BACTOSAC-P^{TM} seem to have a higher *Lactobacillus* in their gut than instead of shedding in the feces.

Acknowledgement: This work was supported by a grant from Mahidol University and K.M.P. Biotech. Co. Ltd., Thailand.

References:

[1] C. G Buffie and E. G Pamer, "Buffie CG, Pamer EG.. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 13: 790-801," *Nat. Rev. Immunol.*, vol. 13, 2013.

Prac-018

Identification of Genotype and Phenotype of Antibiotic Resistance of *Escherichia coli* Isolates from Pigs in Southern Vietnam

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Introduction: At present, the resistance of *E. coli* changes very fast and the phenomenon of multiple resistance to multiple antibiotics at the same time accounts for quite high [4]. The increasing prevalence and diversity of antibiotic resistance (AMR) genes of *E. coli* are of concern to the world's current use of antibiotics. Many AMR genes on *E.coli* have been identified, in which genes have been used extensively in prior studies such as aadA1, dfrA12, strA, strB, sul1, sul2, sul3, CatA, CatB, tetA, tetB, blaTEM, blaSHV, blaOXA2, cmlA and other related gene groups. The most commonly encountered antibiotic resistance also corresponds to the most commonly used antibiotics [2]. The study was conducted to determine the characteristics and correlation between genotype and phenotype of AMR of *Escherichia coli* isolated from commercial pig farms..

Materials and Methods: Ninety pigs' fecal samples were collected from 3 age groups such as weaners, growers and finishers from 10 pig farms in the two Southern regions of the South East and Mekong Delta. 3 stool samples were collected for each age group of pig in a farm. A random sample (25 g faeces) was randomly collected from 3 pigs of the same age group and then pooled into 1, called a sample. The presence of seven AMR genes (blaTEM, aadA1, strA, dfrA12, Sul3, cmlA and tetA) were determined by PCR [1,3,5] and AMR phenotypes to antibiotics (common-used 20 antibiotics) were identified by the method of determining the minimum inhibitory concentration (MIC).

Results: The prevalence of seven AMR genes of *E. coli* was very high, including weaned pigs (95.71%), growers (97.62%) and finishers (94.76%). In general, this prevalence by region was 93.02% in the South East and 97.78% in the Mekong Delta. Otherwise, the ratio of phenotypic antibiotic resistance was hugely varied among 20 antibiotics (0 to 100%), average of 43.0% (weaners), 39.5% (growers) and 36.3% (finishers). 42.7% phenotypic AMR was showed in the South East region while 38.4% in the Mekong Delta. The multi-resistance based on identification of genotypic and phenotypic evidences were revealed in several *E. coli* isolates however the poor correlation was found between the genotype and phenotype of the AMR of *E. coli* in this study.

Table 1. The correlation between AMR genotype and phenotypic of *E. coli*

Rate of AMR genes identified [n (%)]		Rate of AMR identified		Correlation (Kappa)
		Antibiotics	Rate [n (%)]	
blaTEM	87 (96,67)	Ampicillin	89 (98,89)	K= 0,492
		Amoxicillin	89 (98,89)	K= 0,492
		Amox/clav	6 (6,67)	K= 0,005
		Cephalexin	4 (4,44)	K= 0,003
		Cefpodoxime	4 (4,44)	K= 0,003
		Ceftiofur	4 (4,44)	K= 0,003
		Imipenem	4 (4,44)	K= 0,003
		Piperacilin	53 (58,59)	K= 0,094
aadA1	89 (98,89)	Amikacin	0	
		Gentamicin	36 (40)	K= 0,015
		Tobramycin	25 (27,78)	K= 0,009
strA	74 (82,22)	Amikacin	0	
		Gentamicin	36 (40)	K= 0,252
		Tobramycin	25 (27,78)	K= 0,154
dfrA12	86 (95,56)	Trime/sulfa	63 (70)	K= 0,196
Sul3	89 (98,89)	Trime/sulfa	63 (70)	K= 0,051
cmlA	89 (98,89)	Chloramphenicol	88 (97,78)	K= 0,662
tetA	88 (97,78)	Tetracyclin	87 (96,67)	K= 0,795

Conclusions: The results indicated that the incidence of genotypic and phenotypic antimicrobial resistance is high in surveyed isolates of *E. coli.*

Acknowledgement: This study was conducted by NLU's research team and White Ocean Vet Co., Ltd, Hochiminh city, Vietnam under the funding of Huvepharma (Thailand) through project number No. 201901.

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Prac-019

Improvement in Production and Reproductive Performances after Replacing Type I (EU) PRRS Vaccine with Whole Herd Ingelvac® PRRS MLV.

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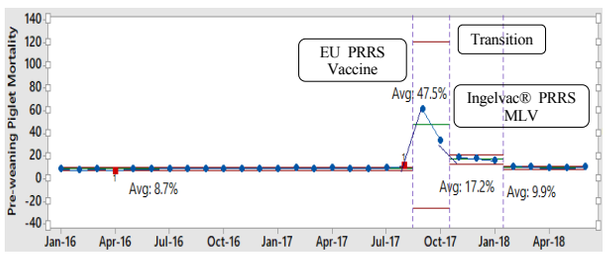
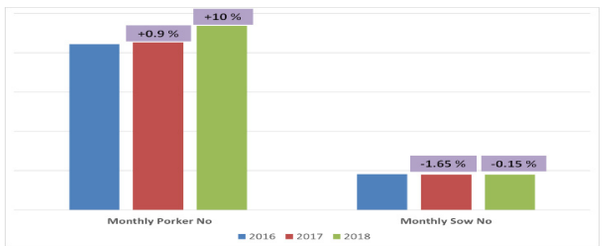
Introduction: It has been shown that pigs vaccinated with type 2 (US) PRRS modified live vaccine (MLV) has the highest survival rate in experimental HP-PRRS challenge; while type 1 (EU) killed vaccine has the lowest survival rate as compared to other vaccines. Furthermore, immunization by EU PRRSV only provide protection against secondary homologous EU PRRSV challenge but not heterologous US PRRSV. This study reports a case of PRRS outbreak, followed by improvement of farm production and reproductive performance after replacing EU PRRS vaccine with whole herd Ingelvac® PRRS MLV (US strain).

Materials and Methods: The farm is a single site, farrow to finish farm in Malaysia. In September and October 2017, the farm experienced a spike in pre-weaning piglet mortality as high as 47.5% in one unit; increased nursery mortality at 20%; high abortion rate at 15%; and increased mummified fetus percentage to 5%. Necropsy was performed with findings of lymphadenopathy and starvation. Lymph nodes and serum samples were sent for laboratory diagnosis. Rt-PCR results showed that the samples were negative for classical swine fever virus; serum samples of 1 and 4 weeks old (to be weaned) piglets were tested positive for US and EU strain PRRSV (54% positive for US strain PRRSV). While ELISA results showed that the farm is negative for Aujeszky's disease. With the diagnosis, the farm decided to stop EU PRRS MLV in breeders and EU PRRS inactivated vaccine in porkers, and started to use Ingelvac® PRRS MLV whole herd (breeders and piglets) mass vaccination in November 2017, followed by sow booster vaccination a month later and revaccinate every 3 months. Farm performance data were collected and followed up from Jan 2016 to June 2018. Serum samples were taken every quarter after change of vaccination program to test for PCR against PRRSV.

Results: The average pre-weaning piglet mortality has declined drastically from 47.5% during outbreak to 9.9% during the first half of 2018 **(Graph 1)**. After using Ingelvac® PRRS MLV, total porker number increased by 10% in 2018 despite sow number reduced by 0.15% as compared to 2017 **(Graph 2)**. Reproductively, farrowing rate improved from 78.1% to 83%; piglets born alive per litter increased from 10.2 to 10.8; while pigs weaned per sow per year improved from 20.02 to 23.41. Furthermore, rt-PCR results showed that there is reduction in percentage of PRRS positive pig from the initial 54% in October 2017 to 0% in June 2018. Results are summarized in Graph 1 & 2.



Graph 1: Pre-weaning piglet mortality.



Graph 2: Total porkers vs sows no by year.

Conclusions: The drastic reduction of pre-weaning piglet mortality in this case once again shows that Ingelvac® PRRS MLV provides better protection as compared to EU PRRS vaccine in where US PRRSV is prevalent. While the increase in number of porker despite reduction in sow population indicates that farm efficiency has been increased together with the improvement in sow reproductive performance and porker survivability. These results shows that whole herd mass breeder and piglet vaccination with Ingelvac® PRRS MLV provides high level of protection, and improving overall farm performance and efficiency besides being safe to be mass-vaccinated in breeders.

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Prac-020

Macrolide resistance and related genes of *Streptococcus suis* from clinically healthy pigs

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Introduction: *Streptococcus suis* is an emerging zoonotic pathogen associated with pigs and can also cause severe systemic infections in humans. The two large outbreaks of human infection in 1998 and 2005 in China caused 52 deaths out of 229 cases [1]. Since 2005, *S. suis* infection in pigs becomes very common, and very diverse *S. suis* isolates have been identified from disease pigs in different geographic regions in China. Among these isolates, high rate of antimicrobial resistance (AMR) even multi-drug resistance (MDR) is becoming an important concern for disease control and public health. Resistance to tetracycline and macrolide-lincosamide-streptogramin B (MLSB) was much prevalent among the *S. suis* isolates from disease pigs [2]. However, it is not clear about the situation in clinically healthy pigs. Our previous investigation revealed more prevalence and diversity of *S. suis* isolates from the clinically healthy pig herds than those from disease pigs [3]. Macrolides are one of the most frequently used antibiotics in pig industry. This study aimed to analyze antimicrobial susceptibility of 186 *S. suis* isolates from clinically healthy pig herds to four common-used macrolide antibiotics, and to identify the corresponding antimicrobial resistance genes (ARGs) by whole genome sequencing.

Materials and Methods: The 186 *S. suis* isolates used in this study were obtained from ten clinically healthy pig herds in five large-scale and five small-scale pig farms in central and southern China, and genomes of the isolates were sequenced and analyzed. Antimicrobial susceptibility tests of 4 commonly used macrolide antibiotics were performed on the 186 isolates by trace broth dilution method according to the CLSI-2017 (Clinical and Laboratory Standards Institute). The 4 macrolides including erythromycin (ERY), tiamulin (TIA), tylosin (TLS) and tilmicosin (TMS) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *Streptococcus pneumoniae* ATCC 49619 was used as a quality control.

Results: *S. suis* isolates from healthy pig herds were 59.1% sensitive to tilmicosin, 28.5% , 21.2% and 5.4% sensitive to tiamulin , erythromycin and tylosin respectively. The antimicrobial susceptibility results indicated that the AMR rate was highest for tylosin (94.6%), followed by erythromycin (78.5%), tiamulin 38.7%), and tilmicosin (35.5%) (Table 1). The ARGs distributions among the isolates were shown in Table 2. The 167 isolates were detected with at least a erythromycin resistance gene *erm*B (65.15%), *mef*A (1.6%), *erm*T (0.5%), and with two genes or three genes, *erm*B + *erm*A (16.1%), *erm*B + *mef*A (1.6%), *erm*B + *erm*T (0.5%), *erm*B + *mef*E + *msr*D (2.2%), *erm*B + *erm*A + *erm*T (0.5%) and at most 4 genes *erm*A + *erm*B + *mef*A + *msr*D (0.5%), while another 21 isolates were detected none corresponding ARGs. The results revealed that erythromycin resistance genes *erm*B is the most prevalent ARGs, and multiple ARGs exist in the S. suis isolates from healthy pigs.

Table 1. MIC of macrolide antibiotics in 186 *S. suis* isolates from clinically healthy pigs

Antibiotic	MIC50 (μg/mL)	MIC90 (μg/mL)	Isolates (%)		
			S	I	R
ERY	≥64	≥64	40(21.5)	0	146(78.5)
TIA	32	64	53.(28.5)	61(32.8)	72(38.7)
TLS	≥512	≥512	10(5.4)	0	176(94.6)
TMS	2048	≥2048	110 (59.1)	106(57)	66(35.5)

Table 2. The distributions of macrolide resistance genes in 167 S. suis isolates in healthy pigs

Resistance genes	No of isolates (%)
*erm*B	121 (65.1)
*mef*A	3 (1.6)
*erm*T	1 (0.5)
*erm*B + *erm*A	30 (16.1)
*erm*B + *mef*A	3 (1.6)
*erm*B + *erm*T	1 (0.5)
*erm*B + *mef*E + *msr*D	4 (2.2)
*erm*B + *erm*A + *erm*T	1 (0.5)
*erm*A + *erm*B + *mef*A + *msr*D	1 (0.5)

Conclusions: This study revealed that the AMR situation of *S. suis* from clinically healthy pigs to macrolides is serious, especially to tylosin and erythromycin. The corresponding ARGs may be an important source of AMR of pathogenic bacteria.

Acknowledgement: This work was supported by the International S & T Cooperation Program of China (2013DFG32360).

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Prac-021

Oestrus synchronization of sexually mature gilts by an altrenogest oral solution and effect on reproductive performances in a Chinese herd

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Introduction: Altrenogest is a synthetic analog of progesterone widely used in gilts to synchronize oestrus. Increased prolificacy of gilts treated by altrenogest has also been shown in Europe and Korea [1-2-3]. This study was performed to confirm safety and efficacy of an altrenogest oral solution in a Chinese herd according to a controlled, blinded and randomized design.

Materials and Methods: Study was performed in a Chinese farm owning 5,000 sows (American pure bred Yorkshire, Landrace and Duroc). One hundred twenty six sexually mature replacement healthy gilts were randomly allocated to 2 groups of 63 gilts each. Group T received an altrenogest oral solution at the posology of 20 mg/d for 18 days (5 ml/d of Virbagest®, Virbac) while group C received a placebo. Clinical examination was performed daily during treatment. Oestrus was detected twice daily with a boar (as standing reflex) during 6 weeks from start of treatment. Artificial insemination was performed twice respectively 12 and 24 h after standing reflex detection. Pregnancy diagnosis was performed by ultrasonography 4 weeks after insemination. Pregnant gilts were followed till farrowing with individual weighing of piglets at birth and weaning. Return to oestrus was checked during 10 days post weaning. Gilts oestrus range was defined as the delay between the first and last gilts coming into heat. Oestrus grouping over 3 days was defined as the maximum oestrus rate over 3 consecutive days among the gilts showing heat. Conception rate was the rate of pregnant gilts among inseminated gilts. The farrowing rate was calculated among pregnant gilts. Groups were compared by the t test for quantitative data and by the chi-square test for categorical data.

Results: No clinical disorder was noticed during treatment. The rate of gilts showing oestrus was not significantly different between groups (95% in group T and 98% in group C). However oestrus range was 3-fold shorter in group T, grouping over 3 days being significantly higher (occurring between 6 and 8 days after the stop of treatment). Conception and farrowing rates were similar between groups. The number of born alive/weaned piglets per litter and the birth weight were significantly higher in group T. Post weaning return to oestrus over 10 days was numerically higher in group T.

Table 1. Reproductive performances of gilts

Group	T	C
Oestrus range (days)	5	15
3 days oestrus grouping	85%[a]	34%[b]
Conception rate	96.7%[a]	93.6%[a]
Farrowing rate	98.3%[a]	96.6%[a]
Born alive per litter	11.7±2.2[a]	10.5±2.9[b]
Birth weight (kg)	1.5±0.1[a]	1.4±0.1[b]
Weaned per litter	11.6±1.6[a]	10.6±1.5[b]
Weaning weight (kg)	6.7±0.6[a]	6.5±0.6[a]
Ten days post weaning return to oestrus rate	96.3%[a]	90.4%[a]

[a, b]: Different superscripts indicate significant differences

Conclusions: Safety and efficacy of the tested drug was confirmed in the synchronization of gilts oestrus. Higher prolificacy was also proven. This effect was previously explained by increased ovulation rate without change of foetal survival [4-5]. Increased weight of piglets issued from altrenogest treated gilts should be confirmed by further investigations.

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Prac-022

Pharmacodynamic Study of Chlortetracycline 20% Water Soluble in Thai Pigs

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Introduction: Pharmacodynamic (PD) of antimicrobials (AMBs) is their effects on microorganisms. For an AMB to be effective, its concentration at the target site of action plays an important role. The AMB concentration(s) can be determined through pharmacokinetic (PK) studies. The relationship between PK and PD, thus, is of importance for clinical outcomes when treating microbial infections. Minimum Inhibitory Concentration (MIC) is commonly used as the PD measurement [1, 2]. Tetracyclines (TCs) are bacteriostatic antimicrobials which their concentrations above the MIC level over time are useful measurement for their antimicrobial efficacy [2]. For chlortetracycline (CTC), the ratio of AUC:MIC is also used for measurement [3]. However, the data of TCs PD study is still lack [4]. Our study aimed to determination PD of CTC 20% Water Soluble product (Farmcare® CTC Soluble Powder 20% & Omnisol200® water soluble) for *Actinobacillus pleuropneumoniae* (APP), *Haemophilus parasuis* (HP), *Pasteurella multocida* (PM), and *Streptococcus suis* (SS) isolates from Thai pigs during 2017-2018.

Materials and Methods: PK parameters of CTC studied in nine Landrace pigs following single oral dose of CTC20%WS equivalent to 40 mg CTC/kg bw (Table 1) and the MIC_{50} and MIC_{90} of APP, HP, PM, and SS (Table 2) were used for the determination of PD by using the ratio of C_{max}:MIC and AUC:MIC [1].

Table1. Pharmacokinetic parameters of chlortetracycline in plasma

Parameters	Units	Mean ± SD
C_{max}	µg/mL	3.61 ± 0.77
AUC_{∞}	µg-hr/mL	27.43 ± 5.26

Table 2. MIC of chlortetracycline studied from 2017-2018 isolates (N_{total}=40).

MIC (µg/ml)	APP	HP	PM	SS
MIC_{50}	2	2	2	1
MIC_{90}	8	2	2	2

Results: PD of CTC20%WS are illustrated in Table 3. The concentrations of CTC in plasma and the MIC_{50} level are illustrated in Figure1.

Table 3. PD data of CTC20%WS

PD	C_{max}:MIC		AUC:MIC	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
APP	1.81	0.45	13.72	3.43
HP	1.81	1.81	13.72	13.72
PM	1.81	1.81	13.72	13.72
SS	3.61	1.81	27.43	13.72

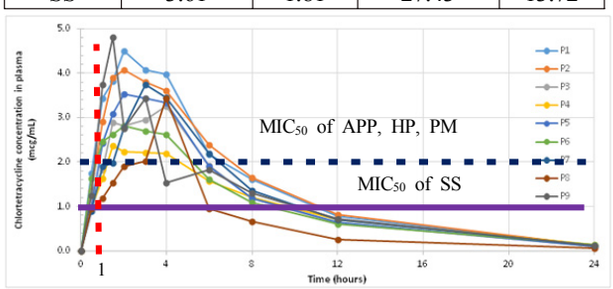

Figure 1. Concentration of CTC in plasma of each pigs (N=9) and the MIC_{50} level of APP, HP, PM, and SS.

Conclusions&Discussion: According to the PK and PD data, plasma concentration of CTC from CTC20%WS at the dosage of 40 mg CTC/kg bw exceeds the MIC_{50} of APP, HP, PM at 1.0 hour and of SS at 0.5 hour post administration. In addition, the plasma concentration of CTC exceeds the MIC_{90} of HP, PM, and SS at 1.0 hour post administration. This indicates that CTC20%WS can start its activity within an hour against studied pathogens. Even though the ratio of C_{max}:MIC and AUC:MIC is not quite high, but the preliminary PD data demonstrate the *in vivo* efficacy of CTC20%WS on APP, HP, PM, and SS. Further study of PD on lung and other tissues such as tonsil and lymph node are recommended since concentration of chlortetracycline in lung and other tissues should normally be greater than in plasma [2, 5].

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Prac-023

Pharmacokinetic Study of 20% Water Soluble Chlortetracycline in Thai Swine

Kanjana Imsilp[1], *Amnart Poapolathe*[1], *Saranya Poapolathep*[1], *Phanwimol Tanhan*[1], *Usuma Jermnak*[1], *Narumol Klangkaew*[1], *Napasorn Phaochoosak1*[1] , *Netchanok Malingam*[2], *Waraporn Poomngam*[2], *Montri Rodpaitoon*[2], *Rachen Khusakul*[3], *Orada Mora*[4], *Piyakorn Thongsuk*[4], *Metta Makhanon*[*5]

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Introduction: Antimicrobial administration in infected animals play a crucial role in swine diseases containment. The administration in water soluble (WS) forms of antimicrobials currently becomes more efficient than in-feed medication for the prudent and successful treatment. Pharmacokinetic (PK) parameters have been used to assure that delivered antimicrobials can reach systemic circulation and its effective concentration become available at the target organs. Chlortetracycline (CTC) is a broad- spectrum antimicrobial used in the swine industry. One study reported that mean plasma concentrations for days 1-6 following in-feed administration were 0.14 ± 0.06, 0.36 ± 0.12, and 0.75 ± 0.19 µg/mL for 100, 400, and 1000 mg CTC/kg of feed, respectively [1]. Our study aimed to determine chlortetracycline PK parameters following single oral administration of a 20% WS CTC product (Farmcare® CTC Soluble Powder 20% & Omnisol200® water soluble) in a Thai commercial farm.

Materials and Methods: Nine Landrace pigs withdrawn from antibiotics for 14 days were used in this study. All animals were fasted for 12 hours prior to CTC administration. Each animal was orally given the 20%ws CTC at the dosage of 40 mg CTC/kg body weight. Blood samples were collected from every animal at time 0, 30, 60, 90 minutes and 2, 3, 4, 6, 8, 12, 24, and 32 hours. Each blood sample was centrifuged and plasma was collected and stored at -80℃ until analysis (*Total plasma samples of 126 samples*). The analysis was performed using a method modified from Prado *et al*. (2015) [2] and Sunderland *et al*. (2003) [3]. An Agilent 1260 HPLC system (Agilent Technologies, USA) was used to determine CTC concentration in plasma sample analysis. PK parameters, were calculated using PK solutions 2.0x software. Statistical determination was performed using Microsoft Excel 2013 software.

Results: Concentrations of CTC in plasma and PK parammeters after oral administration are shown in Figure 1 and Table 1, respectively.

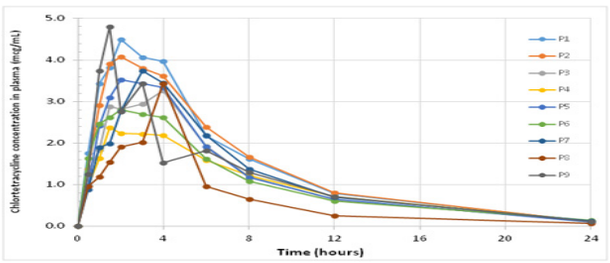

Figure 1. Concentrations of chlortetracycline (CTC) in plasma of pigs (n=9) receiving single oral dose of 20%WS CTC equivalent to 40 mg CTC/kg bw.

Table 1. Pharmacokinetic parameters (Mean ± SD) of chlortetracycline following single oral dose of 20%WS CTC equivalent to 40 mg CTC/kg bw in pigs (n = 9).

Parameters	Units	Mean ± SD
C_{max}	µg/mL	3.61 ± 0.77
T_{max}	Hr	2.44 ± 0.98
$t_{1/2}$	Hr	4.88 ±0.62
$AUC_{(0-t)}$	µg-hr/mL	26.63 ± 5.26
AUC_{∞}	µg-hr/mL	27.43 ± 5.26
$AUMC_{\infty}$	µg-hr*hr/mL	191.59 ± 34.53
MRT	Hr	7.00 ± 0.51
CL	mL/hr/kg	1,514.99 ± 343.01

Conclusions: The average maximum concentration (C_{max}) of chlortetracycline (3.61 ± 0.77 µg/mL) was reached at 2.44 ± 0.98 hours following single oral administration of 20% WS CTC at 40 mg CTC/kg bw. The elimination half-life ($t_{1/2}$) was 4.88 ± 0.62 hours with mean residence time (MRT) of 7.00 ± 0.51 hours. The result shows that this water soluble CTC product is a better choice for swine drug administration than in-feed medication based on its PK.

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Prac-024

Pig borne zoonoses in bangladesh; a neglected public health concern

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Introduction

Pigs are raised in the South-Asia (India, Nepal, Bhutan, Bangladesh) mainly by poor peoples as a means of poverty alleviation (Nahar et al. 2012). Pig raising is getting importance due to easy rearing with natural resources, high number of piglet born, high disease resistance and low production cost. Pigs play an important role in the livelihood of rural communities. A significant percentage (20-30%, even up to 41%.) of rural household income is contributed by pigs. Pigs are source of zoonoses as intermediate host and potential reservoirs for various disease agents and cause fatal disease. Zoonotic diseases transmitted by pigs include taeniasis or cysticercosis/ neurocysticercosis, trichinellosis, swine influenza, Nipah virus infections, Japanese encephalitis etc. Pig borne zoonoses is not only the local health problem of pig raisers, but also emergence of a new disease can also appear as a global threat, as like swine flu. Pigs as a zoonotic reservoir have not been explored in detail in Bangladesh. It has a great value to know details about pig borne zoonoses as it has significant socioeconomic impacts on rural ultra poor people of Bangladesh. In this review, pig raising in Bangladesh, human pig interactions, pig borne zoonoses with emphasis on intervention strategies is briefly described.

Pig raising in Bangladesh

Pig raising has traditionally been part of the lifestyle of the ethnic/tribal communities living in different regions of Bangladesh. In Bangladesh, the domestic breeds of pig are reared on garbage, kitchen waste and human excreta. Small-scale pig rearing (1-3 pigs) in the backyard is the most predominant practice in Bangladesh. There are some small-medium size farms which houses 10-500 pigs. Pigs are raised by certain rural people who are educationally, economically and socially most backward. Traditional pig raising in Bangladesh increases the risk of exposure to pig borne zoonoses (Nahar et al. 2012; Nahar et al. 2013. The humans live very close to their pigs and often touch, caress, and feed them, exposing themselves to their saliva and feces (Nahar et al. 2013).

Pig borne zoonoses in Bangladesh

The pig raisers in Bangladesh are completely unaware that disease can transmit from pigs to humans. They are frequently come in close contact with sick pigs, sometimes even slaughter and consume the sick pig, increasing the risk of pig-to-human transmission (Nahar et al. 2012). There is very limited evidence of pig borne zoonoses in Bangladesh although several pathogens are present which can infect both pigs and people. Japanese encephalitis is endemic in pigs (Khan et al. 2014) thus might have possibility to transmit in pig raisers. Neurocysticercosis (NC) cases were recorded from human (Biswas and Debnath, 2015).

Conclusion

Traditional pig raising has cultural and economic implications to the minority community of Bangladesh. This may put them at risk of transmission of fatal diseases from their pigs. Intervention that reduces the risk of zoonotic disease is indeed a necessity for poor pig raisers. The traditional pig husbandry practices might be crucial with disease transmission. Thus, a good pig husbandry intervention that emphasizes the health benefits to both pigs and people could help reduce the risk of zoonotic disease in minority community of Bangladesh.

Acknowledgements

We acknowledge all the authors of articles related to pig raising and pig-borne zoonoses in Bangladesh.

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Fig 1 Feeding on garbage, kitchen waste.

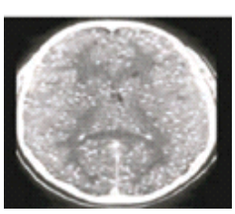

Fig 2 Brain with NCC (Debnath, et al., 2015).

Prac-025

Plasma disposition kinetics and distribution of toltrazuril and its main metabolite in intestinal tissues and contents of piglets after oral and intramuscular administrations

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Introduction:
Porcine coccidiosis caused by *Cystoisospora suis* is a major cause of diarrhea and poor growth in piglets worldwide. The only effective chemotherapeutic drug available for the control is oral toltrazuril. Iron deficiency anaemia (IDA) is also an important cause of poor growth, and is prevented by intramuscular injections of iron complexes. Recently, the first toltrazuril-iron based combination for injection has been developed and registered for the concomitant prevention of coccidiosis and IDA in piglets (Forceris®, Ceva, France). This study aimed to evaluate, the disposition kinetics of toltrazuril and its main metabolite in the plasma and predilection tissues of *Cystoisospora suis* after oral (Baycox® 5%) and intramuscular (Forceris®) application of toltrazuril in piglets.

Material and methods:
56 piglets from 4 litters were included and randomly allocated to two treatment groups.
Group A, 29 piglets were treated with Forceris® on the second day of life (24h+) at the recommended label dose (1.5 ml per piglet).
Group B, 27 piglets were treated with intramuscular iron dextran (Uniferon® 200) on the second day of life (24h+) at the recommended label dose (1 mL per piglet) and oral toltrazuril (Baycox® 5%) on the fourth day of life (72h+) (0.4 mL/kg bodyweight). Animals (minimum 4 piglets per time point and per treatment) were sacrificed at 1, 5, 13 and 24 days post-treatment. From each piglet, the following samples were collected: Blood, jejunum, ileum, jejunum content, ileum content. Concentrations of toltrazuril and its active metabolite (toltrazuril sulfone) were determined using validated HPLC-UV method.

Results:
On overall, intramuscular application resulted in significantly higher and more sustained concentrations in plasma, intestinal tissue (ileum and jejunum) and intestinal content in our study. Higher tissue concentrations after oral dosing were observed only immediately after dosing (Day 1): jejunum (6.29 µg/g) and ileum (3.67 µg/g). Remarkably, toltrazuril and toltrazuril sulfone accumulated more in proximal intestinal segment (jejunum), independently of the administration route.

Conclusions:
Drug concentrations at the predilection site of the parasite are important for its pharmacological effects. *C. suis* is an intracellular parasite affecting enterocytes in the jejunum and ileum. Higher and more sustained concentrations were observed following IM application, which may be responsible for its higher anticoccidial activity (significant reduction of oocyste excretion).

Prac-026

Porcine Model of Peritoneal Fibrosis and Its Applications for Compound Efficacy Evaluation and Regenerative Medicine

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Introduction: Patients with kidney failure rely on life-saving peritoneal dialysis (PD) to facilitate waste exchange and maintain homeostasis of normal physical conditions. However, PD itself often results in peritoneal fibrosis (PF) and organ adhesions that compromise the efficiency of PD or normal functions of visceral organs[1,2,3]. Upon PF, mesothelial cells from the peritoneum were destroyed and resulted in ultrafiltration failure, or in the worst scenario, the development of encapsulating peritoneal sclerosis[4, 5]. Although rodent models had delivered useful clues on the pathogenesis of PF, their physiological and anatomical dis-similarities to human limit their further applications on the evaluation of therapeutic efficacy. In this study, we established for the first time, porcine model of peritoneal fibrosis by the use of sodium hypochlorite (SHC).

Materials and Methods: Peritoneal fibrosis and visceral organ adhesion were induced in 5-week-old piglets by intraperitoneal injection of 30 ml/kg B.W., 0.05%-0.2% of SHC. Antemortem (laparoscopy examination, biopsy) and post-mortem analyses (pathology and cytokine analyses) were performed to monitor the progression and the severity of peritoneum/visceral organ fibrosis and adhesion.

Results: From both laparoscopy examination and biopsy, we observed a dose-dependent severity of PF induced by SHC. Histological analyses of abdominal wall, liver, omentum and duodenum showed increased thickness of submesothelial compact zone with significant collagen deposition. Immunofluorescent studies revealed the fragmentation of ventral peritoneum, the proliferation/ accumulation of myofibroblasts. Moreover, acute inflammatory cytokine, IL-1β and TNF-1β , but not IL-10 were elevated in day2 post injection and decrease at day4 to day7.

Conclusions: No current PF animal model shares high physiological and anatomical similarities with human. Therefore, our pig model could provide an alternative and more suitable platform for the study human PF and intra-abdominal organ adhesions. It can also be used to evaluate the efficacy of potential candidates on the prevention (e.g. compounds) and treatments (e.g. stem cells) for peritoneal fibrosis.

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Prac-027

Quantitative Determination of flumethasone in Pig liver by surrogate analyte-based LC-MS/MS method

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Introduction: Flumethasone is one of the most administered corticosteroids to treat inflammatory reactions, disorders of the musculoskeletal, respiratory, and gastrointestinal disorders in farm animals. Residues of corticosteroids in farm animals cause a public health risk since they may have pharmaceutical and toxicological effects for consumers. Therefore, we need to monitor its residue. In addition, application of rapid and effective samples preparation process and analytical method are necessary. The purpose of this study aimed to establish the analysis method of residual residual flumethasone in pig liver.

Materials and Methods: liver tissues were homogenized (10g) and spiked with flumethasone at the concentration 1ng/g (n=3). The modified QuEChERS extraction method was used for samples preparation of liver tissues. Surrogate standards were employed to generate calibration curves. Analysis was performed by Shimadzu Nexera LC interfaced to an ABsciex QTrap 6500 mass spectrometer (LC-MS/MS). The chromatographic column was a C18 (2.1 mm x 100 mm, 3.5 ㎛). The mobile phase consisted of 5mM ammonium acetate and 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), and the gradient was used as mentioned below: 30% B (0~3 min) - 60% B (7~16min) - 30% B (16.1 min - 17min). The source conditions were optimized to obtain two identification points (precursor: 411.3, product: 253.2/ 121.2 m/z).

Results: Concentration response showed linearity within the concentration range (R2 > 0.998). Recoveries of flumethasone were between 92.26%. The coefficient of variation observed was 5%. The limit of quantification (LOQ) and detection (LOD) were 1.79 and 0.59ng/g with external standard curve.

Conclusions: Quantitative determination of flumethasone with surrogate standard was successfully applied in pig liver when the analytical recoveries were not sufficiently high. According to the obtained results, this method may be applied for the analysis in residues monitoring for flumethasone in pig liver tissues.

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Prac-028

Reduction of Pseudorabies Sero-prevalence Using Ingelvac® Aujeszky Modified Live Vaccine in Two Malaysia Farms

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Introduction: The swine industry worldwide has successfully used Bartha-K61 (inactivated/live) vaccines to control pseudorabies virus (PRV) for more than three decades [1]. However, there are still reports of sporadic PRV cases in areas of high pig density in Malaysia since 1998 [2,3]. Most Malaysia farmers practice breeding herd vaccination using inactivated PRV vaccine and eradication has not been successful so far. Intranasal (IN) piglet vaccination using gE-deleted Bartha vaccine was demonstrated to hasten eradication of PRV as compared to conventional inactivated PRV vaccines [4]. This study aims to evaluate the efficacy of Ingelvac® Aujeszky MLV in reducing the sero-prevalence of PRV in 2 farrow-to-finish swine farms in Malaysia.

Materials and Methods: This study involved two farms located in the densest pig farming area in Malaysia.
Farm 1: 300 sows, previously mass vaccinating PRV inactivated vaccine 4 times annually in breeding herd only, changed to Ingelvac® Aujeszky MLV in April 2018 and period of comparison of 6 months.
Farm 2: 300 sows, previously mass-vaccinated with PRV inactivated vaccine 4 times annually in breeding herd and porker (8th week old), changed to Ingelvac® Aujeszky MLV in February 2018 and followed up for 6 months. Both farms decided to implement mass vaccination 4 times annually for the breeding herd and in 1-3 day-old piglets using Ingelvac® Aujeszky MLV.
The percentage of seropositive animals before and after intervention was evaluated using Pseudorabies Virus gpI Antibody ELISA Test kit (IDEXX Laboratories, Inc., Westbrook. US).

Results: After using Ingelvac® Aujeszky MLV, the percentage of animals that tested seropositive for pseudorabies gI antibody declined in both farms.

Table 2. Percentage of pseudorabies seropositive animals in Farm 1 before and after the use of Ingelvac® Aujeszky MLV.

Stage		Before	After 6 Mths	Difference 6 Mths
Breeding Herd		43% (n=14)	7% (n=14)	-83%
Grower-Finisher	8 weeks	40% (n=5)	0% (n=6)	-100%
	12 weeks	40% (n=5)	0% (n=6)	-100%
	16 weeks	0% (n=5)	0% (n=5)	NA
	20 weeks	0% (n=5)	0% (n=5)	NA

Table 3. Percentage of pseudorabies seropositive animals in Farm 2 before and after the use of Ingelvac® Aujeszky MLV.

Stage		Before	After 6 Mths	Difference 6 Mths
Breeding Herd		42% (n=12)	0% (n=12)	-100%
Grower-Finisher	8 weeks	50% (n=4)	0% (n=6)	-100%
	12 weeks	25% (n=4)	0% (n=6)	-100%
	16 weeks	25% (n=4)	0% (n=5)	-100%
	20 weeks	0% (n=4)	0% (n=5)	NA

Conclusions: This study demonstrated the efficacy of Ingelvac® Aujeszky MLV in reducing sero-prevalence of PRV in all age groups including breeding herds. Eradication of pseudorabies by vaccinating breeding herd only using inactivated PRV vaccine was shown to be ineffective. Eradication of PRV within half a year is achievable by breeding herd mass-vaccination and intranasal route application of Ingelvac® Aujeszky MLV to piglets 1-3 day of age.

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Prac-029

Swine veterinary practice trends in the philippines

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Introduction: The swine segment is the biggest contributor among the food animal/ livestock sector of Philippine agriculture [1]. The swine veterinary practitioner plays an important role in the management and technical support services for this industry and the allied service industries, so it is important that the industry had enough veterinary complement. The need for the swine industry to prepare for the future and trends are important to consider so the right actions be implemented [2].

Materials and Methods: This paper will report on demographic data gathered from the PVMA or the Philippine Veterinary Medical Association's [3] annual convention and scientific conference and the PCSP or the Philippine College of Swine Practitioners' technical seminar - the Pinoy Pork Challenge [4].
Attendees were required to fill up on-line registration forms that required demographic data. The availability of automated systems to gather information has allowed for the faster gathering and ensuring the integrity of the data.

Results: It is noted that 25-27% of attending veterinarians to the PVMA indicated that they are into swine practice whether as a single or mixed practice. In both the PVMA and PCSP data, the highest trends in Types of Practice were in Farm (27-35%), Corporate (26-27%) and Government (9-23%). Data on School Graduated From showed trends that majority of the universities offering veterinary medicine courses do have a percentage of their graduates pursuing swine practice. Trend in Gender show there are more males (53-57%) than females (43-47%). The biggest Age Groups are the Millennials, participants born from 1980 to 1994 (39-47%), followed by Generation X, born from 1965 to 1979 (28-35%) and the Baby Boomers, born from 1946 to 1964 (21-24%).
The high trend of swine veterinarians practicing in Regions of Practice - Central Luzon, CALABARZON and National Capital Region - coinciding with the areas with the top two biggest swine populations and the headquarters of most business companies [4].

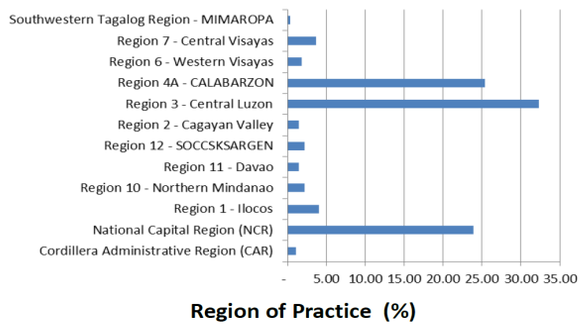

Figure 1: Percentage of Swine Veterinarians practicing in selected swine producing regions in the Philippines.

The data trends show that swine practice is still a popular choice for most veterinarians in the Philippines. The big number of technologically adept age groups, have moved the professional associations to adopt technology for data gathering and for faster information dissemination. The top two swine production areas have a good number of swine practitioners.

Conclusions: The data indicates the need to for professional organizations to formulate programs and activities to encourage veterinarians and veterinary graduates whose career choices are in swine practice.

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Prac-030

The adhesion ability of probiotic bacteria to swine intestinal mucus; In vitro test

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Introduction: The swine intestinal mucus covering the epithelial cells is explain the transient pattern of colonization characteristic for most bacteria. An in vitro evaluation of probiotic bacteria adhesion to swine intestinal mucus provides a good additional model for studying the ability of probiotics to adhere to intestinal surfaces. In the present study, we examined a number of probiotic strains for their ability to adhere to intestinal mucus isolated from different parts of pig small intestines. Adhesion ability should be considered when selecting the strain and dose for the most effective probiotic product for practical use. Materials and Methods: All the bacterial strains used in this study were prepared by microbial laboratory of K.M.P. BIOTECH Co., LTD (Thailand). The strains (i.e. *Lactobacillus acidophilus, Lactobacillus plantarum, Pediococcus pentocaseus, Bacillus subtilis and Bacillus licheniformis*) were selected mainly on the in vitro of inhibition ability on Enterotoxigenic *E. coli*, Enterohemorrhagic *E. coli* and enzyme production as shown in previous study. Swine intestinal mucus was isolate from different parts of weaned pig small intestine (i.e. duodenum, jejunum and ileum). The quantitation of the bacterial adhesion to the swine intestinal mucus was determined as earlier descript[1][2]. Mucus layer was prepared by incubating 100 µL of the clarified mucus suspension for 18-24 h at 4℃ on Eppendorf tube. The swine intestinal mucus in Eppendorf tube was mixed with 100 µL of each bacterial strains, the tube incubated at 35±2℃ for 1 h and the mucus washed 3 times with 200 µL of PBS to remove any unbound bacteria. To release and lyse the adhered bacteria. 100 µL of PBS was added to each tube and shake by vortex. The proportion of adhered bacteria was assessed as the percentage of remaining cells from each tube as compared to 100 µL of initial bacterial suspension.

Results: The adhesion ability to swine intestinal mucus varied between the different bacterial strains. Depending on the source of the mucus type: the highest 88.67% of adherence on duodenal mucus was found for *Pediococcus pentocaseus* 16Avpd02 compared with other strains. *Lactobacillus plantarum* CU20 showed the highest adherence proportion on Jejunal (96.46%) and ileal (84.65%) mucus than the rest of the strains. Considering the spore formation of *Bacillus* spp., *Bacillus* spp. in spore form showed a higher adherence score than their vegetative form. For example, spore form of *Bacillus licheniformis* KMP-TN001 had a higher adherence capacity to duodenal, jejunal and ileal mucus compared with their vegetative cell (77.86 vs 31.12%, 80.31 vs 41.49% and 77.8 vs 34.85%, respectively).

Discussion and Conclusion: The present results clearly showed that probiotics candidate bacteria (i.e. *Lactobacillus acidophilus, Lactobacillus plantarum, Pediococcus pentocaseus, Bacillus subtilis and Bacillus licheniformis*) have different percentage of adherence in different part of the small intestine. This implied that probiotics candidate has their own residence area in the pig intestine. Thus, to achieve the highest benefit from using probiotic for pig, one should combine multi-strains of probiotics in particular product.

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Prac-031

Validation of LC-MS/MS Method for ochratoxin A in pellet feed by QuEChERS-based Extraction

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Introduction: Ochratoxin A (OTA) is a widely spread mycotoxin that contaminates a great variety of feedstuffs. It is nephrotoxic in all of the mammalian species tested, the pig is the most sensitive one. Residues of mycotoxin in feedstuff cause a toxicosis of livestock. Therefore, we need to control mycotoxin in feedstuffs. In addition, application of rapid and effective samples preparation process and analytical method are necessary. The purpose of this study aimed to establish the analysis method of ochlatoxin A contamination in pellet feed.

Materials and Methods: Pellet feed were homogenized (5g) and spiked with ochlatoxin A at the concentration 10ng/g (n=3). The modified QuEChERS extraction method was used for samples preparation. Analysis was performed by ABsciex QTrap 5500 mass spectrometer(LC-MS/MS). The chromatographic column was a C_{18} (2.1 mm x 150 mm, 5㎛). The mobile phase consisted of 5mM ammonium acetate and 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The source conditions were optimized to obtain two identification points (precursor: 403.9, product: 239.1/358.3 m/z).

Results: Our Concentration response showed linearity within the concentration range (R^2 > 0.999). Mean of recoveries of OTA was 99.96%. The coefficient of variation observed was 8.8%. The limit of quantification (LOQ) and detection (LOD) were 0.73 and 2.21ng/g with external standard curve.

Conclusions: The proposed method was successfully applied to the determination of OTA in pellet feed. The QuEChERS-based extraction method was applied to save time and effort in the sample preparation process of ochlatoxin A analysis. According to the obtained results, this method may be applied for the analysis in residues monitoring for OTA in pellet feed. The method can be used for a wide variety of feeds.

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Prod&Wel-001

Administration of fercobsang® to increase weaning weight of piglets

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Introduction: As a number of hyperprolific sows are introduced in Korean farms, the number of live born piglets per sow has increased. But still, many of the farms have difficulties on increasing the number of piglets weaned per sow. Selection for litter size is negatively correlated with birth weight, which in turn decreases the survival rate of piglets. Eventually, this leads to a reduction in the number of weaning piglets per sow. Since the weaning number and weaning weights of piglets are important parameters in pig farm productivity, using of supplements or additives are needed to improve them. The aim of this study was to assess the effects of FERCOBSANG® on the weaning weight of piglets with injection to pre-farrowing sows and 2-day-old piglets.

Materials and Methods: In total, 6 litters were used for this study. Three litters were randomly selected for treatment group, and rest of three litters were allocated to control group. Sows in the treatment group received 10 ml of FERCOBSANG® on 7 days before farrowing, and 1 ml of FERCOBSANG® was injected to the 2-day-old piglets (n=39) that were born from sows in treatment group. For the control group, same volume of sterile saline were injected to the sows and their piglets (n = 40) in the same manner as the treatment group. Body weight of each piglet was measured at birth, 7 days and 20 days of age and the mean values were calculated. The differences of body weight between treatment and control group were analyzed by using t-test. The statistical analysis was performed using SAS 9.4, and the difference in body weight was considered to be significant when the *p*-value was <0.05.

Results: Although the mean body weight of piglets at birth was higher in treatment group (1.55 kg) than in control group (1.47 kg), the difference was not significant (p=0.339, t-test). The mean body weight of 7-day-old was significantly higher in treatment group (2.65 kg) than in control group (2.35 kg) (p= 0.0291, t-test) and it was identified that the average body weight of the 7-day-olds was increased by 300 g in the group injected with FERCOBSANG®. Lastly, the mean body weight on the 20th day after birth was also significantly higher in treatment group (6.01 kg) than in control group (5.16 kg) (p= 0.0088, t-test) and the difference of the mean body weight between the 2 groups was found to be 850g. (Table 1)

Table 1. Comparison of average body weight of piglets in 2 groups

	Treatment group	Control group	Differences	P-value
At birth (kg)	1.55	1.47	0.08	0.339
At 7 days old (kg)	2.65	2.35	0.3	0.0291
At 20 days old (kg)	6.01	5.16	0.85	0.0088

Conclusions: Treating FERCOBSANG® on sow and their piglets significantly increased the body weight of 7 days and 20 days old piglet. The body weight at weaning is important parameter for survival rate of piglets as well as the productivity of pig farm. FERCOBSANG® leads to increase body weight of piglet would be very helpful to increase farm productivity.

Acknowledgement: This work was supported by a grant from the Sung-Jin GGP Farm in Darby Genetics.

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Prod&Wel-002

Administration of PGF2a in postpartum primiparous sows improved milk yield and litter weight gain of the suckling piglets

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Introduction: Prostaglandin F2α (PGF2a) is an important substance regulating the function of corpora lutea in female pig [1]. In practice, PGF2α is commonly used for the farrowing induction in order to facilitate cross fostering management [2]. PGF2α also stimulate myometrial contraction [1]. Thus, administrations of PGF2α in postpartum sows is able to eliminate residual products and infectious debris that occur during farrowing. Administration of PGF2α in sows postpartum increased piglet body weight at weaning and increased litter size at subsequent farrowing [3]. To our knowledge, the influence of multiple administrations of PGF2α in postpartum sows has not been investigated. Therefore, the aim of the present study was to determine the influence of multiple administration of PGF2α in postpartum sows on milk yield and their litter performances.

Materials and Methods: The study was conducted in a 1500-sow commercial swine herd in Thailand from June to July 2018. In total, 33 Landrace x Yorkshire crossbred sows were included in the experiment (12 primiparous and 21 multiparous sows). The sows were classified into 3 groups, i.e., Control (n=10), Treatment 1 (n=12) and Treatment 2 (n=11). Sows in the treatment groups were administered 2 mL of PGF2α intramuscularly (5 mg/ml Dinoprost, Enzaprost® Vet, CEVA Animal Heath Ltd., Libourne, France). The first PGF2α treatment was done within an hour after the end of farrowing and, in treatment 2 group, the sows also received repeated doses of PGF2α at 7 and 14 days postpartum. The piglets were identified by using ear tattoo and their body weight was measured at 0, 1, 5 and 20 days of age. Milk yield of the sows were calculated by using Bayesian hierarchical model previously reported by Hansen et al. [4]: Milk yield (kg) = 2.23 + 0.05 (litter size − 9.5) + 0.23 x (average litter daily weight gain (kg/day) − 2.5. Milk yield and litter weight gain of the piglets were compared among groups by using general linear models.

Results: On average, the milk yield of sow was 10.0 ± 1.72 kg/day (range 6.0 to 13.2 kg/day). Milk yield in primiparous and multiparous sows after single and multiple doses of PGF2α administration is presented in Table 1. On average, litter sizes at days 5 and 20 of lactation were 10.0 and 9.3 piglets/ litter, respectively. Litter weight gain, average daily weight gain and body weight of the piglets at weaning were 46.7 ± 11.5 kg, 336 ± 58.4 gram/day and 7.2 ± 0.92 kg, respectively. The litter weight at day 20 of lactation in treatment 2 group was higher than control (71.3 and 61.9 kg, respectively, P=0.057) and tended to be higher than treatment 1 group (63.0 kg, P=0.079).

Table 1 Milk yield in primiparous and multiparous sows after single and multiple dose of PGF2α administrations in postpartum sows (least square means ± SEM)

Group	Primiparous	Multiparous
Control	7.61 ± 0.72^{a}	10.95 ± 0.59
Treatment 1	8.98 ± 0.64^{ab}	10.69 ± 0.54
Treatment 2	10.25 ± 0.83^{b}	10.51 ± 0.51

Conclusions: Administration of PGF2α at days 0, 7 and 14 postpartum significantly improved milk yield of primiparous sows and also increase litter weight of the piglets at 20 days of lactation.

Acknowledgement: Financial support for the present study was provided by Chulalongkorn University.

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Prod&Wel-003

Assessment of claw lesions of sows in Korean swine farms

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Introduction

Claw lesion is one of the major causes of lameness in sows and the lameness is one of the reasons for remove sows from the swine heard. Economic loss due to lameness in the US swine herds was over $24 million. In addition, the lameness is one of the factors that measures animal welfare in the European Union. This survey investigated prevalence of sow claw lesions in Korean swine farms according to parity and farms' reproductive performance. Lesions of claws were classified into horizontal and vertical cracks of claws, and length difference of claws and dew claws.

Materials and methods

This survey was conducted for a year on sows in total of 5 farms and the subjects were a total of 931 pigs. The lesions observed in the study were horizontal and vertical claw cracks, differences in the length of claw and in the length of dew claws of each foot. Scores were assigned on 0, 1, 2, and 3 at each foot depending on the severity of the claw lesions. The sows' parity was classified into 1 to 2, 3, 4, 5 and more than 6.

The farms were surveyed based on their reproduction performance. The reproduction performance of the farms was based on pigs weaned/sow/year (PSY).

To evaluate claw lesions and its affect, we compared claw lesion scores for different parity, foreleg and hind leg, and reproductivity in Korean swine farm.

Results and conclusions

In terms of reproductive performance of farms, the most severe claw lesion in the lowest reproductivity farm was vertical crack in right foreleg and the second lowest was the vertical crack in left foreleg.

The most severe claw lesion in the highest reproductivity farm were vertical crack in left foreleg and dew claws length difference of right hind leg.

In terms of sow parity, parity more than 6 had the highest claw lesion score. Parity 1 had the lowest claw lesion score and parity 3 had the second lowest claw lesion score.

The most severe claw lesion for parity more than 6 was dew claw length difference of hind leg. For parity 1 sow, vertical crack was the most severe claw lesions, and the claw length difference of forelegs were the least severe. The claw lesion with the highest score for all sow groups were vertical crack in right foreleg and left foreleg. The two least scored claw lesion were claw length difference in forelegs.

The two farms with worst reproduction results among 5 farms had the highest claw lesion scores. However, since only five farms were under investigation, further investigation is needed to see more accurate relationship between reproductive performance and claw lesions. These results can be basic data to establish preventive measures for losing sows due to lameness and reduce economic loss in swine farms.

Prod&Wel-004

Comparative body morphometrics of native pig (*Sus domesticus*) grown during the wet and dry seasons in Marinduque, Philippines

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Introduction: This study takes a look at the growth performance of the native pig (*Sus domesticus*) grown during the wet and dry seasons expressed in body morphometrics.

Materials and Methods: Two native pig breeds: Black (B) (N=180) and White Feet (WF) (N=56) were considered in this study. Body morphometrics were taken after 3 months (90 days) of grow-out. Native piglets used in this study were weaned out from the sow 35 days after birth and were ready for dispersal after 90 days. The grow-out periods for the different cohorts were from February 2017 to June 2018. Body morphometrics include: length of the snout, head, forehead, ear, neck, heart girth, mid girth, rump, body, tail, foreleg, hind leg, pelvic, foreleg (height), hind leg (height), hair and weight (at birth and 90 days). Wet season in the Philippines usually starts from June and ends in October while the dry season is from November to May.

Results: Average temperature for the study period during the dry season (27.09+1.30℃) did not significantly differ (p=0.051) with that of the wet season (28.4+0.55℃). The amount of rainfall significantly differed (p=0.037) between the dry and wet seasons. Average rainfall during the dry season was 68.20+33.70 mm and for the wet season was 108.94+30.86 mm. Results of the Discriminant Function Analysis for the morphometrics of the B breed showed that the morphometrics in general differed significantly (Wilks' Lambda: 0.599, approx. F =1.652, p< 0.004). The difference was significantly influenced by the weight at 90 days (p=0.049). Results of the Tukey's HSD test showed that the weight at 90 days of the female cohort (11.32+2.93 kg) was significantly higher compared to the rest of the cohorts. The rest of the cohorts did not differ significantly in terms of weight at 90 days (female-dry season: 9.57+2.98 kg; male-wet season: 9.75+2.64 kg; male-dry season: 9.25+2.98 kg).For the WF breed, results of the Discriminant Function Analysis for the general morphometrics did not yield any significant difference between the different cohorts (Wilks' Lambda: 0.242, approx. F = 1.187, p < 0.2253).

Conclusion: The results of the morphometric analysis although preliminary in nature may provide useful information on the best season and the choice breed for native pig grow out operation.

Acknowledgment: The Department of Science and Technology - Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST - PCAARRD) funded this study under the Multi-Agency Research and Development Program on Conservation, Improvement and Profitable Utilization of the Philippine Native Pig.

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Prod&Wel-005

Coping with drawback period by using 42 Degree® in nursery pigs: its effect on pro-inflammatory cytokines levels and anti-pyretic

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Introduction: In nursery house, one of the most important problem is a drawback period (setback) which occurs during the first 2 weeks of entering the nursery house, which result in growth retardation and respiratory and gastrointestinal diseases [1]. There are many factors that cause a drawback period, for example, stress from weaning, from transportation, from nutritional changes, environmental changes and also social stress from fighting [2]. During nursery period, it has been shown that there are many pro-inflammatory cytokines release, such as IL-1, TNF alpha and IL-6, with respond to the inflammatory process and stressors. In practice, if one can find a product that can handle this drawback period, subsequently there is no negative effect on growth performance during the nursery period. The **42 Degree®** is a natural product, compose of willow bark, horse tail, string nettle and propandiol which claims to provide quick recovery from elevated body temperature, alleviates pain and mobility problem, keeping animal active, maintain fed intake and therefore prevent body weight losses. Therefore, this study aimed to test the product, **42 Degree®**, for minimizing the effect of drawback on pro-inflammatory cytokines levels (IL-1 and IL-6), anti-pyretic effect (PGE2 level) in nursery pig.

Materials and Methods: 60 nursery pigs were allocated into 2 groups as follows: Treatment, the 30 nursery pigs, were fed with **42 Degree®** by water dripping at the recommended dose for nursey pig for a period of 6 weeks (age between 4 and 10 weeks old); Control: 30 nursery pigs, were fed with normal feed and water dripping for a period of 6 weeks (age between 4 and 10 weeks). They were vaccinated against PCV-2, CSF, FMD at 32, 41 and 61 days old, respectively. Data recording and sample collection: 1) Clinical signs of respiratory infection and diarrhea: present or absent. These clinical signs were recorded during the study period. 2) Body temperature was measured by using infrared ear thermometer at 21, 43, 56, 71 days old. 3) Body weight recording (n=30 in each group). 4) Serum samples of 10 pigs in each group were taken before the experiment and at 43, 56, 71 days old (after intake **42 Degree®**), kept at -20℃ and analyzed for pig IL-1 beta, pig IL-6 and PGE2 using ELISA kit according to manufacturer's instruction (Abcam plc, Cambridge, UK).

Results: Clinical signs of respiratory and diarrhea are absent. The body temperature in both groups are varied between 37.38-38.73℃ (control) and 37.82-38.78℃ (treatment), respectively. There is no significant different for the IL-1 levels between groups. The levels of IL-6 are varied within groups and among times and fluctuated across the time. During the course of experiment, the level of IL-6 was higher in control group than in treatment group, however, at 56 days old a dramatically increased in the level of IL-6 was found in both groups with a higher level found in control group than in treatment group. For PGE2, the levels of PGE2 are varied within groups and among times and fluctuated across the experiment.

Discussion and Conclusion: During 2-3 weeks of nursery period, pigs are exposed to many stressors such as environmental stress, nutritional changes, social stress from fighting. These stressors may increase pro-inflammatory cytokines such as IL-6 and PGE2 in serum, consequently affect pig behavior such as reduce feed intake, get sick and subsequently poor growth performance in pig [1]. However, this is not the case in treatment group. This implied that 42 Degree, containing active ingredient such as salicin, flavonoid and tannin, with its anti-inflammatory and anti-fever action, not only able to minimize the negative effect of those pro-inflammatory cytokines during a drawback period and promote growth performance in treatment group, but also lessen the adverse effect of vaccination as directly shown by a higher body weight gain and ADG in treatment group.

Acknowledgement: This work was supported by a grant from Innovet Corporation Co., Ltd., Samutprakarn, Thailand.

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Prod&Wel-006

Effect of a synergistic blend of feed additives on performance and carcass yield of grow-finishing pigs raised in commercial farm conditions

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Introduction: Traditionally, antibiotic growth promoters (AGP) have been used in commercial pig production to stimulate growth and maintain the health of animals. However, in the post AGP era, alternatives like the synergistic blend of feed additives (FA) has been regarded as a novel antibiotic replacement because of their health protecting and growth promoting properties [1, 2]. Synergistic FA has a broad antimicrobial effect and support a functional gut necessary to enhance animal performance [1, 2, 3]. As the search for effective alternatives to antibiotic continued and the preference for pork raised without antibiotics increases, the use of a synergistic blend of FA could be a viable solution to achieve optimum performance. The current study aims to validate the efficacy of a synergistic blend of FA on growth performance and carcass yield of grow-finishing pigs under commercial farm conditions.

Materials and Methods: This study was conducted in a commercial farm in Vietnam with 440 pigs housed in pens with 11 pigs each and allocated to one of four treatments including negative control (NC, a basal diet without AGP), antibiotic (AGP, NC + Colistin at 20 ppm/T feeds), synergistic blend of FA added in feeds (PFX, NC + PFX at 1 kg/T feeds) and as PFX plus a synergistic blend of FA given via drinking water (SPH) (PFX+SPH, NC + PFX at 1 kg/T feeds + SPH at 1.2 L/1000 L water). PFX is a synergistic blend of medium chain fatty acids, slow release C12, target release butyrates, organic acids (OA) and a phenolic compound, while SPH is a blend of free and buffered OA (mainly formic acid, acetic acid, and ammonium formate). Pigs were given standard corn-soya diets during the growing (d1-52) and finishing period (d53-112). The zootechnical performance was recorded at d1, 52 and 112, while the carcass yield was evaluated at the end of the experiment.

Results: Pigs given a synergistic blend of additives in feed and water (PFX+SPH) showed equal performance as AGP in terms of final body weight, ADG, ADFI and FCR; and carcass yield (P>0.05, Table 1). Compared to the NC, FA supplementation either alone or combination significantly increased ADG by 18 g/d when the single product was used (PFX) and 42.4 g/d when the combination was supplemented (PFX+SPH). The FCR significantly improved by 9 points with PFX and up to 17 points when PFX+SPH blend was fed over the entire fattening period. At the end of the finishing period, PFX+SPH significantly enhanced pig market weight by 5 kg and consequently increased carcass yield compared to the NC group (*P*=0.01). ADFI was similar in all treatment groups (*P*>0.05, Table 1).

Table 1. Growth performance and carcass yield of grow-finishing pigs supplemented with AGP or synergistic blend of feed additives during the fattening period.

TREAT	NC	AGP	PFX	PFX+SPH
ADG, g	668^{c}	713^{a}	686^{b}	710^{a}
ADFI, g	2034	2049	2029	2045
FCR	3.05^{a}	2.87^{c}	2.96^{b}	2.88^{c}
End weight, kg	98^{c}	104^{a}	100^{b}	103^{a}
Carcass, %	74.9^{b}	75.9^{a}	75.6^{a}	75.8^{a}

^{a,b}Means within a row having different letter differ significantly (P<0.05).

Conclusions: Supplementing a synergistic blend of FA either alone or in combination improved performance of grow-finishing pigs raised in antibiotic-free production system. In addition, the approach of supplementing synergistic blend of FA in feed and water (PFX+SPH) is as effective as AGP in promoting growth and improving carcass yield of fattening pigs. Thus, this strategy could be an effective alternative to antibiotics in commercial pig farming conditions.

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Prod&Wel-007

Effect of Butaphosphan and Cyanocobalamin supplementation in semen extender on boar sperm quality

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Introduction: Butaphosphan is an organic phosphoric acid compound that is an energy metabolism for ATP synthesis in sperm cell [1]. Cyanocobalamin is a co-enzyme involved in the TCA cycle [2], gluconeogenesis [3] and decreased lipid-peroxidation of sperm membranes from free radicals. Therefore, butaphosphan supplementation in semen extender may increase energy and motility of boar sperm. The objective of the present study was to determine the effect of butaphosphan and cyanocobalamin supplementation in extender on boar semen quality.

Materials and Methods: A total of 22 semen samples from 5 mature boars were collected. The boars were kept in an open-housing system in Livestock Animal Hospital, Chulalongkorn University, Nakhon Pathom province, Thailand. The semen collection was performed by gloved hand method. The ejaculates were sent to the laboratory for semen evaluation immediately after collection. The semen with at least 70% subjective sperm motility and 90% morphologically normal were used. They were diluted in short term semen extender. Diluted semen (sperm concentration 3,000 million/100 ml) was divided into four groups i.e., Group I (control) semen extenders without supplementation. Groups II, III and IV semen extenders were supplemented with butaphophan and cyanocobalamin (Octafos®, Octa Memorial Co. Ltd., Thailand) supplementation at 0.1, 0.4 and 0.5 ml/100 ml of extender, respectively. All samples were kelp at 16℃ and investigated on days 0, and 3 after storage (semen collection day = day 0). The sperm kinematic parameters (i.e., total motility, progressive motility, VCL, VSL, VAP, LIN, STR, WOB, ALH and BCF) were evaluated at 37℃ by using a computer assisted sperm analysis system (CASA) (SCA®, Proiser S.L., Valencia, Spain). The sperm kinematic parameters were regarded as a dependent variable and were analyzed by using the general linear models (GLM) procedure of SAS. $P < 0.05$ was regarded to be statistically significant.

Results: On average, the total motility was 81.8 ± 10.0 % and the progressive motility was 51.3 ± 11.4 %. The total and progressive motility in each group are presented in Table 1.

Table 1. Total and progressive motility in control and groups II, III and IV

Group	Total Motility		Progressive motility	
Days	0	3	0	3
Control	82.8	72.0	50.0	37.3
II	84.9	79.1	53.0	46.6
III	81.8	79.8	54.0	54.2
IV	75.3	73.9	51.2	56.0

In day 0 and 3, group II and III had higher total and progressive motility than control group ($P > 0.05$). Moreover, group IV had the total motility lower than control group in day 0 but both total and progressive motility at day 3 after collection in group IV was higher than control group ($P > 0.05$).

Table 2. The sperm kinematic parameters in control compared with groups II, III and IV in day 0.

	Group			
	Control	II	III	IV
VCL	48.9	50.6	53.2	55.4
VSL	15.1	16.2	16.8	16.0
VAP	31.4	32.2	33.1	33.1
LIN	31.5	33.3	32.3	31.1
STR	49.4	50.5	49.7	48.9
WOB	59.5	60.7	59.5	58.1
ALH	2.2	2.3	2.4	2.5
BCF	4.8	5.1	5.3	5.3

It was found that groups II, III and IV had higher the all sperm kinematic parameters than control group but did not differ significance ($P > 0.05$).

Conclusions: Butaphosphan and cyanocobalamin supplementation in extender improve boar semen quality.

Acknowledgement: Financial support for the present study was provided by National Science Technology and Innovation Policy Office and Office of the Higher Education Commission (Talent Mobility program).

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Prod&Wel-008

Effect of gestation length on body weight at birth of the newborn piglets

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Introduction: Piglet birth weight is the most important factor for survival and growth performance of neonatal piglets [1]. Furthermore, piglets born with a low birth weight also have a significantly lower colostrum consumption compared to those with a higher birth weight [2]. Small piglets also have a reduced ability to maintain their body temperature [3]. Thus, body weight is an important survival indicator and the thermoregulation ability of the piglet. Genetic selection for sows with increased litter size has resulted in a reduction in piglet body weight, due to a decreased uterine space for fetal development and decrease amount of nutrient available per fetus [1]. The present study determined factors influencing body weight at birth of the piglets with special emphasis on the effect of gestation length in hyper-prolific sows.

Materials and Methods: The study was conducted in two commercial swine herds (A and B) in the Northern part of Thailand from August 2018 to March 2019. Both herds belong to the same breeding company. A total of 2,461 newborn piglets from 203 Landrace x Yorkshire crossbred sows were included in the experiment. The sows were classified according to parity into 4 groups, i.e., 1, 2-4, 5-6 and ≥7. The piglets were classified according to the control of parturition protocol into 2 groups, i.e., induction of farrowing using PGF2α (n=548) or using Altrenogest+ PGF2α (n=1862). Gestation length were classified into 5 groups, i.e., 112-113, 114, 115, 116 and 117 days. Body weight of each individual piglet was determined within 24 h after birth. Coefficient of variation (CV) of the piglet body weight within litter and body weight data were analyzed by using general linear model procedure of SAS. The statistical model included effect of herd, parity, treatment and gestation length. The total number of piglets born per litter (TB) was included in the model as regression. Least square means were obtained and were compared using least significant difference test. $P<0.05$ was regarded to be statistically significance.

Results: On average, TB was 13.4±2.2 piglets/ litter (range 8 to 19). Body weight of the piglets at birth and the within litter variation of the piglet birth weight were 1513 ± 313 g and 21.3 ± 15.5%, respectively. Body weight at birth of the piglets by gestation length of sows are presented in Table 1. Gestation length of sows influenced both the body weight at birth and the variation of piglet birth weight within litter ($P<0.05$). Piglets born from sows having gestation length of 117 days were heavier than piglets born from sows with a shorter gestation length (Table 1). Likewise, the CV of within litter birth weight of piglets in sows having gestation length 112-113 days was higher than sows with gestation length of 114, 115, 116 and 117 days (34% vs 21%, 21%, 20% and 21%, respectively, $P<0.05$). Neither treatment ($P=0.418$) nor parity groups ($P=0.860$) of sows influenced body weight at birth of the piglets. An increase of TB by 1 piglet resulted in a decreased of piglet birth weight of 38.3 g ($P<0.001$).

Table 1 Body weight at birth of the newborn piglets by gestation length of sows.

Gestation length (days)	N	LSmean ± SEM
112-113	166	1520 ± 547[a]
114	905	1462 ± 295[a]
115	733	1440 ± 332[a]
116	278	1522 ± 487[a]
117	328	1675 ± 488[b]

[a,b] different superscript differ significantly ($P<0.05$).

Conclusions: Gestation length of sows influenced both the body weight at birth and the variation of piglet birth weight within litter. An increase of 1 born piglet per sow resulted in a decrease in birth weight of 38.3 g.

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Prod&Wel-009

Effect of Paracetamol in oral solution on antipyretic effect and growth performance of 4 weeks old pig after vaccination

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Introduction: Vaccination is a situation that induces stress in pig. It may develop fever and result in feed intake reduction. This process is similar to setback or growth check status in weaning pig in aspect of poor growth performance. Meanwhile, paracetamol has potential on antipyretic and anti-inflammatory. Thus, the objective of this study was to determine the effect of paracetamol on antipyretic effect and growth performance, including glucocorticoid metabolite level and malondialdehyde (MDA) level which has strong relation with stress in pigs. Materials and Methods: The study was performed in 4 weeks old pig after vaccination. A total of 1300 pigs were divided into 16 stables and separated into two groups: a treatment group (8 stables) was given medicated (Parcetam®) water with 30 mg/kg/day for 5 days, while a control group (8 stables) was given the water without medicine. The study was conducted over a period of 21 days. Fecal collection, blood collection and also measuring weight were done at 0, 7, 14 and 21 day of experiment for detecting glucocorticoid metabolites level, MDA level and growth performance respectively. Moreover, body temperature of both groups was measured since day 0-7 after treatment.

Results: Glucocorticoid metabolites level, MDA level and growth performance shown in table 1-3.

Table 1 comparison of mean rectal temperature of pigs administered with paracetamol in drinking water (treatment group), and without paracetamol (control group) after vaccination.

Indexes	Treatment gr.	Control gr.
Initial weight (day 0)	8.79 (±0.87)	8.40 (±0.67)
Body weight (day 7)	11.52 (±0.86)	11.05 (±1.07)
Body weight (day 14)	12.13(±1.51)	11.56(±1.23)
Final weight (day 21)	15.26(±1.92)*	13.20(±1.81)

Table 2 comparison of mean glucocorticoid metabolites level of pigs administered with paracetamol in drinking water (treatment group), and without paracetamol (control group) after vaccination.

Indexes	Treatment gr.	Control gr.
ADG 0-7	390(±93.5)	378.57(±136.82)
ADG 7-14	86(±212.60)	71.80(±207.20)
ADG 14-21	447.14(±261.33)	235.36(±222.92)
ADG 0-21	307.74(±80.12)	228.57(±82.80)
FCR 0-21	1.91 (±0.41)**	2.71(±0.83)

Table 3 comparison of mean MDA level of pigs administered with paracetamol in drinking water (treatment group), and without paracetamol (control group) after vaccination.

Indexes	Treatment gr.	Control gr.
Day 0	3.12(±0.98)	4.05(±1.18)
Day 14	2.44(±0.73)	2.54(±0.50)
Day 21	2.33(±0.89)	2.10(±0.51)

Conclusions: There were no significantly difference between 2 groups on rectal temperature ($p>0.05$). They also had no significantly difference between 2 groups on weight. However, the treatment group had a significantly higher weight at day 21 of experiment than the control group and had significantly lower FCR ($p<0.05$). Moreover, growth performance, glucocorticoid metabolite and MDA levels were no significantly difference between treatment and control group ($p>0.05$).

Acknowledgement: We would like to thank CEVA Animal Health Thailand Co,.Ltd and Faculty of Veterinary Medicine, Chiang Mai University, For their financial support and for providing products and facilities for use in this study.

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Prod&Wel-010

Effect of sow vaccination during lactation on sow and piglet performance in the Philippines

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Introduction: Vaccination is an important tool for the control and prevention of infectious diseases in pig farms. However, aside from stimulating protective immunity, vaccines must also have no or minimal adverse effects that can potentially impair the pigs' performance. In this study, the impact of vaccinating lactating sows on body temperature, daily feed intake (DFI), and their piglets' performance was evaluated by comparing 2 commercial Parvo/Lepto/ Erysipelas (PLE) vaccines with non-vaccinated controls.

Materials and Methods: The study was done in a farrow-to-finish farm with 900 sows in the Philippines. A total 135 apparently healthy multiparous sows from 4 consecutive weekly batches were selected and randomly assigned to 1 of 3 groups (45 per group) taking into consideration a similar parity distribution between groups. At 14 days of lactation, sows in groups 1 and 2 were injected intramuscularly with 2ml of ERYSENG® PARVO/LEPTO (EPL) (Hipra) and 5 ml of vaccine B, respectively, following manufacturer's instructions. Sows in group 3 were left unvaccinated. Rectal body temperatures of all sows were taken at 24h before vaccination (-24h), at vaccination (0h) and at 6h, 24h and 48h post-vaccination. Individual sow feed intake was recorded daily for 4 consecutive days starting a day before vaccination until 2 days post-vaccination. Piglets from selected sows were weighed at birth, 14 and 26 (weaning) days of age. Statistical analysis software (SAS) was used to check for differences between groups.

Results: The mean rectal body temperatures (MRT) at -24h and 0h were similar for all groups (Table 1). At 6h post-vaccination (+6h), the MRT of group 1 was significantly higher than the control group but significantly lower than group 2 (P<0.05). By 24h post-vaccination the MRT of group 1 had returned to levels similar to controls. The MRT of group 2 was significantly higher than groups 1 and 3 at +6h and continued to be significantly higher than controls until +24h (P<0.05). By +48h, the MRT of group 2 sows had decreased to levels similar to group 3. The mean DFI at D-1 was similar for all groups (Table 2). For the 3 succeeding days (D0, D+1, D+2), the mean DFI of group 2 sows was significantly lower than group 1 by 0.84, 1.32, 0.77 kg and group 3by 1.27, 1.08, 0.75kgs, respectively (P<0.05). There was no significant difference in mean DFI between groups 1 and 3 for the duration of the study. No significant differences in mean piglet weights between the 3 groups were observed at birth, on the day of sow vaccination (14 days of age) and at weaning.

Table 1. Mean rectal temperatures (Co) of sows at different time points before and after vaccination.

Group	-24h	0h	+6h	+24h	+48h
1 (EPL)	39.26^{a}	39.06^{a}	39.82^{b}	39.24^{ab}	38.99^{a}
2 (Vaccine B)	39.21^{a}	39.09^{a}	40.46^{a}	39.42^{a}	38.98^{a}
3 (Control)	39.18^{a}	39.10^{a}	39.40^{c}	39.07^{b}	39.00^{a}

Table 2. Mean daily feed intake (kg) of sows at different time points before and after vaccination.

Group	D-1	D0	D+1	D+2
1 (EPL)	5.43^{a}	4.74^{a}	5.26^{a}	5.34^{a}
2 (Vaccine B)	5.35^{a}	3.90b	3.94^{b}	4.57^{b}
3 (Control)	5.48^{a}	5.17^{a}	5.02^{a}	5.32^{a}

Conclusions: This study shows that PLE vaccines can differ on their impact on lactating sows. The lack of significant difference in MRT and DFI between group 1 and the control group suggests that this vaccine (ERYSENG® PARVO/LEPTO) does not induce significant adverse post-vaccination effects in lactating sows. In contrast, the significant increase in MRT and consequent decrease in DFI in group 2compared to controls and group 1suggest that caution should be taken when administering this vaccine to lactating sows. An increase in MRT following PLE vaccination has been reported to negatively influence sow DFI and consequently linked to decreased milk production and reduced piglet growth over a 21-day lactation period1. In the present study, no significant effect on piglet performance over 26 days of lactation was noted for both vaccines.

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Prod&Wel-011

Effects of Improvac® vaccination of female pigs in the finisher period: Meta-analysis of parameters most relevant for pig producers

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Introduction: Improvac® was originally developed as an alternative to physical castration of boars and is marketed in more than 60 countries. This anti-GnRF (gonadotropin-releasing factor) vaccine has recently emerged also as an alternative for rearing gilts up to heavier slaughter weights [1,2]. Its ensuing suppression of oestrus during late finisher phase can avoid the heat associated reduction in feed intake, the behavioral problems such as mounting and aggression, as well as the risk of unwanted pregnancy if reared with uncastrated boars [3,4]. The aim of the current meta-analysis was to analyze the effect of immunological castration of gilts on growth performance parameters most relevant for pig producers.

Materials and Methods: A comprehensive database served as source of studies on the effects of Improvac® immunocastration in female pigs in the grower-finisher period (known as gilts), summing up to 37 studies at the time of data search. Studies were included if they reported the effect of immunological castration of gilts on average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and/or final live weight (LW) compared to untreated/control gilts. Studies using the feed additive ractopamine were excluded. Meta-analysis techniques were used to estimate the mean differences between immunologically castrated gilts versus entire female pigs, thereby also differentiating between conventional pork production and pigs destined for the production of high quality cured products (HQCP).

Results: Thirteen papers met the inclusion criteria. The numbers of comparisons and gilts for each parameter are presented in Table 1. Compared with control gilts, ADG was on average +45.1 g/day higher ($p<0.001$) in immunocastrated gilts in the overall analysis (combining both production systems). There was no significant difference between conventional pork production: +45.4 g/day ($p<0.001$) and pigs destined for the production of HQCP: +43.6 g/day ($p=0.005$). Compared with control gilts, ADFI was on average +0.19 kg/day higher ($p<0.001$) in immunocastrated gilts. There was also no significant difference between conventional pork production: +0.19 kg/day ($p=0.003$) and pigs destined for the production of HQCP: +0.19 kg/day ($p=0.005$). FCR was not different between immunocastrated and control gilts in the overall analysis ($p=0.556$) as well as in conventionally produced pigs ($p=0.916$). However, FCR was slightly higher in immunocastrated gilts destined for the production of HQCP: +0.093 g/g ($p=0.034$). In overall analysis, final LW was on average +4.0 kg higher ($p<0.001$) in immunocastrated gilts than control gilts. There was no significant difference between conventional pork production: +3.8 kg ($p<0.001$) and pigs destined for the production of HQCP: +6.1 kg ($p=0.001$).

Table 1: No. of comparisons and gilts by live performance parameter and pork production system

Parameter	Number of comparisons (gilts)	
	Conventional	HQCP
ADG	9 (2,509)	2 (80)
ADFI	6 (1,574)	2 (80)
FCR	4 (1,502)	2 (80)
Final LW	9 (2,665)	2 (80)

Conclusions: Immunocastration with Improvac® is a productive and reliable alternative for pig producers to raise female pigs or market gilts, leading to higher ADG and final LW by suppressing oestrus at the end of finisher phase.

Acknowledgement: This work was supported by an unrestricted grant from Zoetis.

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Prod&Wel-012

Evaluating the efficacy of Panacur® deworming through slaughter checks

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Introduction: *Ascaris suum* is a common nematode parasite which causes serious economic loss in pig farms[1]. The main manifestations are a decrease of feed conversion rate and daily gain. Larval migration can lead to secondary complications[2], such as pneumonia. Previous studies have also shown its effect on the immune response toward *Mycoplasma hyopneumoniae* vaccine[3]. Common diagnostic methods are clinical fecal egg detection and slaughter inspections for milk spots on the liver. Slaughter checks can be used to adjust the farm deworming program. Panacur (5% fenbendazole) is an anthelmintic which can act on all developmental stages of *Ascaris suum*, including the egg stage. Deworming programs should take the prepatent period of the parasite into consideration (7-8 weeks).

Methods: Two farm level trials were conducted to demonstrate the efficacy of worm control by using Panacur® Control efficacy was assessed by slaughter checks on the livers. Farm 1 - A two-site farm of 5,000 sows in northern China, with no deworming in the fattener stage, with high levels of liver condemnation at slaughter due to milk spot lesions. Panacur® was implemented at the age of 80days and 120days at a dosage rate of 1g/10kg bodyweight in feed. Farm 2 - A one-site farm of 1,400 sows in southeast China, Ivermectin was used previously, but there was no fixed deworming scheme. Historically nearly 98% of livers were discarded due to milk spots. Panacur® was implemented at 70days, 120days, 150days respectively, at a dosage of 1g/10kg bodyweight in feed. Rating criteria for milk spot liver was as follows - Grade 0: no lesion, Grade 1: <10 white spots, Grade 2: more than 10 white spots

Results: Farm 1 - The proportion of healthy livers of the treatment group(n=120) was 93.3%, compared with the control group (n=123,63.4%). The proportion of grade 1 and grade 2 milk spot livers in the treatment group was 2.5% and 4.2%, compared to 26.8% and 9.8% respectively in the control group. A significant difference was observed. Farm 2 - The proportion of healthy livers after three treatments with Panacur (n=123) increased from 1.89% to 78.05% compared with that before treatment (n=53). The proportion of grade 1 and grade 2 milk spot livers decreased from 26.42% and 71.7% before treatment to 17.07% and 4.88% after deworming.

Discussion: The results in both farms indicate that two or three single administrations of Panacur® at a dose rate of 1g/10kg bodyweight, in feed can significantly reduce the proportion of milk spots in the liver at slaughter and hence condemnations. Based on the above results, Farm 1 appeared to perform better than Farm 2 and we speculate that this was also due to husbandry issues. Farm 1 was newly built with a fully slatted floor while in Farm 2, pigs were on older cement floors. Secondly, drinking water in Farm 1 is from a borehole, whereas water from Farm 2 is from a dam, possibly increasing the risk of *Ascaris suum* infection through recirculation of pathogens.

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Prod&Wel-013

Impact of antibiotic's (marbofloxacin) originality on abnormal meat incidence in korean pigs

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Introduction: Since 2011, after the outbreak of foot-and-mouth disease (FMD) in Korea, the government has implemented a national vaccine policy [3]. There have been increased side effects ever since, especially the induction of abnormal meat (formation of abscesses and granulomas) at the vaccination site and the change in incidence is 2.3% to 31.8% in farms of Dodram Pig Farmers Cooperative [3]. However, there are another causes for abnormal meat, because the abscess and granulomas are formed as an immune response to a foreign material [1, 2]. While attempting various methods to reduce the incidence of abnormal meat, we suspected that generic antibiotic injections for treatment might have an impact on inducing abnormal meat and conducted this study.

Materials and Methods: The experiment conducted in a commercial farm of Dodram Pig Farmers Cooperative and 440 fattening pigs were randomly assigned to 7 groups including 6 experimental (E, marbofloxacin-injected) groups with 40 pigs each and 1 control (C, non-injected) group with remaining 200 pigs. Each of the pigs in 6 experimental groups injected one of the 6 marbofloxacin(MAR) products being sold in Korea (2 originals and 4 generics) according to producer's instructions and the details of each group and product are shown in Table 1.

Table 1. Experimental groups and information of injectables

Groups	Products	Marbofloxacin Content	Dosage
C	None	-	-
Oa	Original, A	16%	4 cc/pig/1 day
Ob	Original, B	10%	2 cc/pig/3 days
Ga	Generic, A		
Gb	Generic, B		
Gc	Generic, C		
Gd	Generic, D		

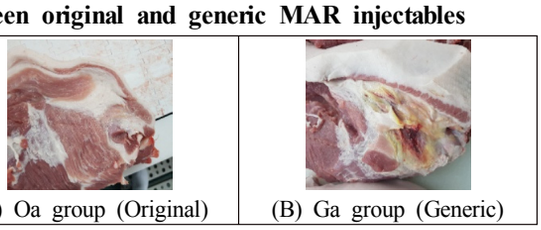
About one month after the injection, the pigs were sent to the slaughterhouse and the presence of gross lesions at the injection site was recorded for the individual pigs that were able to track the group.

Results: As shown in Table 2, abnormal meat lesions were most frequently observed in all of Generic 10% MAR injected groups (Ga, Gb, Gc, Gd) and the incidence of Gc group was the highest among them. However, the incidence of Original MAR injected (Oa, Ob) groups were significantly lower than all of generic 10% MAR injected groups and Oa group's incidence was the lowest of all the experimental groups. Furthermore, differences were found in both the incidence and appearance of abnormal meat. Inflammatory lesions including Abscess and hematoma were confirmed in Ga ~ Gd groups, whereas only mild inflammation localized in fascia was confirmed in Oa ~ Ob groups (Fig.1).

Table 2. Abnormal meat incidence of 7 groups

Groups	C	Oa	Ob	Ga	Gb	Gc	Gd
Incidence(%)	3.06	3.85	23.08	61.54	57.69	80.65	53.85

Fig. 1. Difference of abnormal meat appearance between original and generic MAR injectables



(A) Oa group (Original) (B) Ga group (Generic)

Conclusions: Antibiotic injections are inevitable in raising pigs. Through this study, it was identified that there was a difference in the incidence and the appearance of abnormal meat between the original and the generic MAR injectables. Based on the results, it is concerned that use of original MAR injectables is recommendable for improving the productivity of the farm.

Acknowledgement: This experiment was sponsored by Vetoquinol Korea.

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Prod&Wel-014

Impact of litter size in first parity on sow lifetime reproductive performance

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Introduction: One of the most critical factors driving the reproductive performance of sow herd is gilt development and management. The ability to assess sow performance at first parity is necessary when making selection and culling decisions for the breeding herds, as early performance of the sow has been shown to be indicative of later lifetime performance. Studies conducted in Europe and Japan have shown that sows with a high number born alive in parity 1 have subsequent litters with a higher than average number born alive as well. Therefore, the objectives of this study were to compare the gilt management and number of piglets total born across parities.

Materials and Methods: The study was conducted in a 1,000 sow farm in Southern Korea. I applied the system to the gilt who were inseminated from September in 2017. First of all, the role of gilt acclimation in the control of PRRS is the important because the naïve gilts introduced into a PRRSV positive herd are susceptible to be infected with the virus. The gilts were vaccinated and revaccinated 4 weeks later. Secondly all gilts age at first estrus were recorded and were to be first served at or after second estrus cycle when attained target weights over 135kg. all gilts were fed altrenogest at 20mg/day for 18 days to be synchronized and increase total born.

Results: A total of 1,313 gilts and 7,418 sows were included in the statistical analysis. The total born of 1st parity sows in 2018 increased 1.4 and 1.1 respectively compared to 2016 and 2017 (Table. 1). The sows that had the most litter size in 1st parity continued to produce the most litter size throughout all the subsequent parities (Table. 2), and also had higher farrowing rate up to second parity.

Table 1. Quarterly total born in first parity for 3 years

1st TB	First	Second	Third	Fourth	Ave
2016	11.8	11.9	11.3	12.8	12.1
2017	12.7	11.6	12.5	12.4	12.4
2018	13.4	14.0	13.3	13.4	13.5

Table 2. Sow parity and associated total born for 3 years

Parity	1	2	3	4	5	6	7	8	Ave
2016	12.1	12.1	12.4	12.7	11.9	12.0	12.4	12.0	12.2
2017	12.4	12.4	13.5	13.1	12.8	12.4	11.7	11.6	12.7
2018	13.5	13.2	14.3	15.0	15.1	14.7	14.3	14.0	14.2

Conclusions: It is important how to manage the gilts because the more gilts have piglets, the higher litter size in the farm continue to rise.

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Prod&Wel-015

Improving sow productivity through poly-gamma-glutamate (γ -PGA) treatment: a pilot study

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Introduction: Poly-γ -glutamic acid (γ -PGA), produced by biosynthesis of Bacillus subtilis, is an unusual and promising agent, of which attractive properties made it to be applied in many fields [1]. As previous studies have demonstrated immune stimulation effect of γ -PGA using mouse and pig models [2], the current study was conducted as a pilot study to investigate the effect of γ -PGA in sows and newly born piglets when it is treated to sows.

Materials and Methods: This trial was performed in a conventional one-site Grand Parent (GP) breeding pig farm. Out of a total of 88 Pregnant sows, 46 pregnant sows were randomly selected and treated intramuscularly (IM) with 5 ml of commercial 20 mg/ml γ -PGA (Dongbang Inc., Seoul, Korea) at 5 days before parturition, and 42 sows were treated with 5 ml of PBS as a control group. The sows were monitored for dystocia, stillbirths, deformed fetuses, underweight and live born piglets. Average birth weight (ABW) of live born piglets was measured and correlated with the number of live born piglets. Diarrhea incidence rate of piglets was also recorded and analyzed for the duration of farrowing until weaning. Diarrhea incidence (DI) was calculated as described in Sayan et al. [3] as:

DI (%) = (Number of litters with diarrhea × diarrhea days) / (Total litters in group × experiment days) × 100%.

Results: Sows treated with γ -PGA displayed less dystocia compared to untreated sows (3/46, 6.5% versus 4/42, 9.5%), and piglets born from treatment group presented a lower rate of stillbirth (47/644, 7.3% versus 54/571, 9.4%), underweight (49/644, 7.6% versus 52/571, 9.1%), and a higher number of total live born piglets without any congenital defect (529/644, 82.1% versus 450/571, 78.8%). γ -PGA treated sows only showed higher rate of deformed fetus compared to untreated sows (19/644, 3.0% versus 15/571, 2.6%). Higher ABW was observed among live born piglets of γ -PGA than those of control group (1.38 ± 0.03kg, 529 piglets versus 1.34 ± 0.05kg, 450 piglets). Correlation data between number of live births and ABW demonstrates that the γ -PGA treated sows produced increased number of live born piglets with higher ABW when compared to control group sows. Overall DI and day-by-day diarrhea rate of treatment group (DI 19.2%) were significantly lower than control group (DI 30.8%).

Conclusions: γ -PGA treatment against pregnant sow at late pregnancy showed overall improvement on reproductive performance especially shown by the increased number of live born piglet with higher ABW, and low incidence of neonatal diarrhea. The immune-stimulating and antiviral effects of γ -PGA can be held responsible for the better reproductive performance of the sows.

Acknowledgement: This work was supported by the Technology Development Program for Bioindustry (Project No. 116102-3) of the Ministry for Food, Agriculture, Forestry and Fisheries of the Republic of Korea.

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Prod&Wel-016

Milk yield and milk compositions in primiparous and multiparous Danish Landrace x Yorkshire crossbred sows in Thailand

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Introduction: Piglet survival during suckling period depends on 3 different sources of energy including colostrum, transient milk and mature milk [1]. Piglets are generally having a high energy requirement due to their suckling activity at birth and thermoregulation. Danish Landrace and Yorkshire sows are commonly imported to Thailand for producing Landrace x Yorkshire crossbred F1 females for commercial herds. However, extremely difference in environmental temperature and humidity between Denmark and Thailand may cause a high impact on milk yield and milk compositions. The objective of the present study was to determine milk yield and milk composition of Danish Landrace x Yorkshire crossbred sows in a commercial swine herd in Thailand.

Materials and Methods: A total of 109 sows and their offspring were investigated. The piglets were weighed at 0, 1, 3, 10 and 17 days after birth. The milk yield was estimated by a formula previously published by Hansen et al. (2012) [3]. The estimated milk yields on day 3 to 10 and day 10 to 17 can be calculated as: Milk yields on day 3-10 = 1.93+0.07 x (litter size-9.5) + 0.04 x (litter gain, kg/day-2.05) and Milk yield on day 10-17 = 2.23+0.05x (litter size-9.5) + 0.23 x (litter gain, kg/day-2.05). Milk composition were analyzed for fat, lactose, protein and dry matter concentration by infrated spectroscopy (MilkoScan FT2 instrument, Foss MilkoScan, Hillerød, Denmark). Milk yield were analyzed by using general linear model procedure. Milk composition were analyzed by using general linear mixed model procedure. The statistical model include parity, day in milk and interaction between parity and day in milk. Sow ID was included as random effect. Sow parity was classified into four groups including 1,2,3-4 and 5-6. $P < 0.05$ was regarded to be statistically significant.

Results: The colostrum/ milk yield of sows at 0, 3-10 and 10- 17 days of lactation were 6.1 ± 1.5, 10.2 ± 2.2 and 12.8 ± 2.1 kg/day, respectively. Milk composition (i.e., fat, protein, casein and dry matter) in each day of lactation was presented in Table 1.

Table 1 Composition of sow milk in each day in lactation (g/100 g)

Composition	0	3	10	17	P
Fat	4.9^{b}	6.2^{a}	6.2^{a}	6.7^{a}	<0.001
Protein	15.2^{a}	10.8^{b}	5.2^{c}	5.5^{c}	<0.001
Lactose	2.8^{c}	3.7^{b}	4.9^{a}	4.8^{a}	<0.001
DM	23.5^{a}	21.8^{b}	18.2^{c}	19.2^{c}	<0.001

Milk composition in each sow parity number were presented in Figure 1. As can be seen from the figure, no effect of sow parity number was found on milk composition.

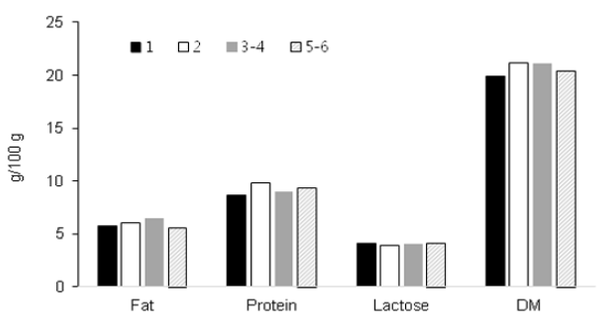

Figure 1 Milk composition in primiparous and multiparous sows

Conclusions: Sow parity number had no impact on milk composition in Danish sow reared in tropical climate.

Acknowledgement: Financial support for the present study was provided by a grant for International Research Integration: Chula Research Scholar, Ratchadaphiseksomphot Endowment Fund. M. Nuntapaitoon is granted by a Postdoctoral Fellowship Ratchadaphisek Somphot Fund.

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Prod&Wel-017

Mycotoxin contamination of pig feeds in Korea

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Introduction: Mycotoxins produced by fungi can cause toxic and/or carcinogenic effects in pigs. Mycotoxin consumption in pigs is likely to cause morbidities such as decreased productivity, chronic damage to organs, weight loss, immune suppression, and reduced reproduction [1]. However, it is difficult to measure the economic impacts of mycotoxins in pig feeds. The purpose of this study was conducted to investigate the occurrence and levels of common mycotoxin [aflatoxin (AF), ochratoxin A (OT), deoxynivalenol (DON), fumonisin (FUM), T-2 toxin (T-2), and zearalenone (ZEA)] in commercial dry feeds for pigs in Korea.

Materials and Methods: A total of 77 pig feed samples were collected from feed silo (n = 40) and hopper (n = 37) in nine pig farms in Korea, 2018. For determination of mycotoxins, feed samples were milled with an analytical mill (Ika, Germany). The ground samples were extracted with water or methanol-water according to the Veratox ELISA kits (Neogen, Austria) and absorbance was measured at 630 nm.

Results: All of the tested samples were positive with OT, T-2, and ZEA in ELISA kits (Table 1). The positive rate was also detected with 97.4% (n = 75) for DON, 92.2% (n = 71) for FUM, and 89.6% (n = 69) for AF, respectively. The highest concentration of OT was 22.7 μg/kg, below the 200μg/kg value which was the maximum allowed level in Korea. And also, the highest concentration was 800 μg/kg for DON and 4,100 μg/kg for FUM, below 900 and 5,000 μg/kg which were the maximum recommended levels in Korea. Only three samples were contaminated with AF, T-2, or ZEA at the levels above the regulation limit. However, it was obvious that the levels of these toxins in feeds were not seriously high. The prevalence of mycotoxins produced by *Fusarium* spp. was significantly different ($p<0.05$) according to the feed companies (data not shown). Although the number of samples was relatively low, these data indicated that the need for more intense monitoring for mycotoxins in pig feeds in Korea.

Conclusions: Although most of the pig feeds were contaminated with mycotoxins in Korea, only three samples were contaminated with AF, T-2, or ZEA at the levels above the regulation limit. And, the levels of these toxins in feeds were not seriously high.

Acknowledgement: This research was supported by the Animal and Plant Quarantine Agency of the Ministry of Agriculture, Food and Rural Affairs of Korea [grant number B-1543018-2017-19-02].

Reference: [1] Bohm J, et al. 2010. Mycotox Res. 23: 147-153

Table 1. Mycotoxin occurrence and levels in pig feeds (silo, n = 40: hopper, n = 37) in Korea

Mycotoxin	Source	n Positive samples (%)	Min	Max	Mean ± SD	n Above levela	Regulation level (μg/kg)
Aflatoxin	Silo	35 (87.5)	0	5.5	1.22 ± 1.13	0	10c
	Hopper	34 (91.9)	0	12.8	1.51 ± 2.07	1b	
Ochratoxin	Silo	40 (100)	0.6	22.7	7.79 ± 4.05	0	200c
	Hopper	37 (100)	0.4	21.2	6.83 ± 4.15	0	
Deoxy-nivalenol	Silo	39 (97.5)	0	700	370 ± 150	0	900d
	Hopper	36 (97.3)	0	800	370 ± 173	0	
Fumonisin	Silo	37 (92.5)	0	4,100	1,140 ± 889	0	5,000d
	Hopper	33 (89.2)	0	3,600	940 ± 911	0	
T-2	Silo	40 (100)	7.1	77.2	30.0 ± 15.9	0	250d
	Hopper	37 (100)	5.5	466.1	40.4 ± 73.2	1	
Zearalenone	Silo	40 (100)	13.0	101.1	50.3 ± 22.5	1	100d
	Hopper	37 (100)	19.6	102.3	47.2 ± 20.5	1	

[a] Number of samples that exceeded the maximum regulation levels
[b] One sample was positive at aflatoxin and T-2, simultaneously
[c,d] Maximum allowed levelc and recommended leveld in Korea

Prod&Wel-018

Prevention of early parturition in sows by using altrenogest

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Introduction: The gestation length in modern genetic sow averages 115.4 ± 1.62 days and 10% of sows farrowed before 114 days of gestation [1]. Sows with early parturition had significantly more stillborn piglets compared to sows with normal gestation length (i.e., 114-117 days) [1]. A previous study has demonstrated that administration of altrenogest between 110-113 days of gestation could effectively prevent early parturition in sows without any negative effect on their offspring [1]. In Thailand, gestation length of a modern high prolificacy sows varied from 110 to 124 days, in which 5.2% farrowed before 114 days and 10.5% farrowed after 118 days of gestation [2]. The high variation of gestation length lead to the difficulties of farrowing supervision and cross-fostering. Therefore, the present study aims to minimize the variation of gestation length in sows by using altrenogest to prevent early parturition in combination with the use of farrowing induction by using double administration of prostaglandin F2alpha at 114 days of gestation.

Materials and Methods: The study was conducted in four commercial swine herd in the Northern part of Thailand from August 2018 to March 2019. In total, 601 Landrace x Yorkshire crossbred sows were included in the experiment (parity 4.9 ± 1.9). The sows were classified into 2 groups, i.e., CONTROL (n=158), TREATMENT 1 (n=443). Sows in the treatment groups were administered 20 mg of altrenogest orally between 106 and 114 days of gestation (Regumate®, Merck Animal Health, Madison NJ, USA). Additionally, the sows were administered PGF2alpha twice within a 6-hr interval (Planate®, Merck Animal Health, Madison, NJ, USA) at 1 day after withdrawal of altrenogest. Newborn piglets (n=2,461) were randomly selected to determine their individual body weight at birth. The data were analyzed by using SAS. The sow traits, i.e., gestation length, total born and born alive were compared between groups by using general linear model (GLM) procedure. The body weight at birth of the piglets were compared between group by using GLM. Least square means were obtained from the models and were compared by using least significant difference test. $P<0.05$ was regarded to be statistically significance.

Results: Gestation length of sows in the TREATMENT group was longer than CONTROL groups (115.1 and 114.6 days, $P<0.001$). Distributions of gestation length in CONTROL and TREATMENT groups are presented in Table 1. The proportion of sows farrowed ≤114 days in TREATMENT group was lower than CONTROL group (27.8% and 46.8%, $P<0.001$). The total number of piglets born per litter in TREATMENT was higher than CONTROL groups (13.5 and 13.0, $P=0.013$). However, number of piglets born alive did not differ between CONTROL and TREATMENT groups (12.7 and 12.9, $P=0.352$). Body weight at birth of the piglets did not differ between CONTROL and TREATMENT groups (1459 and 1460 g, $P=0.973$).

Table 1 Distribution of gestation length in CONTROL (n = 158) and TREATMENT (n = 443) groups

Gestation length (days)	CONTROL (%)	TREATMENT (%)
110-113	8.9	4.3
114	38.0	23.8
115	41.1	45.4
116	9.5	13.3
117	0	12.4
118–119	2.5	1.1

Conclusions: Administration of 20 mg altrenogest orally from 106 to 114 days of gestation significantly extended the gestation length in sows.

Acknowledgement: Financial support for the present study was provided by Chulalongkorn University.

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[1] Vanderhaeghe C. et al., 2011. Reprod Dom Anim. 46, 428–433.
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Prod&Wel-019

Synchronization of parturition in sows by using altrenogest and double administrations of PGF2alpha

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Introduction: In swine industry, a high proportion of piglet mortality occur during pre-weaning period [1]. Interestingly, up to 40% of the piglet pre-weaning morality occur within 1 day postpartum [2]. Farrowing supervision is one of the most important key to assist the sow and piglet during perinatal period. Since the variation of gestation length and the onset of parturition in sows is common [2], the farrowing supervision is difficult to be well planned. A combination of altrenogest and PGF2α administrations in sows has been proved to be an efficient method to avoid early parturition and reduce the proportion of sows with delayed parturition [3]. However, this protocol has not been implemented under field conditions. We investigated the efficacy of three farrowing synchronization protocols including the use of PGF2α, altrenogest and a combination of altrenogest and PGF2α on the onset of parturition in sows.

Materials and Methods: The study was conducted in four commercial swine herds in the Northern part of Thailand from August 2018 to March 2019. All the herds belong to one breeding company. A total of 671 Landrace x Yorkshire crossbred sows were included in the experiment. On average, the parity number of sows was 4.9 ± 1.9 (range 1 to 9). In these herds, it is a common practice to induce farrowing at 114 days of gestation. Therefore, the natural gestation length was not known. The sows were classified according to the synchronization of parturition protocols into 3 groups, i.e., PGF2α (n=158), Altrenogest (n=62) and Altrenogest+PGF2α (n=451). Sows in the PGF2α group were treated with double administrations of PGF2α (Planate®, Merck Animal Health, Madison NJ, USA) at 114 days of gestation. Sows in the Altrenogest group were treated with altrenogest 20 mg/day (Regumate®, Merck Animal Health, NJ, USA) from 106 to 114 days of gestation and sows in the Altrenogest+PGF2α were treated with altrenogest from 106 to 114 days and followed by double administration of PGF2α at 115 days of gestation (8h00 and 16h00). The data were analyzed by using general linear model procedure of SAS. $P<0.05$ was regarded to be statistically significance.

Results: Frequency distribution of gestation length in PGF2α, Altrenogest and Altrenogest+PGF2α groups are illustrated in Table 1. The proportion of sows farrowed on a single day of the week (i.e., on Saturday) in Altrenogest+PGF2α group was higher than PGF2α and Altrenogest groups (85.4%, 20.9% and 11.3%, respectively; $P<0.001$). The total number of piglets born per litter in Altrenogest+PGF2α was higher than PGF2α and Altrenogest groups (13.5, 13.0 and 11.4, respectively; $P<0.05$). Gestation length in Altrenogest+PGF2α and Altrenogest were higher than PGF2α groups (115.2, 115.1 and 114.6 days, respectively; $P<0.001$).

Table 1 Distribution of weekday when the onset of parturition occurs in PGF2α (n=158), Altrenogest (n=62) and Altrenogest+PGF2α (n=451) groups.

Weekday	PGF2α (%)	Altrenogest (%)	Altrenogest+ PGF2α (%)
Sunday	0.6	22.6	3.1
Monday	0	25.8	0.4
Tuesday	0	8.1	0
Wednesday	5.1	8.1	0.2
Thursday	29.1	11.3	0.4
Friday	44.3	12.9	10.4
Saturday	20.9	11.3	85.4

Conclusions: Synchronization of parturition in sows could be performed by using 20 mg altrenogest from 106 to 114 days of gestation in combination with double administrations of PGF2α at 115 days of gestation. This protocol is able to synchronize the onset of parturition to be occur within one day for 85.4%.

References:

[1] Nuntapaitoon et al., 2018. Asian-Australas J Anim Sci. 31:237-244.
[2] Sasaki and Koketsu 2007. Theriogenology 68:123-127.
[3] Gaggini T.S. et al., 2013. Reprod Dom Anim. 48:390-395.

Prod&Wel-020

The administration of an isotonic protein drink to piglets in early-life improves the intestinal microbial profile and reduces E. coli occurrence pre-weaning

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Introduction: The functional maturation of the pig's digestive and immune systems immediately post-birth is largely mediated by the colonization with maternal and environmental microorganisms. Optimal microbial colonization is known to improve the piglet's lifelong immune status, reduce pathogen presence and maximize the pig's digestion and growth performance [1]. Early interventions which modulate the intestinal microbiome can result in healthier and more productive pigs by harnessing the "window of opportunity" that exists early in life. The aim of this study was to assess the effects of early-life administration of an isotonic protein solution on the gut microbiome and on the presence of *E. coli*.

Materials and Methods: 15 gilts (Yorkshire x Landrace x Duroc) and their litters (161 piglets) were enrolled in the study. Litters were allocated to one of two treatments, Group A (Control) - no pre-weaning supplementation, and Group B - piglets were supplemented from day 2 to 8 of life with 500 mL/litter/day of an isotonic protein solution in an open pan. Group B litters also received the same solution 3 and 2 days pre-weaning, followed by a mixture of creep feed and solution from 1 day pre-weaning to 2 days post-weaning. Both groups had *ad libitum* access to normal feed after weaning (at 21 days). Pigs from each litter were randomly selected and euthanized at trial days 9 (n=13 vs 12), 17 (n=13 vs 14) and 30 (n=10 vs 8). Feces were collected for *16S rRNA* gene sequencing (MiSeq Illumina). Samples for semi-quantitative *E. coli* culture were taken from the ileal mucosa.

Results: ***Effects on beneficial bacteria***. Group B piglets had a 3.5-fold higher faecal *Lactobacillus* at day 9 of life compared to Control (4.0% vs 1.2% of all gut bacteria, respectively; *P=0.0001*), amounting to a 233% increase. *Lactobacillus* are beneficial gut bacteria which modulate gut and immune maturation, displace potential pathogens and improve digestion and growth. Gut *Lactobacilli* specialize in using simple carbohydrates. Therefore, the presence of easily-digestible ingredients in the isotonic protein solution provided them with the substrate they needed to thrive.

Bacteroides were 2-fold more abundant at day 17 in Group B piglets compared to the Control group (8.5% vs 4.3% of all gut bacteria; *P<0.05*). The *Bacteroides* are also beneficial, linked to improved immune function and more rapid gut maturation [2]. Their abundance is inversely correlated to that of *Lactobacillus*, however, both bacteria are beneficial.

Potential pathogens. Members of the *Prevotellaceae* family are carbohydrate fermenters in the intestine. However, they are also linked to intestinal pathology due to weaning stress in pigs, especially as they are known mucus degraders. *Prevotellaceae* were significantly lower in Group B pigs compared to Control at days 9 and 17 (20.5% and 27.6% vs 29.0% and 37.0%, of all gut bacteria, respectively; *P<0.01*). These differences represent a 29% reduction at day 9 and a 25% reduction at day 17. No differences were seen at day 30 of life.

E. coli. Fewer of the Group B pigs (7/30, 23%) were positive for *E. coli* pre-weaning (day 9 and 17 combined) compared to Controls (14/26, 54%) (*P = 0.01*; Figure 1). This represents a 57% reduction.

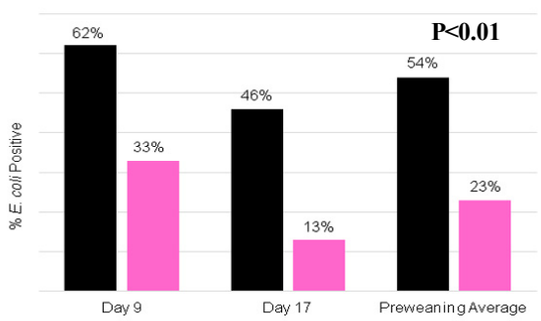

Figure 1. Percentage of Pigs Positive for E. coli

Conclusions: Administration of an isotonic protein solution early in life stimulated beneficial bacteria while reducing potential pathogens and reducing the number of *E. coli*-positive pigs. Since most pre-/peri-weaning intestinal pathology in pigs is linked to *E. coli*, administration of this isotonic protein solution provides an alternative to antimicrobials.

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Prod&Wel-021

The evaluation of minimum inhibitory concentrations of amoxicillin for various swine pathogens isolates from Thailand

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Introduction

Amoxicillin is one of the most common antibiotic that has been used in pig industry. It was established as the drug of first choice for certain indications in individual and group treatments[1]. At present, there are a number of amoxicillin products used in veterinary medicine for swine. Emergence and spread of the microbial resistance to antibiotics in veterinary medicine increase the importance of the selection of optimal treatment choice and right quality product. Antibiotic susceptibility testing (AST) is the best way to monitor the sensitivity/resistance and its development of the pathogens circulating on farm. The aim of this study was to evaluate a sensitivity pattern of amoxicillin for various swine pathogens isolates from Thailand.

Materials and Methods

143 isolates of various swine pathogens were obtained from diseased pigs submitted to National Institute of Animal Health (NIAH) of Thailand during 2017 to 2018. The minimum inhibitory concentrations of amoxicillin were determined by the micro dilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2018)[2]. The test results were reported as MIC_{50} and MIC_{90} values that reflected the minimal inhibition concentration for 50% and 90% of the pathogens tested, respectively.

Result

The range of MIC values of amoxicillin against *Streptococcus suis* (n=40) was ≤0.0625 - 2 µg/ml, *Streptococcus suis* type 2 (n=16) was 0.0625 - 1 µg/ml, *Actinabacillus pleuropneumoniae* (n=18) was 0.125 -1 µg/ml, *Haemophillus parasuis* (n=12) was 0.5 - 2 µg/ml, *Actinobacillus suis* (n=4) was 0.5 - 2 µg/ml, *Pasteurella multocida* (n=36) was 0.125 - 8 µg/ml, *Erysipelothrix rhusiopathiae* (n=4) were 0.0625 - 0.25 µg/ml, and *Burkholderia pseudomallei* (n=13) was 32 - 128 µg/ml. The MIC_{50}, MIC_{90} and the MIC breakpoint for resistance (µg/ml) are presented in table 1.

Table 1 Comparison of MIC_{50} and MIC_{90} values (µg/ml) of amoxicillin and MIC breakpoint for resistance (µg/ml) for swine pathogens

Pathogen	MIC50	MIC90	Break-point	% sensitive
Streptococcus suis, n=40	0.25	1	1	92.5
Streptococcus suis type 2, n=16	0.25	0.5	1	100
Actinabacillus pleuropneumoniae, n=18	0.5	1	2	100
Haemophillus parasuis, n=12	0.5	1	2	100
Actinobacillus suis, n=4	0.5	2	2	100
Pasteurella multocida, n=36	0.5	4	2	86.1
Erysipelothrix rhusiopathiae, n=4	0.0625	0.125	2	100
Burkholderia pseudomallei, n=13	32	64	32	53.9

Conclusion

The result showed the high sensitivity of amoxicillin against various swine bacterial pathogens including Streptococcus *suis, Actinabacillus pleuropneumoniae, Haemophillus parasuis, Actinobacillus suis, Pasteurella multocida, and Erysipelothrix rhusiopathiae.* This study confirmed that amoxicillin is an optimal choice of antibiotic to be a first line treatment.

Reference

[1] Vetrimoxin® registration document
[2] CLSI, 2018. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals, VET01/08. Clinical and Laboratory Standards Institute, 2018.

Prod&Wel-022

Using super antioxidant substances (Viusid Vet) for treatment of azoospermia, oligospermia and asthenospermia boars

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Introduction: Boar spermatozoa are not only sensitive to cold shock and heat shock but also to the fluctuation of temperature in their house. Although, most of boar station used evaporative cooling system (EVAP) some purebred boar are very susceptible to the environmental changes. In 2018, we found 9 purebred boars (1-2 years of age) showed characteristic of azoospermia, oligospermia, asthenospermia and high percentage of proximal and distal cytoplasmic droplet in their semen. The farmer gave them some supportive therapy for 2 months; however, no boar responds to the treatment. It has been reported that the oxidative stress can have a negative effect to the testis especially the spermatogenesis and during maturation in the epididymis, subsequently showed poor quality of semen [1]. We, therefore, aimed to test the efficacy of Viusid Vet, in which the company used the technology for molecular activation to produce extremely high antioxidant capacity, i.e. 1 gram of product provide antioxidant capacity of 11,587 ORAC which is 1.9, 7.8, and 29.8 times higher than vitamin C, tocopherol and ascorbic acid, respectively.

Materials and Methods: 9 purebred boars (6 Duroc and 3 Berkshire), age between 1-2 years, were used in this study. 7 boars (Duroc and Berkshire) were diagnosed as azoospermia (no spermatozoa), oligospermia (< 100 x 10^9 spermatozoa/ml), asthenospermia (< 60% of progressive motility) and 2 Berkshire boars as high percentage of proximal and distal cytoplasmic droplet (> 15% of droplet). All boars were daily supplemented 8 grams of Viusid Vet (powder form) by top dressing. The sperm parameters such as concentration, progressive motility and tail abnormality, were evaluated before and after feeding boars with Viusid Vet starting at 3, 4, 5, 7 and 9 wk [2]. Feed of boar was also analyzed for Fumonisin, Aflatoxin and Zealarenone by using ELISA method. Proximate analysis of boar feed was also performed.

Results: The sperm parameters before and after feeding boar with Viusid Vet are presented in Figures 1 and 2. The levels of Aflatoxin, Fumonisin and zearalenone are in a normal level. The proximate analysis is met with the NRC recommendation.

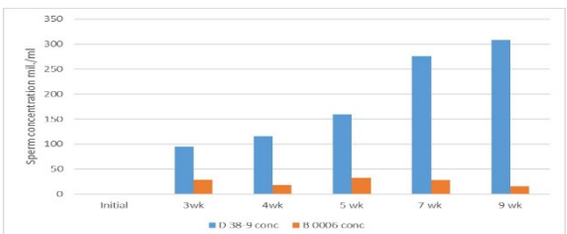

Figure 1 Sperm parameters before and after feeding azoospermia boars with Viusid Vet.

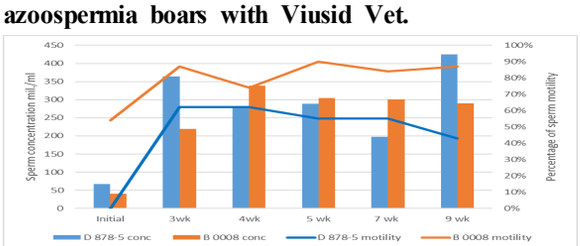

Figure 2 Sperm parameters before and after feeding oligospermia and asthenospermia boars with Viusid Vet.

Discussion and Conclusion: The present results clearly showed that Viusid Vet with its extremely high antioxidant capacity, diminished the detrimental effect of oxidative stress to the testis, and consequently improve boar semen qualities in all 9 boars that had problem with azoospermia, oligospermia, asthenospermia and other problems. However, a period of improvement is different, depending on particular problem.

Acknowledgement: This work was supported by a grant from Innovet Corporation Co., Ltd., Samutprakarn, Thailand.

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[1] Chanapiwat C and Kaeoket K., 2016. Thai J. Vet. Med. 46, 155-160.
[2] Kaeoket K et al. 2010. Asian J. Androl. 12, 760-765.

Prod&Wel-023

Using super antioxidant substances (Viusid Vet) for treatment of boars with high percentage of cytoplastic droplet in semen

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Introduction: Boar spermatozoa are not only sensitive to cold shock and heat shock but also to the fluctuation of temperature in their house. Although, most of boar station used evaporative cooling system (EVAP) some purebred boar are very susceptible to the environmental changes. In 2018, we found 6 purebred boars (1-2 years of age) showed characteristic of high percentage (> 15%) of proximal and/or distal cytoplasmic droplet in their semen. Pig farmer gave them some supportive therapy; however, no boar responds to the treatment. It has been reported that the oxidative stress can have a negative effect to the testis especially during 4 weeks of spermatogenesis and 2 weeks of maturation in the epididymis, subsequently showed poor quality of semen [1]. In mammal, during maturation, spermatozoa undergo denude their cytoplasmic droplet in order to gain fertilization ability. However, this process may be disturbed if the epididymis faces with oxidative stress. We, therefore, aimed to test the efficacy of Viusid Vet, in which the company used the technology for molecular activation to produce extremely high antioxidant capacity, i.e. 1 gram of product provide antioxidant capacity of 11,587 ORAC which is 1.9, 7.8, and 29.8 times higher than vitamin C, tocopherol and ascorbic acid, respectively.

Materials and Methods: 6 purebred boars (4 Duroc and 2 Berkshire), age between 1-2 years, were used in this study. 6 boars (Duroc and Berkshire) were diagnosed as high percentage of cytoplasmic droplet (> 15%). All boars were daily supplemented 8 grams of Viusid Vet (powder form) by top dressing. The sperm parameters such as concentration, progressive motility, tail abnormality, and proximal and cytoplasmic droplet were evaluated before and after feeding boars with Viusid Vet starting at 3, 4, 5, 7 and 9 wk [2]. Feed of boar was also analyzed for Fumonisin, Aflatoxin and Zealarenone by using ELISA method. Proximate analysis of boar feed was also performed.

Results: The percentage of cytoplasmic droplet before and after feeding boar with Viusid Vet are presented in Figure 1. The other sperm parameters are presented separately. The levels of Aflatoxin, Fumonisin and zearalenone are in a normal level. The proximate analysis is met with the NRC recommendation.

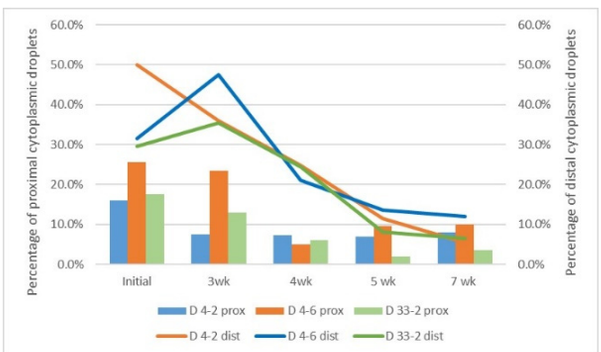

Figure 1 Sperm parameters before and after feeding high-cytoplasmic droplet-boars with Viusid Vet.

Discussion and Conclusion: The present results clearly showed that Viusid Vet with its extremely high antioxidant capacity are able to improve boar semen qualities after 2 weeks of treatment in all 6 boars that had problem with high percentage of proximal and distal cytoplasmic droplet. However, a period of improvement in each boar is different, depending on the severity of the problem. To our knowledge, this is the first report on the treatment of boar with super antioxidant subtances.

Acknowledgement: This work was supported by a grant from Innovet Corporation Co., Ltd., Samutprakarn, Thailand.

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[1] Chanapiwat C and Kaeoket K., 2016. Thai J. Vet. Med. 46, 155-160.

[2] Kaeoket K et al. 2010. Asian J. Androl. 12, 760-765.

PRRS-001

A comparative study of the efficacy of a porcine reproductive and respiratory syndrome subunit and a modified-live virus vaccine against respiratory diseases in endemic farms

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) was first discovered in 1987 in North America. Since then, the disease has become endemic and is one of the most important infectious diseases to the swine industry, resulting in tremendous economic losses worldwide. In Korea, a commercial PRRS modified-live virus (MLV) vaccine has been widely used to control epidemic and endemic PRRSV infection since its first introduction in 1996. Despite the fact that the PRRS MLV vaccine has been efficacious in controlling PRRSV infection, there are increased concerns about its safety because of the possible risk of reversion to virulence [1]. The objective of this study was to evaluate and compare the efficacy of the PRRS subunit vaccine with a PRRS MLV vaccine in endemic PRRS farms.

Materials and Methods: The clinical field trial was conducted on three separate farms. Farms A and B housed 1,000-sow herds and 2 site (farrow-to-nursery and nursery-to finish) production with all-in-all-out system. Farm C housed a 1,000-sow herd and 1 site (farrow-to- finish) production with all-in-all-out system. Pigs in the VacA/Subunit, VacB/Subunit, and VacC/Subunit groups from Farms A, B, and C respectively, were injected intramuscularly on the right side of the neck with 2.0 mL of the PRRS subunit vaccine (PRRSFREETM PRRS subunit vaccine, Reber Genetics Co. Ltd.) at 21 and 42 days of age according to the manufacturer's instructions. Pigs in the VacA/MLV, VacB/MLV, and VacC/MLV groups from Farms A, B, and C, respectively, were administered intramuscularly on the right side of the neck, a 2 mL dose of the PRRS MLV vaccine (Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica.) at 21 days of age, according to the manufacturer's instructions.

Results: In Farm A, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacA/Subunit and UnVacA groups compared to the VacA/MLV group during the 112-168 days period. The overall growth performance (21 to 168 days of age) was also was significantly higher ($P < 0.05$) in pigs from the VacA/Subunit and UnVacA groups compared to the VacA/MLV group. In Farm B, the ADWG was significantly higher ($P < 0.05$) in pigs from the VacB/MLV group compared to the UnVacB group between 70-112 days of age. The overall growth performance (21 to 168 days of age) was significantly higher ($P < 0.05$) in the VacB/Subunit and VacB/MLV groups compared to the UnVacB group. In Farm C, pigs in the VacC/Subunit group had a significantly higher ($P < 0.05$) ADWG compared to the UnVacC group between 21-70 days of age. There was no significant difference on the overall growth performance (21 to 168 days of age) among the VacC/Subunit, VacC/MLV, and UnVacC groups. There was no significant difference between vaccinated and unvaccinated pigs in all 3 farms, in the number of genomic copies of PRRSV-1 or PRRSV-2 detected in the blood samples collected. Vaccine virus was detected in the serum samples of 16/20 pigs at 42 dpv and 9/19 pigs at 70 dpv in the VacA/MLV group, 15/20 pigs at 42 dpv and 9/20 pigs at 70 dpv in VacB/MLV group, and 12/20 pigs at 42 dpv and 13/19 pigs at 70 dpv in VacC/MLV group. No vaccine virus was detected in the serum of pigs from the VacA/Subunit, VacB/Subunit, VacC/Subunit, UnVacA, UnVacB, and UnVacC groups throughout the experiment.

Conclusions: The In our study, vaccination of pigs with either of the PRRS vaccines improved the growth performance significantly in Farm B. We did not see an improvement in growth performance by either of the vaccines in Farm C. Interestingly, in Farm A, vaccination of pigs with the PRRS subunit vaccine resulted in significantly better growth performance compared to the MLV vaccine.

Acknowledgement: This study was supported by contract research funds (Grant no. 550-20160053) of the Research Institute for Veterinary Science (RIVS).

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PRRS-002

A field trial of Type 2 Porcine Reproductive and Respiratory Syndrome virus vaccine (PrimePac PRRS) in the Philippines

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Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) is an economically important swine disease with worldwide prevalence[1]. As the name implies, the disease mainly manifests as reproductive failure in sows and respiratory illness in pigs of all ages[2]. Vaccination is a key tool to reduce PRRS-related disorders alongside other various strategies including improved husbandry and biosecurity[3]. In the Philippines, PRRS is a major disease affecting both breeder and fattener pigs causing severe production losses. The safety and efficacy of a new Type 2 PRRS vaccine (PRIMEPAC® PRRS) were evaluated against other commercially available vaccines in four farms.

Materials and Methods: The field trials were started in July 2017 at four commercial swine farms in Luzon, Philippines. All farms in the trial had 1000-1500 sows, endemic PRRS circulation in the breeder and fattening herds, and were using PRRS vaccine in the fattener pigs and breeder herds. These commercial farms had a known history of PRRS infection.

Fattener pigs

On each farm, a total of 180 2-week-old piglets eligible for admission to the study were weighed, ear tagged, and color-coded for easy identification. These animals were assigned randomly to one of the two groups: TREATMENT (n=90), which were vaccinated with PRIMEPAC® PRRS vaccine 1ml intramuscularly and CONTROL (n=90), existing vaccine on farm.

Breeding sows

On each farm, a total of 180 sows eligible for admission to the study were identified using the existing farm identification system (ear tags). These animals were assigned randomly to one of the two groups: TREATMENT (n=90), which were vaccinated with PRIMEPAC® PRRS vaccine 1ml intramuscularly and CONTROL (n=90), existing vaccine on farm.

Results: With regards to safety, no acute reactions were observed post vaccination in both the treatment and control groups for both sows and piglets across the 4 farms.

Table 1. Production parameters of fattener pigs.
a, b: value with different superscripts in each column represent statistically significant differences ($p<0.05$)

TRIAL FARMS	Mortality (%)	Weaning weights (in Kg)	Finishing weights (in Kg)	Average Daily Gain (in g)
A Farm				
Treatment	0[a]	7.9[a]	82.7[a]	563[a]
Control	5.6[a]	8.4[a]	71.6[a]	538[b]
G Farm				
Treatment	12.3[a]	6.4[a]	91.3[a]	527[a]
Control	16.7b	5.9[a]	90.6[a]	526[a]
F Farm				
Treatment	6[a]	7.0[a]	82.7[a]	631[a]
Control	11[b]	6.9[a]	71.6[a]	547[b]

Table 2. Production parameters of breeding sows.
a, b: value with different superscripts in each column represent statistically significant differences ($p<0.05$)

TRIAL FARMS	Total Born Piglets	Born Alive	Stillbirth	Mummified	Weak
D Farm					
Treatment	11.5[a]	10.8[a]	0.55[a]	0.14[a]	0.07[a]
Control	11.2[a]	10.5[a]	0.39[a]	0.24[a]	0.11[a]
G Farm					
Treatment	11.78[a]	9.4[a]	0.1a	0	0
Control	10.33[a]	9.3[a]	0.04a	0	0
Farm F					
Treatment	11.1[a]	10.6[a]	0.26[a]	0.16[a]	0
Control	9.4[b]	8.6[b]	0.34[a]	0.49[a]	0

Conclusions: PRIMEPAC® PRRS demonstrated efficacy and safety in commercial herds with significant differences over the control pigs for some efficacy parameters (Total Born, Average Daily Gain, Mortality). PRIMEPAC® PRRS is a viable option for producers to combat PRRS-related illness in nursery to finisher pigs as well as breeding herds.

Acknowledgement: MSD Animal Health wishes to thank the staff of the 4 farms involved for participating in the trial toward data collection.

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PRRS-003

Case Report: Improvement of Reproductive Problems in Sow and Reduction of PRRS Virus Vertical Transmission with Ingelvac® PRRS MLV Sow Mass Vaccination

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSv) is one of the major pathogens causing reproductive failures in breeding herd. Infection of PRRSv in sow results in early termination of gestation causing repeat service, late-term abortion and increases number of stillbirths as well as weak-born pigs [1]. Moreover, PRRSv can be transmitted vertically from sow to piglets transplacentally which leads to PRRSv-positive piglets [2]. The present case report evaluates efficacy of Ingelvac® PRRS modified life virus (MLV) in reducing reproductive problems and vertical transmission of PRRSv to piglets during PRRS outbreak in a Malaysia swine farm.

Materials and Methods: A 400-sow farrow-to-finish pig farm was reported with increasing abortion rate (16.2%) and percentage of repeat service (17.8%) in June 2018. All gilts/sows were vaccinated against CSF, FMD, Aujeszky's disease, parvovirus infection and atrophic rhinitis. Previously, PRRS control was done by performing sow mass vaccination using type I (EU) PRRS MLV. Blood samples collected from 1-week old (n=6) and 4-week old piglets (n=6) for PRRS PCR revealed heterologous challenge of both type I and type II PRRSv infection for both age groups. In this case, the farmer changed the sow PRRS vaccination from EU PRRS MLV to Ingelvac PRRS MLV. Farm reproductive parameters (abortion rate and percent of repeat services) were evaluated for the period before (January 2018 - June 2018) and after (July 2018 - December 2018) the implementation of sow vaccination using Ingelvac® PRRS MLV. The statistical analysis was generated using Minitab software version 17. Blood samples were collected once again from 1-week old (n=8) and 4-week old piglets (n=8) from farrowing room 6 months after implementation of sow vaccination and subjected to PRRS RT-PCR to indicate reduction in vertical transmission of PRRSv.

Results: After changing from type I PRRS MLV to Ingelvac® PRRS MLV, the sow abortion rate reduced from 13.7% to 9.7% within 6 months (Figure 1), while percentage of repeat services also reduced from 15.5% to 8.0% (Figure 2). After Ingelvac® PRRS MLV vaccination, PRRSv was not detected from both 1-week old and 4-week old piglets in farrowing room piglets, suggesting the vertical transmission of PRRSv was stopped (Table 1).

Figure 1: Sow abortion rate (%)

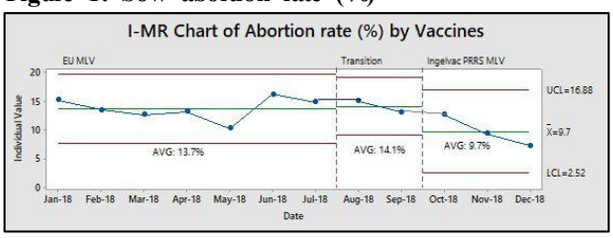
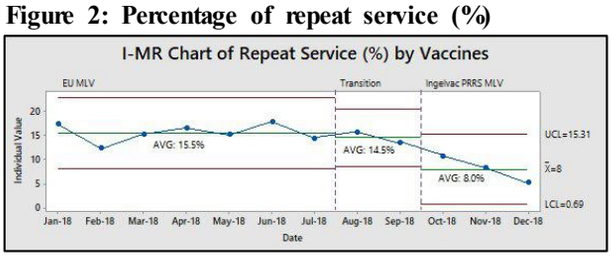


Figure 2: Percentage of repeat service (%)



Conclusions: There was an overall improvement of sow reproductive performance after shifting from type I PRRS MLV to Ingelvac® PRRS MLV sow vaccination under PRRS outbreak situation. PRRS stabilization in breeding herd was achieved as vertical transmission of PRRSv from sow to piglets was eliminated. Furthermore, type I PRRS MLV doesn't provide adequate heterologous protection to the animal herd when there was PRRS outbreak of Type I and Type II strains. This field case provides strong evidence of ability of Ingelvac® PRRS MLV to control both type I and type II PRRSv.

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PRRS-004

Case Report: Rapid control of PRRSV NADC-30 like strain infection by means of management and homogenization of vaccine

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Introduction

This field case demonstrates how to control NADC30-like PRRSV with comprehensive approaches including herd closure, whole herd vaccination and McRebel principles in a Chinese farm.

Materials and Methods

This one site farm is located in Eastern China which has 500 sows. About three weeks after introducing gilts from outside in November 2017, sows aborted, and the proportion of stillbirths and mummies increased, pre-weaning and nursery mortality rate increased as well. Samples collected from aborted fetuses and diseased piglets were sent to the laboratory for testing, where it was confirmed to be PRRSV infection with an NADC30-like strain .

To control disease quickly, following approaches were applied:

1. Herd Closure, stopped introducing any gilts into the farm until being stable.
2. Mass vaccination to all sows boars and gilts with Ingelvac® PRRS MLV twice a month, and 4 times per year afterwards, piglets in the farrowing room were injected with Ingelvac® PRRS MLV at 14 days.
3. Adopted McRebel principles which focused on 4 points:
   - ✓ Foster limitation. Fostering after 24 hours old is strictly forbidden.
   - ✓ Strengthened the management of needles.
   - ✓ Stopped tooth and tail trimming. Employees were not allowed to enter farrowing crates.
   - ✓ Strict AIAO in farrowing and nursery house.
4. Diagnostic Work-up the following samples were taken Placental umbilical cords (PUCs), PRRSV pre-vaccine piglets, 10 week old pigs, 13 week old pigs, 16 week old pigs, and semen from all boars was collected every month, respectively. PCR detection of PRRSV 5 pool 1; positive samples were sequenced.

Table 1. PRRSV antigen detected.

time	PUCs	Pre-vaccine	8weeks	13weeks	16weeks	boars
Dec 2017	4/5[a]	1/1[a]	3/5[a]	2/5[a]	1/5[a]	1/1[b]
Jan 2018	3/5[a]	3/5[a]	4/5[c]	3/5[a]	0/5	0/1
Feb 2018	1/5[a]	1/5[a]	3/5[a]	3/5[a]	2/5[a]	
Mar 2018	0/5	0/5	1/5[a]	1/5[a]	0/5	
April 2018	0/5	0/5	1/5[a]	1/5[a]	1/5[a]	0/1
June 2018	0/5	0/5	2/5[a]	0/5	0/5	

Notes: [a]wild virus; [b]high CT value, can't sequence; [c]wild virus and vaccine virus

Results

After the implementation of these approaches, the production performance of the farm has improved significantly, and the detection rate of PRRSV has also decreased dramatically.

Discussion

PRRSV is one of the biggest threats to pig industry. Vaccination with Ingelvac® PRRS MLV combined with other management procedures can efficiently control clinical signs, improve production performance and reduce virus circulation in herds.

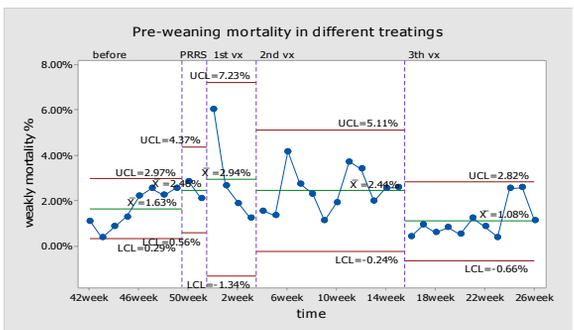

Figure 1: SPC chart of pre-weaning mortality

Keywords: PRRSV, NADC30-like, control, McRebel

PRRS-005

Characterization of porcine tripartite motif genes as host restriction factors againstporcine reproductive and respiratory syndrome virus infection

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Introduction: Members of the tripartite motif (TRIM) family are the important effectors of the innate immune response against viral infections[1]. Porcine reproductive and respiratory syndrome (PRRS) continues to cause substantial economic losses to the pig industry worldwide[2]. However, it is still unknown whether porcine TRIM (pTRIM) genes may restrict the infection of PRRSV. We therefore performed an exhaustive description of pTRIMs, including classification, evolution and expression analysis, to elucidate implication in the anti-PRRSV activitiy.

Materials and Methods: In order to perform a comprehensive study of pTRIM genes, all pTRIM sequences and their related information were obtained from the NCBI. Classification, evolution and genome location of pTRIMs were conducted. The CODEML program from the PAML package was conducted to figure out the TRIM genes under positive selection in mammals, and the model M8 was also used to identify the positively selected sites with the posterior probability more than 0.95. For IFN-β treatment experiment, porcine alveolar macrophages (PAMs) were treated with or without IFN-β (100 ng/mL), and collected in 750 mL of TRIzol reagent at 12 h post-stimulation. For PRRSV infection experiment, PAMs were infected with or without PRRSV a MOI of 0.1, and harvested in 750 mL of TRIzol reagent at 24 h post-infection (hpi). Real-time quantitative PCR (RT-qPCR) was used to detect the relative mRNA level of pTRIMs in PAMs after IFN-β stimulation (non-treated PAMs served as the control) or PRRSV infection (non-infected PAMs served as the control). *ISG15* was used as the positive control. Finally, the relative mRNA expression of pTRIM5, 14, 21, 25 and 38 in eleven healthy porcine tissues (heart, liver, spleen, lung, kidney, stomach, jejunum, colon, bladder, muscle, and lymph node) was detected. The quantity of pTRIMs in these tissues was normalized as compared to that in the muscle.

Results: We firstly defined the entire pTRIM family. 57 pTRIMs were classified into 12 sub-families (C-I to C-XII) based on variable C-terminus, and 17 (TRIM3, 4, 5, 11, 14, 15, 16, 17, 21, 25, 29, 31, 38, 40, 44, 45 and 54) out of them were identified as positively selected genes. 9 (pTRIM5, 14, 21, 25, 26, 34, 38, 39 and 56) porcine IFN-stimulated genes (ISGs) were identified from IFN-β treated PAMs. Then, the PAMs were infected with PRRSV, 11 (pTRIM5, 14, 21, 25, 26, 34, 36, 38, 44, 50 and 56) PRRSV-upregulated genes and one (pTRIM65) PRRSV-downregulated gene were examined. The mRNA expression of the implicated host restriction factors (pTRIM5, 14, 21, 25 and 38) was detected in eleven swine tissues studied, with the higher expression in the spleen and lung tissues.

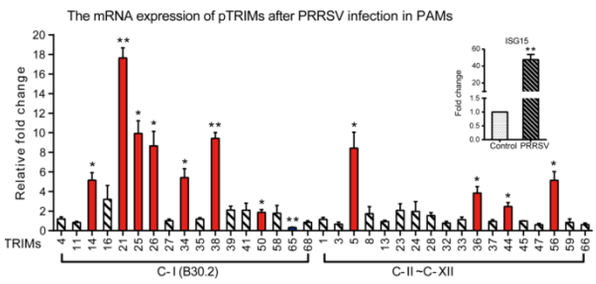

Figure 1. Expression of pTRIMs after PRRSV infection in PAMs.

Conclusions: Our results firstly present the comprehensive characterization of pTRIM genes, and suggest the pTRIM5, 14, 21, 25, and 38 genes as implicated host restriction factors against PRRSV infection.

Acknowledgement: This work was supported by the Natural Science Foundation of Guangdong Province (2014A030312011).

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PRRS-006

Commercial PRRSV-2 MLV vaccine against heterologous single and dual Korean PRRSV-1 and PRRSV-2 challenge

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is classified as a member of the *Arteriviridae* family in the order *Nidovirales*. PRRSV was first isolated in the late 1980s almost simultaneously in Europe and North America and was divided into PRRSV-1 (type 1 genotype and European origin) and PRRSV-2 (type 2 genotype and North American origin) genotypes based on genetic, antigenic, and pathogenic differences [1]. Korean field conditions differ from European and North American continents where there is a predominant PRRSV-1 or PRRSV-2, respectively because both PRRSV genotypes are prevalent. Hence, the objective of this study was to evaluate the protection of the PRRSV-2 MLV vaccine against single or dual challenge with heterologous PRRSV-1 and PRRSV-2 in growing pigs in terms of respiratory disease.

Materials and Methods: At -35 days post challenge (dpc, 21 days of age), the pigs in Vac/Ch1, Vac/Ch2, and Vac/Ch1-2 were injected intramuscularly on the right side of the neck with 2 mL of Ingelvac PRRS® MLV (Boehringer Ingelheim Vetmedica, lot no. 2451088A) according to the manufacturer's instructions. The pigs in UnVac/Ch1, UnVac/Ch2, UnVac/Ch1-2, and UnVac/UnCh were administered an equal volume of phosphate buffered saline (PBS, 0.01M, pH 7.4). At 0 dpc (56 days of age), the pigs in Vac/Ch1 and UnVac/Ch1 were inoculated intranasally with 3 mL of PRRSV-1 inoculums (10^5 $TCID_{50}$/mL of SNUVR090485, second passage in alveolar macrophages). The pigs in Vac/Ch2 and UnVac/Ch2 were inoculated intranasally with 3 mL of PRRSV-2 inoculums (10^5 $TCID_{50}$/mL of SNUVR090851, second passage in alveolar macrophages). The pigs in Vac/Ch1-2 and UnVac/Ch1-2 were inoculated intranasally with 3 mL of each PRRSV-1 and PRRSV-2 inoculums (10^5 $TCID_{50}$/mL of SNUVR090485, second passage in alveolar macrophages and 10^5 $TCID_{50}$/mL of SNUVR090851, second passage in alveolar macrophages). The pigs in UnVac/UnCh were inoculated intranasally with 3 mL of PBS and served as negative controls.

Results: Pigs in Vac/Ch1 had significantly ($P < 0.05$) lower number of genomic copies of challenge PRRSV-1 RNA in their sera at 7, 10, and 14 dpc compared to pigs in UnVac/Ch1. Pigs in Vac/Ch2 had significantly ($P < 0.05$) lower number of genomic copies of challenge PRRSV-2 RNA in their sera at 7, 10, and 14 dpc compared to pigs in UnVac/Ch2. Pigs in Vac/Ch1-2 had significantly ($P < 0.05$) lower number of genomic copies of challenge PRRSV-2 RNA in their sera at 7, 10, and 14 dpc compared to pigs in UnVac/Ch1-2. No PRRSV-1 was detected in the sera of pigs from Vac/Ch2 and UnVac/Ch2 and vice versa. No PRRSV was detected in the sera of pigs from UnVac/UnCh throughout the experiment. Upon PRRSV-1 challenge, pigs in Vac/Ch1 and Vac/Ch1-2 had significantly ($P < 0.05$) higher numbers of PRRSV-1 specific IFN-γ -SC in PBMC compared to pigs in UnVac/Ch1 and UnVac/Ch1-2, respectively, at -21 to 14 dpc. Upon PRRSV-2 challenge, pigs in Vac/Ch2 and Vac/Ch1-2 had significantly ($P < 0.05$) higher numbers of PRRSV-2 specific IFN-γ -SC in PBMC compared to pigs in UnVac/Ch2 and UnVac/Ch1-2, respectively, at -21 to 14 dpc. No PRRSV specific IFN-γ -SC was detected in pigs from UnVac/UnCh throughout the experiment.

Conclusions: The results of this study demonstrate that PRRSV-2 MLV vaccine used in this study confer protection the respiratory disease against heterologous single and dual challenge of PRRSV-1 and PRRSV-2. The vaccinated challenged pigs were protected from subsequent single and dual heterologous challenge of PRRSV-1 and PRRSV-2 as evidenced by the lack of PRRSV-1 and PRRSV-2 viremia, by significantly reduced macroscopic and microscopic lung lesions, and significantly reduced amount of PRRSV-1 and PRRSV-2 RNA in lungs of growing pigs.

Acknowledgement: The author's research was supported by contract research funds (Grant no. 550-20160084).

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PRRS-007

Comparative study evaluating the safety of type 2 porcine reproductive and respiratory syndrome modified-live virus vaccines in pigs

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Introduction: Recently, porcine reproductive and respiratory syndrome (PRRS) modified-live vaccines (MLV) had been used to control PRRSV infection worldwide. The safety of MLVs are of interest due to the potential for vaccinated pigs to continue to shed virus for prolonged periods [1]. Therefore, the study was conducted to evaluate the safety of MLV in terms of virus persistence and shedding pattern using sentinel pigs.

Materials and Methods: Fifty-six, PRRSV-free, weaned sentinel pigs were randomly allocated into 2 groups, G1 (n=21) and G2 (n=21). G1 was vaccinated with Prime Pac® PRRS (MSD Animal Health, The Netherlands). G2 was vaccinated with Ingelvac® PRRS MLV (Boehringer Ingelheim, Germany). Following vaccination, age-matched sentinels were introduced weekly into vaccinated groups (n=1/group) starting from 0 to 42 days post vaccination (DPV). Each batch of sentinel was commingled with vaccinated pigs for 3 weeks and monitored for seroconversion using ELISA. Three vaccinated pigs of each group were also necropsied on a weekly basis from 7 to 42 DPV. Tonsils, feces, nasal swabs, serum samples, urines, and lung lavages were collected and assayed for the presence of virus using virus isolation and PCR.

Results: PRRSV detection and seroconversion in sentinel pigs were summarized in Table 1. Sentinel pigs introduced to G2 at 14 to 42 DPV seroconverted, but sentinel pigs introduce to G1 seroconverted at 28 DPV only. For tonsils, PRRSV was detected in only one pig in G1 at 28 and 35 DPV. In contrast, PRRSV was detected in 2/3, 2/3, and 1/3 of pigs in G2 at 28 to 42 DPV. For serum, PRRSV was detected in 2/3 pigs of G1 at 7 DPV only. Whereas, PRRSV was detected in 3/3 and 2/3 of pigs in G2 at 7 and 14 DPV. For urine, PRRSV was detected in only one G1 pig at 28 DPV but detected in 1/3, 2/3, and 1/3 of pigs in G2 at 21, 28, and 35 DPV, respectively. As well as in lung lavage, PRRSV was detected only one G1 pig at 28 DPV. Meanwhile, PRRSV was detected in 1/3 and 2/3 of pigs in G2 at 21 and 28 DPV, respectively.

Table 1. PRRSV detection and seroconversion in sentinel pigs

Samples		Days post-vaccination (DPV)					
		7	14	21	28	35	42
Sentinel pigs	G1	Neg	Neg	Neg	Neg	Neg	Neg
	G2	Neg	Pos	Pos	Pos	Pos	Pos
Tonsils	G1	0/3W	0/3	0/3	1/3	1/3	0/3
	G2	0/3	0/3	0/3	2/3	2/3	1/3
Feces	G1	0/3	0/3	0/3	0/3	0/3	0/3
	G2	0/3	0/3	0/3	0/3	0/3	0/3
Nasal swabs	G1	0/3	0/3	1/3	2/3	1/3	0/3
	G2	0/3	0/3	3/3	3/3	2/3	2/3
Serum samples	G1	2/3	0/3	0/3	0/3	0/3	0/3
	G2	3/3	2/3	0/3	0/3	0/3	0/3
Urines	G1	0/3	0/3	1/3	1/3	0/3	0/3
	G2	0/3	0/3	0/3	2/3	1/3	0/3
Lung lavages	G1	0/3	0/3	0/3	1/3	0/3	0/3
	G2	0/3	03	1/3	2/3	0/3	0/3

Conclusions: Pigs vaccinated with Prime Pac® PRRS shed vaccine virus shorter than did Ingelvac® PRRS MLV. The persistence of infection in tonsils, nasal swabs, serum, urine, and lung lavages from vaccinated pigs was also lower.

Acknowledgement: This work was supported by Research and Researchers for Industries (Grant No. PHD59I0040).

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PRRS-008

Comparison of the field efficacy of commercial porcine reproductive and respiratory syndrome virus (prrsv)-1 and prrsv-2 vaccines against prrsv-2 in taiwan

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Introduction: Various Modified Live Virus (MLV) vaccines are commercially available, and several studies have shown not only good efficacy of MLV vaccines against challenge with homologous strains but also partial protection against challenge with heterologous strains, whereas others have found poor cross-protection[1]. Genomic homology between the PRRS MLV vaccine and the infecting strain in terms of the ORF5 gene sequence is not a good predictor of vaccine efficacy[2]. The purpose of this study was to compare the efficacy of using 2 commercial PRRS MLV vaccines containing PRRSV-1 and PRRSV-2 strains in a Taiwanese pig farm endemically infected with PRRSV-2.

Materials and Methods: The trial was conducted in a PRRSV-2 infected unstable farm in Taiwan with a PRRS outbreak registered in August 2017 which resulted in severe losses: 25% (200/800) sow herd mortality and respiratory symptoms in nursery pigs.

Whole herd vaccination with different vaccines was performed in 2 groups, mass vaccination on sow herd (700 sows, 4 times/year after basic vaccination) and one shot on one week old piglet:

Group A involved 6510 piglets vaccinated with PRRSV-2 vaccine, from January to June 2018. Group B, involved 7093 piglets vaccinated with PRRSV-1 vaccine (UNISTRAIN® PRRS, (HIPRA) from July to December 2018.

Vaccine efficacy in the PRRS control was evaluated on the basis of these criteria: Viremia-positive rate in weaned piglets, reproductive performance (average weaned piglets and farrowing rate) and survival rate of nursery pigs.

Results: Sow herd PRRSV stability (production of non-viremic piglets at weaning age) was achieved in group B after 3 months of PRRSV-1 sow mass vaccination. In group A, however, the viremia-positive rate in piglets at weaning age was 93.3% after 6 months of PRRSV2 sow mass vaccination. The weaned piglet average after 6 months of mass vaccination was higher in group B (9.35) than in group A (8.52). The survival rate for nursery pigs in group B was higher (94.1%) than in group A (81.49%).

Table 1. Sow herd stability, growth and reproductive performance results in group A and group B. Amv: after sow mass vaccination.

	Group A (PRRSV-2 vaccine)	Group B (UNISTRAIN® PRRS)
Growth performance	Survival rate during nursery period (%)	
	81.49	94.1
Reproductive performance	Average weaned piglets	
	8.52	9.35
	Farrowing rate (%)	
	71.3	77.6
Stability of sow herd	93.3% viremic piglets at weaning (6 months amv)	0% viremic piglets at weaning (3 months amv)

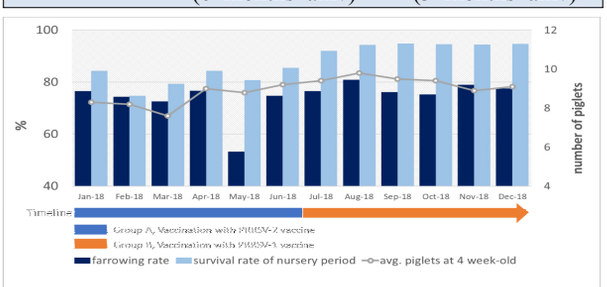

Fig 1. Changes in reproductive and growth performance in group A and group B. Grey line: Average 4 week-old weaned piglet rate.

Conclusions: In the same production system, the heterologous protection conferred by UNISTRAIN® PRRS (PRRSV-1 vaccine) against PRRSV-2 infection was more effective compared with the outcome achieved with the previous PRRSV-2 vaccine, showing a significant improvement in productivity as a result of increases in the farrowing rate, average weaned piglet rate, survival rate during the nursery period and time to achieve sow herd stability.

Acknowledgements: The authors thank managers and farm staff for generous sharing performance record and technical assistance.

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PRRS-009

Control of porcine reproductive and respiratory syndrome virus type2 via the prime-boost concept in a heterologous type 1 on type 2 setup under field conditions

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Introduction: Modified live virus (MLV) vaccines are widely used in sows and piglets to control porcine respiratory and reproductive syndrome (PRRS) in various protocols with various success. The use of an inactivated (INV) PRRS vaccine on top of a PRRS MLV of same type in various MLV-INV alternating protocols is named Prime-Boost Concept (PBC) in all-cycle breeding stock. The PBC is demonstrated to provide better active and colostral protection plus wider PRRS cross-protection in homologous PBC protocols (MLV and INV of same type). Also, improved both PRRS type 1 & 2 neutralizing-antibodies in a heterologous PBC of the type 1 PRRS INV vaccine, Progressis® (Ceva), on top of type 2 MLV vaccine is demonstrated.

The aim of this study is to demonstrate PRRS type 2 control via a Heterologous Progressis® PBC protocol.

Materials and Methods: As a last resort attempt to control PRRS type 2, a Heterologous Progressis® PBC protocol was implemented in a dual PRRS (type 1 and 2) positive Danish 1100 sow herd selling pigs of at 11 weeks old. The farm is in control on type 1 whereas PRRS type 2 has remained out of control, and the farm unstable, since the introduction in the late 1990'ties. The instability is experienced as recurrent PRRS and bacterial disease remaining at high levels, despite several attempts on management optimizations and alternative MLV type 2 vaccination schedules; at present quarterly mass vaccinations.

The Progressis® PBC vaccination protocol implemented: Initiation of PRRSV calm down via double Progressis® mass-vaccination of all breeding stock 4 weeks apart.

Subsequently, Gilts: MLV type 2 priming 6 weeks pre-mating followed by Progressis® boost 4 weeks later, 2 weeks pre-mating. Sows: Progressis® boost 3 weeks prior to each farrowing.

This pre-eradication PBC protocol, was chosen immediately by the farmer encouraged by the positive development following the double mass-vaccinations, despite standard recommendations includes an MLV at 7th week of each gestation.

Cross-sectional serology on groups of 5-10 piglets each age group at 7, 9 and 11 weeks of age (woa): PRRSV type 2 specific IPMA serology to monitor PRRSV circulation. Negative piglets out of nurseries is demonstrated by declining or negative antibodies during nurseries.

Danish official VetStat antimicrobial (AM) all-farms monitoring data expressed in "Animal Daily Doses" (ADD)/100 nursery piglets per day, over pre- and during-PBC periods of both 6 months, were used to measure bacterial disease.

Statistical analysis: Two-tailed t-test.

Results: Age-group PRRSV type 2 IPMA average titres: During MLV type 2 only, titres increased from 83 at 7 woa over 250 at 9 woa to 850 at 11 woa, clearly indicating type 2 circulation and farm instability.

During Progressis® PBC all titers were zero, indicating absence of type 2 circulation and farm stability.

AM use:

Significant reduction from 13.26 ADD during MLV type 2 only to 8.16 ADD during Progressis® PBC ($p<0.05$).

Conclusions: It was demonstrated that Progressis® PBC applied on PRRSV type 2 MLV priming were able to control PPRS type 2 circulation, as well as diminishing secondary bacterial diseases, demonstrated via a significant reduction of AM use in a situation where various PRRS type 2 MLV protocols alone were not.

References:

[1] Spaans et al., Proc IPVS 2016
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PRRS-010

Development T cell-mediated suppressive activity against type1 PRRSV replication in pigs

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) causes serious economic damage to swine industry. PRRSV is a small enveloped virus belonging to the family *Arteriviridae* in the order *Nidovirales*. Due to antigenic variability of field viruses, the live PRRSV vaccines induce partial protection before the appearance of neutralizing antibody, suggesting cell-mediated immunity or other mechanisms may be involved. In a previous study, a suppression of viral replication was examined by viral suppression assay (VSA) using PRRSV-specific T cell enriched PBMC and monocyte-derived macrophages obtained from each of PRRSV infected pigs. This study, we investigated whether PRRSV replication could be effectively suppressed by CTLs in type 1 PRRSV infected pigs.

Materials and Methods: Six three-week-old pigs were challenged with Korean PRRSV strain E38 (type 1 PRRSV-$10^{4.5}$ $TCID_{50}$/ml) through intramuscular and intranasal routes. And then sera and peripheral blood mononuclear cells (PBMCs) were collected every week for 8 weeks. IFN-γ secretion was evaluated by IFN-gamma ELISpot assay using PBMCs. The suppression of viral replication was examined by viral suppression assay (VSA) using PRRSV-specific T cell enriched PBMC and monocyte-derived macrophages obtained from each of PRRSV infected pigs.

Results: The number of PRRSV-specific IFN-γ -secreting T-lymphocytes was increased in all pigs challenged with E38 from 2 weeks post-infection (wpi), reaching at the peak in the number of IFN-γ -secreting T-lymphocytes at 7 wpi and showing drastic decrease at 8 wpi. PRRSV suppressive effect has been observed from 2 weeks post-infection, lasting until the end of the study.

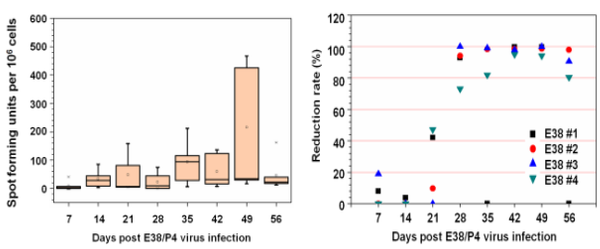

Figure1. Activation of T cell immune response in E38-infected pigs and suppression of E38 replication by stimulated PBMCs in macrophage.

Conclusions: Using Viral suppression assay could be investigate to efficiency of cell-mediated immune response of vaccine candidate PRRSV antigen. These findings will be useful for evaluating CTL responses induced by current and future vaccines, guiding to a novel direction for future vaccine development.

Acknowledgement:
This work was supported by grnat from Animal and Plant Quarantine Agency, Republic of Korea (Project No. QIA A-15043083-2017-20).Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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PRRS-011

Differential diagnostic for porcine reproductive and respiratory syndrome virus by a fluorescence melting curve analysis using peptide nucleic acid probe-mediated one-step real-time RT-PCR

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is an important swine pathogen that causes tremendous economic losses in swine production worldwide. PRRSV can be divided into PRRSV1 (European type) and PRRSV2 (North American type). Genetic distances of ORF6 and ORF7 between Korean PRRSV strains collected at different times were 90.2-100% and 87.3-100% for PRRSV1 and 86.5-100% and 83.7-100% for PRRSV2 respectively. Peptide nucleic acids (PNAs), artificially synthesized DNA analogues, hybridize strongly with DNA and are useful for fluorescence melting curve analyses (FMCA) based on the thermal denaturation of the probe-target duplex. In this study, we developed a FMCA using PNA-based one-step real-time RT-PCR (PNA rRT-PCR) for the differential and qualitative detection of PRRSV1 and PRRSV2.

Materials and Methods: The primers for PNA rRT-PCR were designed on the basis of conservative ORF6 gene sequences of PRRSV1 and PRRSV2 from the NCBI database and Korean PRRSVs isolated strains during 2005-2014. PNA rRT-PCR was performed by following step; 50℃ for 30 min, 95℃ for 15 min, followed by 45 cycles of 95℃ for 30 s, 58 ℃ for 45 s, and 72℃ for 45 s. FMCA began with a denaturation step at 95℃ for 5 min, a stepwise hybridization at 75℃, 55℃ and 45℃ for 1 min, followed by a stepwise temperature increase to 85℃ at 1℃/step with 5 s interval between steps.

Results: The detection limits of PNA rRT-PCR based on these curves were 1.5×10^1 PRRSV1 copies and 1.1×10^1 PRRSV2 copies. The positive Ct values of PNA rRT-PCR showed a defined melting curve with a peak at 64 ± 2℃, suggesting consistency in FMCA over a wide range of target RNA concentrations. The mean Ct values for the multiplex reaction were largely overlapped, differing by ≤1 cycle compared with the single reaction, indicating no interference between primers and probes. To investigate the specificity of the primers and probes, we performed in silico PCR using the PRRSV panels established in this study. The primers and probes designed in this study provided 100% coverage across Korean PRRSVs. However, other primers and probes from previous articles resulted in 29.0-100.0% coverage for Korean RRRSVs. Out of 100 samples from pig farms where wasting and respiratory syndrome was observed in 2018, 14, 26, 7, and 53 were PRRSV1, PRRSV2, mixed, and negative, respectively. The diagnostic sensitivity of PNA rRT-PCR for clinical samples was 100% and the specificity was also 100% compared with conventional RT-PCR (cRT-PCR) combined with nested PCR (Table 1).

Table 1. Comparison of PNA rRT-PCR and cRT-PCR combined with nested PCR using clinical samples.

No clinical of samples	Assay		RT-PCR		Nested PCR	
			+	-	+	-
100	PNA rRT-PCR	+	42	5	47	0
		-	0	53	0	53

Conclusions: The newly developed PCR strategy can be used in parallel with or in place of the existing cPCR, which can reduce the risk of carry-over contamination using a single closed-tube, shorten protocols, and facilitate the discrimination of specific and aspecific amplification by FMCA.

Acknowledgement: This research was supported by a fund (Project Code No. P- 1543069-2017-20-01 and Z-1543069-2014-14-02) from the Animal and Plant Quarantine Agency, Korean Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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PRRS-012

Epidemiological and phylogenetic analyses of porcine reproduction and respiratory syndrome virus in mainland China, 2017-2018

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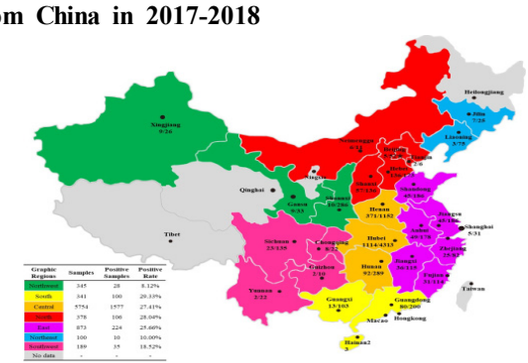
Introduction: Porcine Reproduction and Respiratory Syndrome Virus (PRRSV), including two separate species, PRRSV1 and PRRSV2, is a worldwide leading cause of reproductive failures in sows and respiratory disorders in piglets [1]. Even though pig herds always represent a high PRRSV antibody positive rate which is in part due to an extensive use of attenuated live vaccine, high mortality and abortion rate caused by PRRSV remain big threats to pig industry [2]. Continuously monitoring the circulation and genotype of the prevalent PRRSV strains is beneficial for providing more data for PRRSV prevention and control.

Materials and Methods: A total of 7980 samples (blood, lungs, spleens, lymph nodes, semen, intestinal tracts and swine stillbirths) from pigs with suspected PRRSV infection in pig farms in 27 provinces in mainland China between January 2017 and December 2018 were collected for PRRSV detection with primers listed in the below Table. The primer for Nsp2 can differentiate PRRSV strains into three types: the PRRSV classical strains (1074 bp), HP-PRRSV strain (984 bp) and PRRSV NADC30-like strain (681 bp). ORF5 gene amplified from positive samples were sequenced and were used for phylogenetic analysis. PRRSV VR2332, CH-1a, JXA1, NADC30 and GM2 were included as reference strains.

Gene	Primer Sequence
ORF5	F: 5' GGCGACCGTTTTAGCCTGTCTT 3'
	R: 5' ATCATTATTGGCGTGTAGGTG 3'
Nsp2	F: 5' TTGATTGGGATGTTGTGCTTC 3'
	R: 5' CAATGATGGCTTGAGCTGAGT 3'

Results: Of the 7980 detected by RT-PCR, 2080 (26.07%) samples were positive for PRRSV (Figure 1). The positive rate for each region was different. The Southern China area had the highest positive rate (29.33%), followed by Northern China (28.04%). Monthly, the positive rate of PRRSV in every month was higher than 7.76%; highest positivity was determined in April (55.50%), June (38.39%) and January (28.54%). As for different PRRSV types, the positive rate of PRRSV classical strain in mainland China was only 2.28%, but it had a very high positive rate in Northeast of China (14.29%). In contrast to a low positive rate of PRRSV classical strain in mainland China, the positive rates of HP-PRRSV and NADC30-like strain in mainland China were very high. Central (60.11%), Northern (48.84%) and Eastern (43.59%) China had the highest detection rates of HP-PRRSV. The positive rate of NADC30-like strain was 46.15%, which is much higher than that reported before in mainland China. Southwest (63.64%), Southeast (62.50%) and Northwest (60.00%) had the highest detection rates of NADC30-like. Phylogenetic analysis showed that PRRSVs currently circulating in mainland China were PRRSV2, and they were phylogenetically grouped into four clades, represented by the PRRSV classical strains clade, HP-PRRSV clade, NADC30-like strains clade, and GM2-like strains clade.

Figure 1. The positive rate of PRRSV samples collected from China in 2017-2018



Conclusions: The prevalence of PRRSV remained a worrisome problem in China. While HP-PRRSV is the predominate PRRSV type of circulation, the prevalence of novel strains such as NADC30-like and GM2-like strains are increasing.

Acknowledgement: This work was supported by the National R&D Program of China (grant number: 2018YFD0500800) and Key Laboratory of Prevention and Control Agents for Animal Bacteriosis (Ministry of Agriculture) (KLPCAAB-YTP-1801).

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PRRS-013

Evaluation of the cross protective efficacy of a chimeric PRRSV vaccine against Korean type 2 field strains in a reproductive model

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Introduction: Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV), is the most challenging threat to the swine industry worldwide for over two decades. One of the main obstacles to porcine reproductive and respiratory syndrome (PRRS) vaccinology is the inadequate or no cross-protection conferred by current vaccines. To broaden the cross-protective range of vaccine candidates, in our previous study [1], CB1 chimeric PRRSV was constructed based on two Korean field strains of most prevalent lineages of PRRSV-2 in Korea (K07-2273: Korean lineage C and K08-1054: lineage 5) and was assessed for protection in young pigs. Based on the previously attained results, in the present study, the chimeric virus was evaluated for its safety and cross-protective efficacy in pregnant sows.

Materials and Methods: In the current study, six pregnant sows (J1 to J6) at 60 days of gestation were purchased from a PRRSV free farm. Four pregnant sows, J1 to J4, were vaccinated with CB1 and later, at 30 days post-vaccination (dpv), J1 and J2 were challenged with K07-2273 whereas, J3 and J4 were K08-1054 challenged. Sows, J5 and J6, were kept as non-vaccinated controls and were challenged with K07-2273 and K08-1054 at 90 days of gestation, respectively. Blood samples were collected at 0, 7, 14, 21 (dpv) and 0, 7, 14, 24 day post challenge (dpc). Live born piglets were evaluated for vertical transmission of virus until 28 days after birth.

Results: As compared to non-vaccinated sows, the CB1 vaccinated sows presented lower viremia after challenge. Mean-peak virus titers were detected below 10^2 $TCID_{50}$/ml in CB1 vaccinated sows while those of non-vaccinated sows were detected over 10^3 $TCID_{50}$/ml post-challenge. All of the CB1 vaccinated sows attained seropositivity at 14 dpv, which was maintained up to 24 dpc, while the CB1 non-challenged sows remained seronegative prior to virus challenge. Reproductive evaluation of sows is summarized in Table 1. Furthermore, the piglets from CB1 challenged sow groups attained significantly higher birth weight.

Table 1. Summary of reproductive evaluation

Sow No.	Vaccinated	Infection	Date of farrowing	Nl[a]/nd[b]	Death rate
J1	CB1	K07-2273	113	9/0	0%
J2			115	6/0	
J3		K08-1054	113	12/1	4%
J4			114	13/0	
J5	-	K07-2273	115	12/2	16.67%
J6		K08-1054	113	17/3	17.65%

[a]nl : The number of total live born piglets
[b]nd : The number of dead born piglets

Conclusions: The sows and their piglets vaccinated with the chimeric vaccine exhibited reduced viremia against challenges with two heterologous viruses. Furthermore, the piglets from CB1-vaccinated sows had significantly higher average birth weight. In summary, this study suggests that the structural proteins of Korean PRRSV strains and an immunogenic pFL-12 backbone of CB1 cross-protects the sows against K07-2273 (Korean lineage C) and K08-1054 (lineage 5) challenges. Therefore, CB1 opens possibilities for broadly effective vaccines for reproductive diseases against various Korean PRRSV strains.

Acknowledgement: This study was supported by the Cooperative Research Program for Agriculture Science & Technology Development (PJ012612) in Rural Development Administration, Republic of Korea.

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PRRS-014

Evaluation of the effect of a subunit vaccine on porcine reproductive and respiratory syndrome virus infected pigs

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Introduction: Porcine reproductive and respiratory syndrome (PRRS) causes significant economic losses in most pig-producing countries [1]. Since cellular immunity and neutralizing antibody production were delayed by both virulent and attenuated forms of PRRS virus (PRRSV) [2, 3], it is common in field that PRRSV infects pigs before protective immunity fully established even vaccination. *Pseudomonas aeruginosa* exotoxin A (PE) was able to deliver exogenous antigen for presentation by class I and II MHC molecules [4]. A subunit PRRSV vaccine, 2nd PRRSFREE, consist of (PE), 4 PRRSV antigens and a nuclear export signal fused to an endoplasmic reticulum retention signal, was designed to quickly elicit humoral and cellular immunity. The aim of this study was to determine the effect of PRRSV subunit vaccine on PRRSV infected pigs.

Materials and Methods: Fifteen specific pathogen free pigs were divided into vaccine (10) and control (5) groups. At 7 weeks of age, all pigs were intranasally and intramuscularly challenged with total 10^5 $TCID_{50}$ PRRSV TSYM strain. Pigs were weighted before challenge and for 20 days post-infection (dpi). Vaccine group was injected one dose of 2nd PRRSFREE at 5 dpi. Blood samples were collected at 0, 5, 10, 15 and 20 dpi for real-time PCR. Data were analyzed by t test and $p < 0.05$ was considered as significant difference.

Results: The average viral load of viremic pigs of vaccine group was significantly lower than that of control group at 10 dpi. Since the viremic rates of vaccine and control groups were 50% and 100% at 20 dpi, respectively, the average viremic period of vaccine group was shorter than that of control group. The average daily weight gain of vaccine group was significantly higher than that of control group.

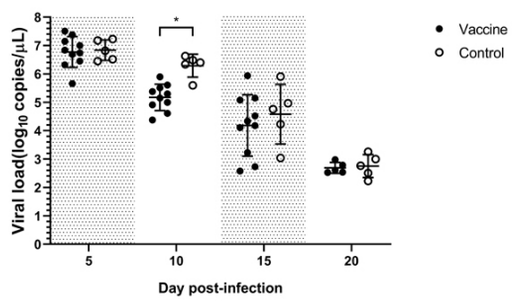

Figure 1. PRRS viral loads in the sera of viremic pigs

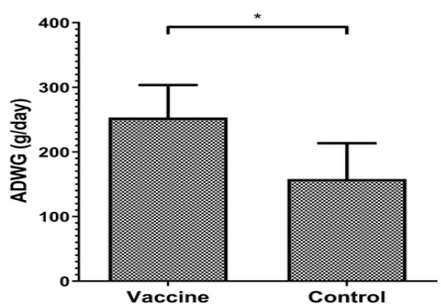

Figure 2. Average daily weight gain

Conclusions: Even immunization is after PRRSV infection, 2nd PRRSFREE is potential for decreasing viral loads of sera, shortening the viremic periods and improving the average daily weight gain. However, the mechanisms of 2nd PRRSFREE is still unclear and should be further investigated.

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PRRS-015

Field performance of unistrain® prrs against type 2 porcine reproductive and respiratory syndrome virus (prrsv2) in pig herds in thailand

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Introduction: PRRS is the disease with the highest impact on the swine industry worldwide. Owing to its endemic distribution and the high mortality caused by both types (PRRSV1 and PRRSV2), immunization of the pigs is required to minimize the economic and productive impact on affected farms [1]. Immunization through modified live vaccines (MLV) has proved to be effective in controlling PRRSV1 and PRRSV2 infection [2]. However, variable results are observed especially when a heterologous PRRSV isolate is circulating [3]. The purpose of this study was to evaluate the field efficacy of UNISTRAIN® PRRS against PRRSV2 strain, widely present in intensive pig herds in Thailand.

Materials and Methods: The performance of 14-day-old piglets from four commercial pig farms in Ratchaburi province (Thailand) was evaluated for 6 months after vaccination. Included farms were positive for the PRRSV2 strain by RT-PCR and by real-time RT-qPCR assay. The piglets were randomly distributed into two groups and vaccinated at 2 weeks of age: group 1 (n=800) was vaccinated intradermally (0.2 ml) with UNISTRAIN® PRRS and group 2 (n=800) was vaccinated intramuscularly (2 ml) with a commercial PRRSV2 MLV vaccine (vaccine A). Safety evaluation consisted in daily monitoring of systemic and local reactions (pyrexia, skin redness and abscesses, clinical respiratory signs, depression and inappetence) for both groups until 3 weeks post vaccination (Fig 1). Productive parameters monitored were: average daily weight gain in the lactation period (ADLWG), and in the fattening period (ADFWG), cumulative mortality rate (until slaughter age), fattening mortality rate (from 10-weeks of age to slaughter age) and feed conversion rate (FCR), (IBM SPSS statistics 22).

Results: Almost no systemic or local reactions were observed in the UNISTRAIN® PRRS group whilst piglets from group 2 showed inappetence (10.6%) and pyrexia (3.75%). All productive performance parameters showed significant differences between groups (Table 1).

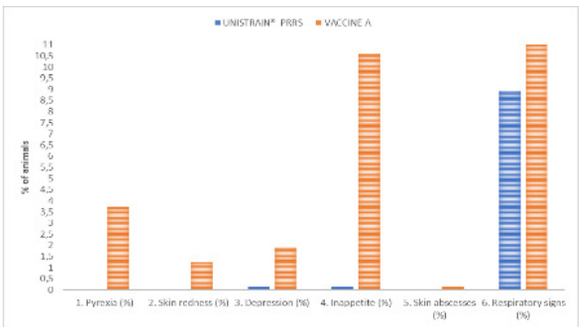

Figure 1. Percentage of animals positive for systemic and/or local reactions in both groups.

Table 1. Productive parameters over a 6-month period after vaccination (mean ± SD)

Productive parameter	UNISTRAIN® PRRS	Vaccine A
ADLWG (kg)	2.5 ± 17.8[a]	2.1 ± 39.9[b]
ADFWG (kg)	0.7 ± 12.9[a]	0.6 ± 15.4[b]
Cumulative mortality rate (%)	10.6 ± 1.7[a]	19.5 ± 3.3[b]
Fattening mortality rate (%)	1.6 ± 0.7[a]	3.9± 1.1[b]
FCR	2.4 ± 5.6[a]	2.6 ± 51.1[b]

Note: *Different letters (a, b) within the same column represent significant differences ($p>0.05$)

Conclusions: In the same production system under PRRSV2 field infection, UNISTRAIN® PRRS (EU strain) showed a significant improvement in productivity, reducing mortality and increasing ADWG. In this case, UNISTRAIN® PRRS was effective against PRRSV2 virus infection compared with the unsuccessful outcome achieved with the previous PRRSV2 vaccine.

Acknowledgement: The authors would like to thank Dr. Sithipon Jongpattanasombut and the HIPRA Thailand staff for their valuable cooperation.

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PRRS-016

Field safety and efficacy of unistrain® prrs against type 2 porcine reproductive and respiratory syndrome virus (prrsv2) in commercial pig herds in thailand

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Introduction: PRRSV affects swine herds, causing a huge economic impact worldwide. Although PRRSV1 is predominant in Europe, genotypes PRRSV1 and PRRSV2 are both disseminated worldwide, in single infected or co-infected herds.

The criteria for MLV vaccine selection should be based on the shorter duration of vaccine virus shedding and the broader heterologous protection [1]. Safety and heterologous protection continue to be the main concerns when using PRRS MLV. Variable results are observed in terms of heterologous protection under field conditions. The objective of this study was to evaluate the safety and efficacy of UNISTRAIN® PRRS on Thai swine farms with an active PRRSV2 infection.

Materials and Methods: This study was carried out from April to December 2018 on 16 farrow to finish farms [500 to 12,000] sows´s census in Thailand. All farms were positive for PRRS and had recorded clinical problems including respiratory distress. PRRSV2 was detected in all of them by PCR. Selected animals were vaccinated with UNISTRAIN® PRRS intramuscularly (IM: 2ml) or intradermally (ID: 0,2 ml). Vaccination was performed in 1,900 gilts; 600 vaccinated ID and 1,300 IM, 11,528 sows; 4,298 vaccinated ID and 7,230 IM and 29,345 piglets; 21,881 vaccinated ID and 7,464 IM.

Safety was evaluated in all sows and piglets for 14 days after vaccination, based on the monitoring of skin redness, breath increase, feed intake (FI) reduction and mortality (Table 1). The efficacy of UNISTRAIN® PRRS was evaluated in groups of 250 sows on two different farms that previously carried out PRRSV2 vaccination. Efficacy evaluation was based on comparative reproductive parameters, 4 months before and after vaccination with UNISTRAIN® PRRS. The parameters evaluated were: farrowing and abortion rates, birth weight, pre-weaning mortality and average daily gain (ADG) of their offspring (Table 2).

Results: No severe adverse reactions were observed in any vaccinated pigs. Vaccinated pigs with skin redness (2/42,773) and FI reduction (3/42,773) returned to normality three days after vaccination without any treatment. In addition, a significant improvement in the reproductive performance was exhibited on Farm 1 and Farm 2.

Table 1. UNISTRAIN®PRRS safety evaluation in gilts, sows and piglets.

Animal groups	Skin redness	Breath increase	FI reduction	Mortality
Gilt (ID)	0/600	0/600	0/600	0/600
Gilt (IM)	0/1,300	0/1,300	0/1,300	0/1,300
Sow (ID)	0/4,298	0/4,298	0/4,298	0/4,298
Sow (IM)	0/7,230	0/7,230	3/7,230	0/7,230
Piglet (ID)	0/21,881	0/21,881	0/21,881	0/21,881
Piglet (IM)	2/7,464	0/7,464	0/7,464	0/7,464

Table 2. Comparative reproductive parameters 4 months before and after UNISTRAIN®PRRS vaccination.

Parameters	Farm 1		Farm 2	
	Before	After	Before	After
Farrowing rate (%)	78.0[a]	92.0[b]	80.0[a]	87.0[a]
Abortion rate (%)	3.0[a]	0[b]	7.0[a]	1.0[b]
Pre-weaning mortality (%)	13.1[a]	5.13[b]	11.51[a]	6.14[b]
Birth weight (kg)	0.95[a]	1.35[b]	1.08[a]	1.21[b]
ADG (g/day)	205.83[a]	275.0[b]	209.17[a]	264.58[b]

Note: different letters (a, b) within the same row represent significant differences (p> 0.05)

Conclusions: In this case, UNISTRAIN® PRRS was shown to be safe and effective in sows and piglets, controlling PRRSV2 clinical problems, reducing the abortion rate and pre-weaning mortality and increasing ADG and the farrowing rate. Therefore, this MLV vaccine should be regarded as a strategic tool in the control of PRRS heterologous infections.

Acknowledgement: The authors would like to thank to Thai swine farm cooperatives for providing the retrospective data and for their encouragement.

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PRRS-017

Importance of analyzing production data to find right timing to change PRRS vaccine

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HIPRA, Amer (Girona), Spain

INTRODUCTION

In 2015 and early 2016, reproductive disorders associated with PRRSV were detected on a farrow-to-finish farm (1,500 sows) located in Korea. At that time, the breeding herd was vaccinated with a live attenuated PRRSV2 vaccine. The aim of this study was to retrospectively assess (from 2015 to 2017) the efficacy of different commercial PRRS vaccines in controlling reproductive disorders on a farm affected by PRRSV1 and PRRSV2.

MATERIAL & METHODS

Due to recurrent reproductive disorders associated with PRRS, in May 2016 it was decided to switch to UNISTRAIN® PRRS (PRRSV1 vaccine). Between 2015 and 2017, different commercial vaccines were used (Table1). Born alive ratio (BAR) and weaned piglets ratio (WPR) from 2015 to 2017 were considered the key performance indicators and were analyzed and used to generate a Statistical Process Control Chart by R statistics. Limits were set at 3σ. Moreover, the ANOVA test was used to compare the time series between the periodic revaccination.

Table 1: PRRS diagnosis by RT-PCR (PRRSV2 and PRRSV1) and vaccine used.

Date	PRRS diagnostic				Vaccine
	Suckling piglet	*45 days old*	*70 days old*	*120 days old*	
15-Nov	PRRSV1 (+) PRRSV2 (+)	PRRSV1 (+) PRRSV2 (+)	PRRSV1 (+) PRRSV2 (+)	(-)	PRRSV2 vaccine 1
16-Mar	PRRSV1 (+) PRRSV2 (+)	PRRSV1 (+) PRRSV2 (+)	(-)	(-)	PRRSV2 vaccine 1
16-May	(-)	PRRSV2 (+)	(-)	(-)	UNISTRAIN® PRRS
16-Jun	(-)	(-)	(-)	PRRSV2 (+)	PRRSV2 vaccine 2
16-Sep	PRRSV2 (+)	PRRSV2 (+)	PRRSV2 (+)	(-)	UNISTRAIN® PRRS
16-Dec	(-)	(-)	(-)	(-)	UNISTRAIN® PRRS
17-Mar	(-)	(-)	(-)	(-)	PRRSV2 vaccine 2
15-May	(-)	PRRSV2 (+)	PRRSV2 (+)	(-)	UNISTRAIN® PRRS
17-Sep	(-)	(-)	(-)	PRRSV2 (+)	UNISTRAIN® PRRS

RESULTS

The moving average of BAR and WPR ranged between 84-90% for almost the whole of 2015. At the beginning of 2016, clinical problems associated with PRRS caused a significant decrease in BAR and WPR. After the application of UNISTRAIN® PRRS, reproductive parameters were brought to in-control levels and circulation of PRRV was not detected. However, each time that UNISTRAIN® PRRS was replaced by the PRRSV2 vaccine, reproductive parameters significantly decreased out of the in-control limits and circulation of PRRSV2 was detected again. Notably, circulation of PRRSV1 was not detected any more during the study period. In fact, the performance of UNISTRAIN® PRRS was significantly associated with higher BAR ($p<0.001$) and higher WPR ($p<0.001$) than both the other vaccines.

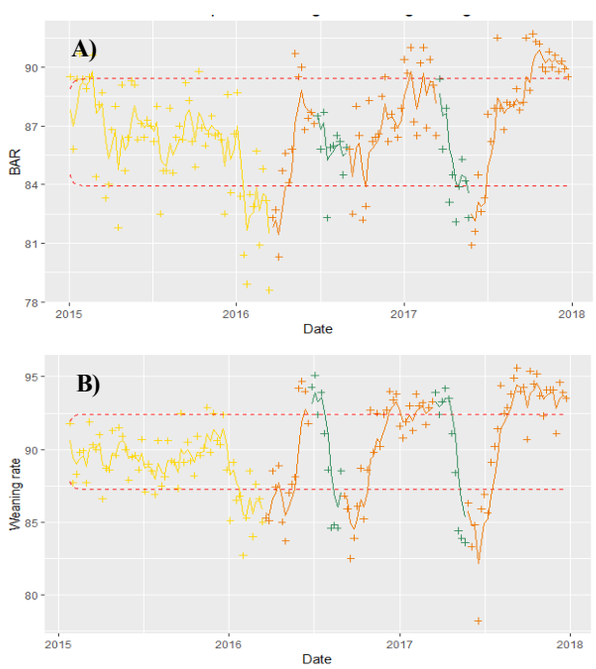

Figure 1. SPC chart of the 2015-2017 data. Results are represented as moving average of BAR (A) and WPR (B). Red lines represent the upper and lower limits of the in-control values. The yellow line represents the period when the PRRSV2 vaccine 1 was used, the orange line UNISTRAIN® PRRS and the green line PRRSV2 vaccine 2.

DISCUSSION & CONCLUSION

UNISTRAIN® PRRS contributed to the control of PRRSV1 and PRRSV2 in terms of improvement of reproductive performance and reduction of PRRSV circulation on the farm.

PRRS-018

Infection of monocytes-derived macrophage with porcine reproductive and respiratory syndrome virus (PRRSV) 2 is modulated by dexamethasone and IFN-γ

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Introduction: Macrophages play critical roles in the immune system and are central in the innate immune system [1]. Porcine reproductive and respiratory syndrome virus (PRRSV) has a restricted cellular tropism, and shows a preference for myeloid lineages, including monocytes, dendritic cells, and macrophages [2]. In particular, macrophages play an important role in PRRSV pathogenesis as sites of early and sustained virus replication. CD163, a cellular glycoprotein in the scavenger receptor cysteine-rich (SRCR) superfamily, has been described to function as a putative cellular receptor for PRRSV [3]. Furthermore, the expression of CD163 in porcine alveolar macrophages (PAMs) and murine macrophage derived cells has been shown that these cells are permissive to PRRSV and support the replication of PRRSV. Therefore, the aim of this study is to investigate the interaction of PRRSV2 with macrophage treated with dexamethasone (DEX), a glucocorticosteroid known to increase CD163 expression on human monocytes, or IFN-γ influencing macrophage biology.

Materials and Methods: Four-weeks-old PRRSV-negative pigs were obtained from a local farm without PRRSV, porcine circovirus 2, and porcine parvovirus. CD14+ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) using a standard cell separation method and CD14 staining, followed by positive magnetic sorting as described in the manufacturer's instrument (Miltenyi Biotec). Monocytes derived-macrophages (MDM) were differentiated from CD14+ monocytes by culture with rhM-CSF. MDM were cultured with DEX or IFN-γ . MDM were infected with Korean PRRSV2 strain KVDL1 and KVDL2 with a multiplicity of infection (MOI) of 1 and the inoculum was removed at 60min post-infection. Cells were washed twice with fresh medium and gentle centrifugation, followed by incubation at 37 ℃ for 20 hrs. Cells pellets were finally collected for flow cytometry (FACS). Expression of CD163 was identified by cell surface markers for CD163 (2A10/11 (Bio-rad)) on MDM by FACS. PRRSV nucleocapsid expression was measured by intracellular staining using SDOW-17A and Cyto Fix/Cyto Perm kit (BD Biosciences).

Results: To investigate that the treatment of DEX on MDM can improve the expression of CD 163, DEX was treated on MDM, followed by the infection of PRRSV, KVDL1 and KVDL2. FACS analysis showed the expression of CD163 was significantly increased. A triple stain was carried out on mock and MDM infected PRRSV at 20hpi to analyze expression of PRRSV2 nucleocapsid protein. The levels of SDOW-17 indicating the expression of nucleocapsid significantly were increased in MDM. In case of the proinflammatory cytokine, IFN-γ , the expression of CD163 was decreased on MDM treated with IFN-γ . IFN-γ pretreated MDM infected with KVDL1 and KVDL2 in IFN-γ containing media showed highly reduction to PRRSV2 infection at 24hpi.

Conclusions: The present study reveals that the treatment of DEX and IFN-γ on MDM is highly responsible for the expression of CD163, affecting the infection of PRRSV. It is necessary to investigate the contribution of two agents in the infection of various genetic and pathogenic PRRSV strains.

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[2] Macatonia I et al., Int Immunol 5, 1119-1128
[3] Chamorro et al., Immunology 2000, 202, 82-9

PRRS-019

New method for evaluating PRRSV vertical transmission using processing fluid

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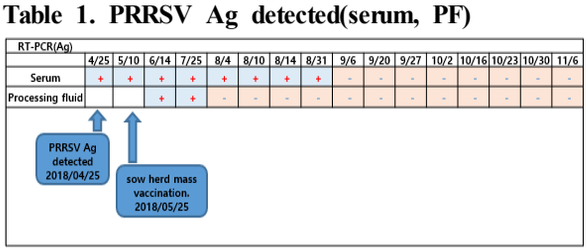
Introduction: Processing fluids (PF), the serosanguineous fluid recovered from piglet castration and tail docking, were used for porcine reproductive and respiratory syndrome virus (PRRSV) infection assessment. PF has been proven to be an effective sample for evaluating the PRRS stability status and vertical PRRSV infection in sow herds. Therefore, many farms around the world are actively using PF for PRRS monitoring. In this case report, we identified the time point when the PRRS vertical and horizontal infection are terminated in breeding herd by monitoring PF and serum of piglet (at weaning) after applying Ingelvac PRRS MLV mass vaccination to the sows which infected with Type 2 PRRSV.

Materials and Methods: This study was conducted in a commercial 500 sows farrow to finish farm. In this farm, PRRSV infection (vertical and horizontal infection in breeding herd) was first detected in 2018, followed by a productivity loss due to increase of stillbirth, mummification and pre-weaning mortality rates of piglets. After the first detection of PRRS virus, we decided to perform vaccination to reduce clinical signs and viremia by PRRSV infection. The farm implemented mass vaccination with Ingelvac PRRS MLV (2ml) once in breeding herd, and then we began monitoring the PF and serum of piglets (4-week-old). Every litter's PF was sampled per 1 ~ 2 weeks from each parities and the piglet's serum of each batch were collected at weaning. All samples were tested by RT-PCR.

Results: In reference to the table 1, both PF and serum of piglets were monitored to determine when PRRSV was eliminated in the breeding herd. As a result of PF monitoring, PRRSV was detected in PF until 8 weeks after sow herd mass vaccination. And no PRRSV was detected in PF after 9 weeks of mass vaccination. On the other hand, PRRSV detected in serum up to 14 weeks after mass vaccination, most likely due to horizontal infection in the farrowing house. After PRRSv vertical transmission was blocked, number of weaned piglets per sow increased (Fig 1).

Table 1. PRRSV Ag detected(serum, PF)



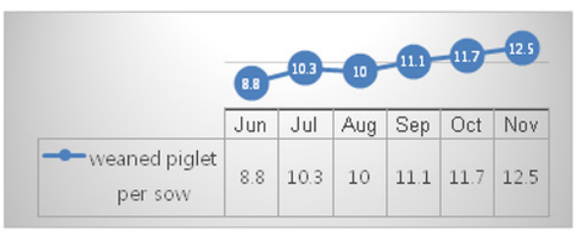


Fig 1. Weaned piglet per sow

Conclusions: Processing fluids is very effective, easy and a relatively cheap method for monitoring vertical PRRSV infection in breeding herd. PF sampling does not require additional man-power or time, as the castration and tail docking are routine tasks on farms. In addition, sensitivity and specificity as high as serum are great advantages. In addition, PF is useful to evaluate the PRRS status in breeding herd. Monitoring of both PF and serum of piglet can be used to determine when vertical PRRSV infection and horizontal PRRSV infection disappear in breeding herd. It is also possible to apply the vaccine program to control the PRRS on the farm by judging whether the infection is vertically transmitted through the PF.

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PRRS-020

Pathological and immunological characterization of two lineage porcine reproductive and respiratory syndrome virus 2 strains in pigs

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of a reproductive failure in sows and respiratory distress in all ages of pigs that resulted in tremendous economic loss in pig farms worldwide [1]. A high genetic diversity exists within PRRSVs, which have been grouped into 9 lineages (PRRSV2) based upon open reading frame (ORF5) phylogenetic relationships [2]. In this study, we sequenced ORF5 of 460 PRRSVs isolated in 2010-2017. The phylogenic analysis showed that Korean PRRSV2 viruses were grouped into lineages 1 and 5 as well as three Korean lineages (kor A, B, and C). The majority of Korean viruses belonged to lineage 5. Therefore, we selected two PRRSV2 strains (KVDL1 and KVDL2) from lineages B and Korea lineage 5 for pathological and immunological characterization.

Materials and Methods: PRRSV-negative pigs with four-weeks-old were randomly divided into four groups of eight animals per group. The pigs were inoculated intramuscularly with KVDL1 strain (group 1), KVDL2 strain (group 2) and prototype strain VR2332 (group 3) with 2 mL (1×10^3 $TCID_{50}$/ml), respectively. Group 4 were used for negative control. All pigs were subjected to daily clinical examination, and their body weights and body temperatures were recorded from 0 dpi (day of post inoculation). Serum and nasal samples were collected at 0, 3, 7, 14, and 28 dpi, respectively. The half of pigs in all group were euthanized at 14 dpi and the rest of pigs were euthanized 28 dpi, respectively. Serum antibodies were tested using the PRRSV ELISA kit (IDEXX PRRS X3 ab test). Populations of T cells, natural killer (NK) cells, and regulatory T (Treg) cells in the PBMCs were analyzed by flow cytometry.

Results: Clinical signs were observed in pigs challenged with the KVDL1, KVDL2, and VR2332 strains. Especially, group 1 showed to severe clinical signs of high fever and low average daily weight gain (ADWG) rate compared to group 2 and group 3. The mean rectal temperatures in group 1 were significantly higher than in the group 2 and group 3 at 6 to 11 dpi. Group 1 had significantly higher mean gross pulmonary lesions (lung consolidation) and microscopical lesions than those of group 2 and group 3 at 14 dpi and 28 dpi, respectively. The viremia of blood of Group 2 and group 3 were higher than that of group 1 at 3 and 7 dpi. Group 1 showed slow increase of specific antibody levels of PRRSV, a high level of CD4+ Treg, γδTCR+ T cell populations at 14 and 28 dpi, and a significantly lower population of NKT and NK cells than group 2 and group 3.

Conclusions: The KVDL1 strain is more virulent than the other strains, showing more severe pathological damage and clinical signs. The KVDL1 strain may inhibit the cellular immune response, which was elevating the level of CD4+ Tregs. Further studies are needed to investigate pathological and immunological characterization of other lineage isolated PRRSVs in animal experiments.

Acknowledgement: This research was supported by a fund (Project Code No. P- 1543069-2017-20-01 and Z-1543069 -2014-14-02) from the Animal and Plant Quarantine Agency, Korean Ministry of Agriculture, Food and Rural Affairs, Republic of Korea

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PRRS-021

Performance improvement after implementation of 3FLEX in a Korean farm.

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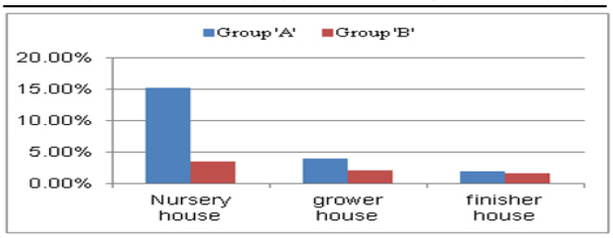
Introduction: Porcine Reproductive Respiratory Syndrome (PRRS) is one of the most important diseases in the swine industry. PRRS virus (PRRSv) can cause respiratory disease like the Porcine Respiratory Disease Complex (PRDC) with coinfection of other pathogens like PCV2, Mycoplasma hyopneumonia(M.hyo), and Swine influenza virus. In addition, genetic, environmental factors and management can also affect the PRDC[1]. PRRSv can predispose pigs to develop more severe clinical symptoms after bacterial infections like Streptococcus suis, Pasteurella multocida, Haemophilus parasuis (HPS) or Actinobacillus pleuropneumonia (APP)[2]. The purpose of this study was to evaluate the efficacy of an additional PRRS MLV vaccine in piglets in a farm with an early PRRSv infection in the nursery and in which piglets were already vaccinated against PCV2 and M.hyo.

Materials and Methods: The field observation was conducted in a farrow-to-finish farm with 200 sows. Pigs are weaned at 25 days of age, and transferred to the nursery house. At about 70 days of age, pigs are transferred to the grower house and at 120 days of age to the finisher house. In this study, piglets in group 'A' are vaccinated with FLEX®combo (Ingelvac CircoFLEX® and Ingelvac MycoFLEX®) once at 3 weeks of age, whereas piglets in group 'B' are vaccinated with 3FLEX® (FLEX®combo mixed with Ingelvac® PRRS MLV) once at the same age. In total, 8 consecutive farrowing batches were included in the study: 4 batches with vaccination protocol 'A' followed by 4 batches with vaccination protocol 'B'. The number of piglets for the 4 batches in each group is 387 in 'A' and 395 in 'B'. There was no all-in/all-out between batches in both groups. Except for the vaccine program, all other factors like genetics, environment, management, ventilation, pig-flow and hygiene were maintained at the same level to evaluate the efficacy of PRRS MLV more correctly. To demonstrate early PRRS infection in the nursery, 4 blood samples were taken from group 'A' pigs at the age of 30, 35, 40, 50 and 60 days, respectively and tested for PRRS by PCR in pools of 4. Pigs of both groups were observed for clinical symptoms in the nursery, and mortality was measured in the nursery, grower and finisher house.

Results: There was a clear difference in mortality between the FLEX®combo and 3FLEX® group (Table.1, Graph.1). Nursery mortality in group 'A' was 15.25% and in group 'B' 3.54%. Grower house mortality in 'A' was 3.96% and in 'B' 2.1%. Finisher house mortality in 'A' was 1.9% and in 'B' 1.61%. Total mortality in 'A' was 20.16% and in 'B' 7.09%. Observed clinical signs in the nursery were wasting, depression and coughing in group 'A', whereas no clinical sign were observed in the nursery in group 'B' (Picture 1). Blood samples of group 'A' piglets were PCR positive for Type 2 PRRSv at 30, 35, 40 and 50 days of age.

Table 1. Mortality in groups 'A' and 'B' for each production stage.

Mortality	FLEX®combo (group 'A')	3FLEX® (group 'B')	Diff. (%)
Nursery house	15.25%	3.54%	-11.71
Grower house	3.96%	2.10%	-1.86
Finisher house	1.90%	1.61 %	-0.29
Total	20.16%	7.09%	-13.07



Graph 1. Mortality in groups 'A' and 'B' for each stage.

Picture 1. Group'A' (left) and 'B'(right) in the nursery house. Depression, wasting and coughing were main symptoms in group 'A', whereas no symptoms were observed in group 'B'.

Conclusions: In this study we demonstrated that the additional implementation of Ingelvac® PRRS MLV via 3FLEX® reduced mortality and clinical signs caused by the early PRRSv infection in the early stage of nursery. Even though the interval between vaccination and infection was short, Ingelvac®PRRS MLV effectively controlled PRRS in the nursery of this farm.

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PRRS-022

Seroprevalence of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) in Peninsular Malaysia from Year 2016 to 2018

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Introduction: Porcine Reproductive and Respiratory Syndrome (PRRS) is a highly contagious disease that causes reproductive problem in sow herds with lowered farrowing rates, increased abortions, mummified, stillborn and weak live born piglets and death. PRRS can also causes respiratory disease in suckling and weaned pigs. This can further make the pigs susceptible to secondary infection and will caused great impact in the performance of the farm. In Malaysia, there is not much studies on the prevelance and disease status on this disease. A genetic characteristic study conducted in 2012 showed seroprevalence of 89.2% against PRRS virus (Vania, 2012). Another study in year 2013 on the status of PRRS virus in Malaysia showed that American strain PRRSV has high prevelance than European strain (Seetha, 2013)

Material and methods: In year 2016, total of 2538 serum samples were collected from 49 farms. While in year 2017, total of 1708 serum samples were collected from 44 farms. In year 2018, 2365 serum samples were collected from 52 farms. The samples were collected from the porkers and breeders and all these samples were collected from farms that are located in five states in the Peninsular Malaysia. These samples were sent to virology laboratory of the Faculty of Veterinary Medicine, University of Putra Malaysia for serological test using IDEXX PRRS X3 Ab test kit.

Results: In the year 2016, 2116 (83.37%) samples from 2538 samples were positive for PRRSV. In 2017, 1529 (89.52%) samples out of 1708 samples were positive. While in year 2018, total of 2365 samples were collected and 2101 (88.84%) samples were positive (Table 1).

Table 1. Total sample and total positive samples from year 2016-2018

Year	Total Sample	Total Positive Samples	Percentage
2016	2538	2116	83.37%
2017	1708	1529	89.52%
2018	2365	2101	88.84%

All 5 states have seroprevalance of at least 80% since year 2016 to 2018, except for Malacca in year 2016 (Figure 1).

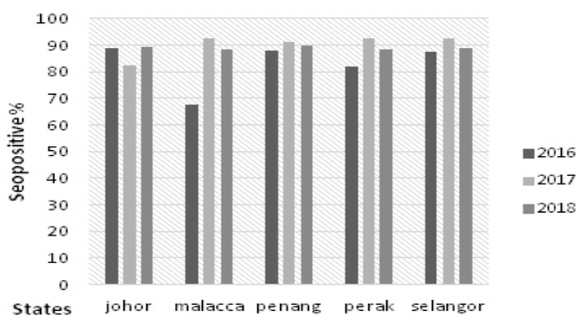

Figure 1. Seroprevalence against PRRS virus according to different states in year 2016 to 2018

As an overview on the farms, in 2016, there were 4 farms out of 48 farms that was 100% seropositive. While in year 2017, there were 13 out of 44 farms with 100% seropositive and in 2018 there were 7 farms out of 52 farms that were 100% seropositive for PRRSV. All farms that were sampled are seropositive with PRRSV in exception that in year 2016, there was a farm that was 100% seronegative with PRRSV.

Discussion and conclusion: This study shows PRRS virus has infected swine herds throughout Peninsular Malaysia. Based on a study on the status of PRRSV in 2013, both American and European strains are found in Malaysia (Seetha, 2013). Thus Peninsular Malaysia is endemic for PRRSV.

References

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PRRS-023

Synthetic-attenuated virus engineering (SAVE) was successfully applied to NSP1 of porcine reproductive and respiratory syndrome viruses for reduction of virulence and induction of protective immunity against heterologous challenge

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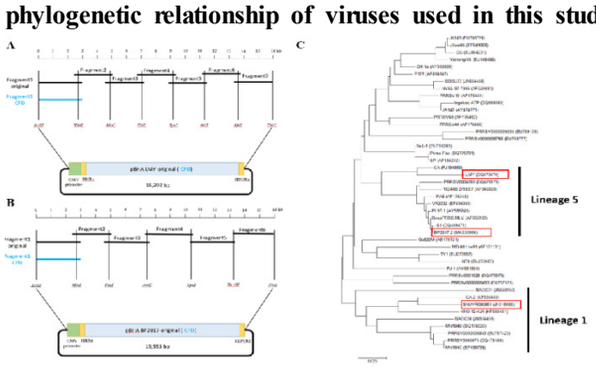
Introduction: A computer-based codon-pairs deoptimization (CPD) technology, synthetic attenuated virus engineering (SAVE), is utilized to control various viruses[1]. The rapid viral attenuation concept by the codon pair deoptimization is to decrease codon pair bias (CPB), which demonstrated the potential usefulness as a technology to develop live attenuated vaccine. For this, we produced field PRRSVs attenuated by codon-pair deoptimization. The affection of SAVE on virulence and immunity of the attenuated strains was confirmed in pigs.

Materials and Methods: NSP1 of two type 2 Korean filed isolates, LMY (Genbank no. DQ473474) [2] and BP2017-2 (Genbank no.MK330996) were subjected to CPD (Fig 1). In the first pig study, 50 commercial cross-bred pigs of 3 weeks old, which were negative for PRRSV antigen or PRRSV-specific antibodies, were randomly divided into 5 groups (10 pigs per group) to examine change of virulence by CPD. In the second pig study, 20 commercial cross-bred pigs of 3 weeks old, which were negative for PRRSV antigen or PRRSV-specific antibodies, were randomly divided into 4 groups (5 pigs per group) to evaluate protective immunity induced by the attenuated viruses, being challenged by a heterologous virus.

Results: In animal infection using 3 weeks-old pigs, the attenuated viruses showed significantly lowered replication ability than the parental viruses without distinct clinical sign and pathological lesions, which were observed in pig infected with the parental viruses. Regarding induction of PRRSV specific immunity, the level of the neutralizing antibodies as well as secretion of IFN-γ -SCs in PBMCs was not different between the attenuated viruses and the parent viruses. More importantly, pigs infected with the attenuated viruses exhibited significant reduction in respiratory scores, viremia, macroscopic and microscopic lung lesion scores, and PRRSV-antigen with interstitial pneumonia against a heterologous challenge with a type 2 virulent strain.

Fig. 1. Designs of infectious clones for CDP viruses and phylogenetic relationship of viruses used in this study



Conclusions: The attenuated viruses generated by SAVE in this study demonstrated the potential usefulness as vaccine strains to provide immune protection against diverse PRRSVs

Acknowledgement: This research was supported by iPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries) (Grant No. 116074-3), and Animal and Plant Quarantine Agency (project no. QIA A-1543083-2017-19), Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

References:

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PRRS-024

Temporal patterns of the adaptive immune responses in local and systemic components of porcine reproductive and respiratory syndrome virus (PRRSV) infected pigs.

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Introduction: In order to formulate efficient strategies against the PRRSV, it is essential to understand the components of host immune responses which are responsible to clear the virus from the host. In this line, the present study was undertaken to compare the trend of humoral and cell mediated immune responses in local tissues and peripheral blood of host during the acute phase of the infection to understand the porcine reproductive and respiratory syndrome virus (PRRSV) clearance mechanism the pigs.

Materials and Methods: Seventy-five, 4-week-old pigs with no previous history of PRRSV infection were infected with type 2 PRRSV JA142 strain and twenty-five pigs were kept as negative control. A total of twenty pigs (infected, n=15 and uninfected, n=5) were euthanized at 3, 10, 21, 28 and 35 days post challenge (dpc) to collect blood (for PBMC and serum) and bronchoalveolar lavage (BAL) cells, lung parenchyma and bronchial lymph node (BLN) samples. For viral growth kinetics, quantification of viral loads in serum and lung tissues was performed using qPCR. Humoral responses were measured using anti-PRRSV IgG and virus neutralizing antibodies (VNA) in serum. Moreover, the adaptive T cell immune responses were compared in PBMCs and local tissues.

Results: The challenged pigs displayed the highest viremia and lung viral loads between 3 and 10 dpc, however, the virus was almost cleared by 28 dpc. The viral proliferation in the host was followed by the appearance of the anti-PRRSV IgG response at 7 dpc which gradually increased upto 35 dpc. However, this response had no role in the clearance of the virus. Further, the VNA response was delayed and was observed after 28 dpc in the challenged pigs. The patterns of cell mediated adaptive immune responses were compared locally and systemically by observing changes in the Th1, Th17, CTL and Treg frequencies in the infected and uninfected pigs. A delayed Th1, Th17 and CTL responses after 21 dpc were observed in peripheral blood. Comparatively, the local tissues displayed early protective Th1, Th17 and CTL responses at 10 dpc. Moreover, no significant upregulation of regulatory T cells was observed systemically or locally in infected pigs.

Conclusions: This study demonstrated that the PRRSV-infected pigs present delayed systemic T cell responses after 21 dpc. Intriguingly, the early adaptive T cell responses were observed in local tissues of the infected pigs which may have some role in viral clearance. Moreover, the delayed appearance of VNA in serum reveals the meager role of the humoral immune response in clearance of PRRSV from host.

Acknowledgement: This study was supported by a grant (No. Z-1543069-2017-20-01) from Animal and Plant Quarantine Agency (QIA), Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea

PRRS-025

The efficacy and overall performance impact of Fostera PRRS in a Vietnamese commercial pig farm naturally challenged by a highly pathogenic PRRS virus

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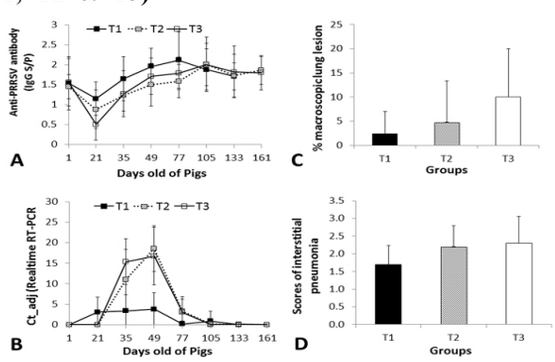
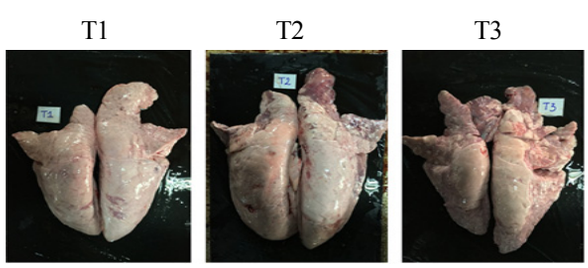
Introduction: A previous study under experimental challenge conditions showed that vaccination of pigs with Fostera PRRS resulted in reduced clinical signs, HP-PRRSV viremia, and severity of lung lesions following the Vietnamese HP-PRRSV challenge [1,2]. However, no in-depth evaluation of the efficacy of this vaccine when administered to 1-day-old or 21-day-old piglets has been performed under field conditions. Therefore, the overall objective of this field evaluation is to provide foundational information required to develop practical and effective vaccination programs for pigs vaccinated with Fostera PRRS under field conditions in Vietnam.

Materials and Methods: In this study we compared the efficacy, safety and overall performance of a modified-live PRRSV-2 vaccine (Fostera PRRS) to an existing PRRSV modified live vaccine in a farm with a recent history of HP-PRRSV-associated respiratory diseases. 351 pigs were randomly allocated to three treatment groups: (T1) vaccinated with Fostera PRRS at 1 day of age (n=118), (T2) vaccinated with Fostera PRRS (n=118) at 21 day of age and (T3) vaccinated with Amervac PRRS (n=115) at 21 day of age. After vaccination, pigs were monitored for local and systemic reactions, and overall health status. Serum samples were collected periodically from vaccination to slaughter, at 1, 21, 35, 49, 77, 105, 133 and 161 days old, to assess antibody response and viremia load [1,2]. Clinical signs (respiration, diarrhea, and death), mean body weight, and daily weight gain were observed daily. Pulmonary lung lesion (gross and microscopy) were assessed at the end of the study (slaughter) [3,4].

Results: The Fostera PRRS vaccinated pigs had milder clinical symptoms, lower levels of HP-PRRSV viremia, and fewer pathological changes in the lung, and higher body weight gain at the end of the study compared to the Amervac PRRS group. Vaccination of pigs with Fostera PRRS at 1 day of age also significantly reduced viral loads in their blood ($P<0.05$) and induced higher anti-PRRSv antibody titers ($P<0.01$) compared to pigs vaccinated with Amervac PRRS at 21 day of age (Figure 1). Fostera PRRS vaccination at 1 day of age can be useful in protecting young piglets from early HP-PRRSV infection because the immunized pigs were marketed 20 days earlier than their peers immunized at 21-day old as they reached the target market weight earlier in this study.

Figure 1: A - Comparison of antibodies against PRRSV (S/P ELISA), B- viremia load of PRRSV, and C & D - pneumonic lung lesions among 3 experimental groups (T1, T2 & T3)





Conclusions: Fostera PRRS MLV vaccine used in this study is effective in improving growth performance from day 1 all the way to marketing age in a farm endemically infected with HP-PRRSV. In fact, Fostera PRRS vaccination at 1 day old can be even more beneficial because the immunized pigs were marketed 20 days earlier than their peers immunized at 21-day old as they reached the target market weight earlier.

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PRRS-026

The impact of PRRSV control in the context of ASF in China

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Introduction:

African Swine Fever (ASF) broke out in China in August 2018; since then it has spread to most of the provinces. With the spread of ASF, live pig transportation across the country has been banned, resulting in redundant finish pigs that can not be properly sold, significantly increasing pig density in local areas. This change has created a major challenge with regard to PRRS control in farms.

Materials and Methods:

Study was conducted on a large breeding farm with 3000 sows in East China, using AIAO weekly batch production mode. The farm has used a "5 step method" for PRRSV control since Nov 2017, with good results. Since September 2018, because of the outbreak of ASF in surrounding provinces, the farm was unable to sell gilts to market, causing significant increase in barn pig density. As a result, AIAO could not be implemented in nursing and fattening houses. Blood from umbilical cords, PRRSV pre-vaccine piglets, 10 week old pigs, 13 week old pigs, 16 week old pigs, and gilts was collected in August and October, respectively. All samples were sent to the laboratory at low temperature for PCR detection of PRRSV 5 pool 1; positive samples were sequenced to determine whether they were wild virus infection.

Results

As shown in Table 1, compared to August, the rates of PRRSV infection in pre-vaccine piglets, 10 weeks old pigs, 13 weeks old pigs, and gilts in October increased dramatically. The CT value of all positive samples in August were higher than 36; thus it was not possible to determine whether they were wild strains by sequencing. The mean CT value in October decreased significantly, and the positive samples were all wild strains according to sequencing results.

The mortality of nursing and fattening houses increased from 3.1% in August to 5.6% in October.

Conclusion

Following the emergence of ASF in China, the production rhythm and internal biosecurity have in the prevention and control of other diseases such as PRRSV. Farms should take steps to strengthen immunization programs via use of a PRRSV vaccine, adjust production, and use the "5-step method" systematically to help prevent and control PRRSV effectively.

Keywords: ASF, PRRS, control, biosecurity, China

References

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[2] McCaw M,(1995). Proc of the A. Leman Conference pp. 161-162.

[3] Morrison B (2014) AASV Annual Meeting pp. 605-612.

Table 1: Results of PRRSV PCR test in August and October

	Umbilical cord blood	Pre-vaccine	10 weeks	13 weeks	16 weeks	Gilts	Mean CT value	CT cutoff value
August	12.50%	25%	10%	10%	0	10%	37.58	38.77
October	0	75%	100%	75%	75%	0	28.89	37

Respi-001

A Case Report: The improvement of porcine pleuropneumonia by change of environmental management

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Introduction: For disease control of piglets, appropriate temperature and humidity control is important. “The amount of heat of air” is an environmental index that is calculated by temperature multiplied by relative humidity. It approximates the change in heat capacity of actual air, so it is used for purpose to evaluate "the state of air acting on the heat metabolism of animals" numerically[1)]. We experienced that the number of deaths suspected porcine pleuropneumonia decreased by change of environment management.

Materials and Methods: This commercial farm has seven finisher buildings. The buildings have liftable automatic ventilation curtains on the north side, south side and skylight, and mist equipment used for keeping humidify. On December 21, 2018, 89 pigs (131 to 146 days old) died in total seven buildings in a day. It means approximately 1.1% of the number of stocks. Clinical symptoms including deep cough, respiratory impulses, and energy loss were observed in this herd. Based on these clinical symptoms of the pigs and *Actinobacillus pleuropneumoniae* was isolated on December 5, it was supposed that caused high mortality porcine pleuropneumonia. As countermeasures against porcine pleuropneumonia, we changed environmental settings. (Table 1). In order to evaluate the environment in buildings after changing the environmental setting index including temperature, humidity, minimum amount of heat of air, number of dead pigs and food consumption were measured, and the values before and after changing the environmental setting were compared.

Table 1. Change point of the environmental setting

	Before	After
Set temperature	18 to 19℃	23℃
Act of mist equipment	45 seconds per 10 minutes	1 minutes per 3 minutes
Setting of curtain open / close	Skylight: kept open North and south: automatically open and close	Skylight: kept closed North: kept closed at night

Results: Since temperature and humidity rose, the minimum heat of air significantly rose after the environmental setting change ($P < 0.05$) (Table 2). The number of deaths decreased significantly ($P < 0.05$), and the variation in the number of deaths per day decreased ($P < 0.05$). The daily food consumption per pig was significantly increased ($P < 0.05$), and the variation in daily food consumption tended to decrease ($P < 0.1$). In addition, the clinical pneumonia symptoms improved after the settings changing.

Table2. Average value and standard deviation of measurement items before and after the environmental setting change

	Before	After
Average maximum temperature	20.5℃	24.9℃
Average minimum temperature	17.0℃	22.5℃
Average humidity	60.60%	67.30%
Average minimum heat of air	1037.5	1513.7**
Average number of daily deaths	18.8	9.3**
Standard deviation of the number of daily deaths	18.2	6.0**
Average daily food consumption per pig	14.3	17.6**
Standard deviation of daily food consumption per pig	2.55	1.61**

* P<0.1 ** P<0.05

Conclusions: In this case, there is a possibility that low heat of air because of low temperature and humidity caused to increase mortality by porcine pleuropneumonia. After the change of environmental setting, the minimum heat of air increased. As a result in the number of deaths decreased and food consumptions increased. Therefore, it is supposed that keeping the minimum heat of air high is effective to prevent porcine pleuropneumonia.

References:

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Respi-002

Antimicrobial Resistance of *Pasteurella multocida*. Isolated From Diseased Swine in Taiwan

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Introduction: *Pasteurella multocida* are gram-negative bacteria which cause progressive atrophic rhinitis and pneumonia in swine. Pasteurellosis may negatively affect growth rate and the efficiency of feed conversion [1]. Although we have vaccines to prevent pasteurellosis, antimicrobial drugs may be a common treatment to control the disease. To decrease drug resistance, this study was performed to establish treatment guidelines for clinical veterinarians.

Materials and Methods: In this study, a total of 141 strains of *Pasteurella multocida* were isolated from diseased swine which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan from January 2015 to March 2019. The minimal inhibitory concentration (MIC) test was evaluated by using the broth microdilution which was performed according to Clinical and Laboratory Standards Institute operating rules. The following antimicrobial drugs were selected for antimicrobial susceptibility testing: ampicillin, ceftiofur, cefazoline, doxycycline, enrofloxacin, florfenicol, kanamycin, lincomycin, lincospectin, erythromycin, tylosin, tiamulin and sulfamethoxazole-trimethoprim.

Results: The results of antimicrobial susceptibility testing of the 141 strains isolated from swine were as follow. 24.8% of *Pasteurella multocida* isolates were found to be resistant to ampicillin, 2.1% to ceftiofur, 9.9% to cefazolin, 66.7% to doxycycline, 2.8% to enrofloxacin, 48.9% to florfenicol, 46.1% to kanamycin, 96.5% to lincomycin, 56.7% to lincospectin, 25.5% to erythromycin, 98.6% to tylosin, 79.4% to tiamulin and 52.5% to sulfamethoxazole-trimethoprim, respectively (Table 1).

Table 1. MIC distribution profile of P. *multocida* isolates from 2015.01 to 2019.03 (n = 141)

Antimicrobial drugs	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Resistance rate (%)
ampicillin	0.5	512	24.8
ceftiofur	0.0625	0.25	2.1
cefazoline	0.5	4	9.9
doxycycline	2	4	66.7
enrofloxacin	0.125	0.125	2.8
florfenicol	2	64	48.9
kanamycin	16	512	46.1
lincomycin	64	512	96.5
lincospectin	32	64	56.7
erythromycin	4	128	25.5
tylosin	64	512	98.6
tiamulin	64	128	79.4
sulfamethoxazole-trimethoprim	128	128	52.5

Conclusions: Based on the result, it suggested that *Pasteurella multocida* isolates in this study were resistant to many antimicrobial drugs. Therefore, clinical veterinarians should only select and use antimicrobial drugs effective to *Pasteurella multocida* to reduce the occurrence of drug resistance in bacteria.

Acknowledgement: Thanks to the Animal Disease Diagnostic Center of National Chiayi University for providing the bacteria. Also thanks to my teacher and seniors for supporting the MIC test.

References:

[1] Davies RL et al., 2003. J. Med Microbiol. 52, 59-67.

Respi-003

Antimicrobial susceptibility testing of *Glaesserella parasuis* from Taiwanese diseased pigs

Ni-Jyun Ke[1], *Wei-Hao Lin*[1,2], *Albert Shu-Wei Chang*[3], *Ming-Tang Chiou*[1,2], ***Chao-Nan Lin***[*1,2]

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[3]*Intervet Animal Health Taiwan Limited, Taipei, Taiwan*

Introduction: *Glaesserella (Haemophilus) parasuis (G. parasuis)*, is the causative agent of Glässer's disease, which induces sudden death, polyserositis, polyarthritis, meningitis, pneumonia and poor production performance, causing severe economic losses in the swine industry [1, 2]. One of the main strategies to control and prevent Glässer's disease is using antibiotics. If pigs suffered Glässer's disease don't be treated with effective antibiotics in time, the lesions would process from acute fibrinous serositis to chronic fibrous serositis which impairing the production performance. However, *G. parasuis* grows slowly so it takes longer time to perform antimicrobial susceptibility testing than other common porcine bacterial pathogens. Therefore, the aim of this study is to screen out effective antibiotics for initial Glässer's disease treatment.

Materials and Methods: Total 338 *G. parasuis* isolates were collected from Taiwanese diseased pigs from December 2016 to February 2019. The 18 examined antimicrobial agents included trimethoprim/sulfamethoxazole (1.25/23.75μg), lincospectin (109μg), amoxicillin (25μg), ampicillin (10μg), enrofloxacin (5μg), flumequine (30μg), cephalexin (30μg), cephalothin (30μg), ceftiofur (30μg), cefquinome (30μg), doxycycline (30μg), oxytetracycline (30μg), florfenicol (30μg), gentamicin (10μg), spiramycin (100μg), tilmicosin (15μg), streptomycin (10μg), kanamycin (30μg) and apramycin (15μg). The agar disk diffusion method was employed in antimicrobial susceptibility testing according to the procedures outline in the Clinical and laboratory standards institute document M31-A3 [3]. Some antimicrobial agents were not examined for all isolates due to lack of antimicrobial disks.

Results:
Our results showed that Taiwanese *G. parasuis* isolates were sensitive to cephalosporin, florfenicol and doxycycline. Besides, Taiwanese *G. parasuis* isolates were highly resistant to trimethoprim/sulfamethoxazole, flumequine, oxytetracycline, spiramycin and streptomycin. The dosage forms of cephalothin, ceftiofur and cefquinome were only allowed for intramuscular injection in Taiwan.

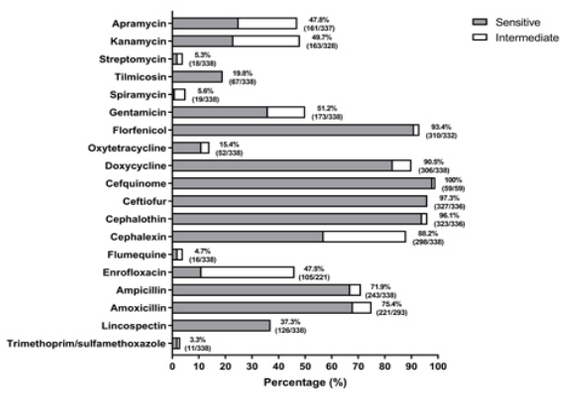

Figure 1. Antimicrobial disk susceptibility

Data were shown as percent of sensitive and intermediate isolate (number of sensitive and intermediate isolate/ examined isolate)

Conclusions: For initial treatment, cephalexin, florfenicol and doxycycline can be added in feed before pigs are stressed to prevent Glässer's disease or used for herd treatment. Cephalothin, ceftiofur and cefquinome can be used for individual treatment of acute phase diseased pigs.

References:

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Respi-004

Atrophic Rhinitis: A Potential Risk Factor for Lung Damage in Fatteners

Huan-Bin Liang*[*1], *Chen-Fei Zhang*[1], *Yi-Min He*[1], *Qing Li*[2], *Wen-Jing Liang*[2], *Jin-Bo Liang*[2]

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Introduction: Atrophic rhinitis (AR) is a widespread and economic cost disease in pigs, which is caused by *Pasteurella multocida and Bordetella bronchiseptica* and is characterized by atrophy of the turbinates[1]. A diseased pig is more susceptible to get infected by other respiratory pathogens, which in turn can lead to more severe lung damage. This study shows the correlation between AR lesion grades and lung damage through the statistical analysis of turbinate and lung lesion scores.

Materials and Methods: Twenty fattening pigs were selected from a commercial farm with 1000 sows in Guangdong province, south China. Turbinate atrophy (Fig. 1a and Fig. 1b) and lung lesion (Fig. 1c) were evaluated in the slaughter house for AR grade and lung damage, respectively. Linear relationship of these two variables was analyzed by Microsoft Excel 2010.

Results: The results of the turbinate atrophy examination showed that 19 pigs had some kind of lesion, meaning that the prevalence of AR in this single farm could be of up to 95%, and most nasal cavities were badly destroyed. Lung lesion examination showed 0.78% to 44.6% damage in the lungs; linear relationship between turbinate atrophy and lung damage is shown in Fig. 2. In this study, 65% of pigs suffered moderate-to-severe injuries in the nasal cavity, yet no clinical signs were found before they went to the slaughter house. This may be due to the mass administration of antibiotics in these pigs, which is a common way for farmers to deal with diseases. Nevertheless, our findings suggest that antibiotics should not be the ultimate way to solve disease-related problems, since pigs' turbinates can be damaged without showing any deformity in their nasal appearance. In this study, the Correlation Coefficient value (R) was 0.7167 (Fig. 2), which is not extremely high concerning the relationship between the two variables. This is mainly because our study is a field trial, and lots of factors may impact lung lesion scores (air flow, temperature, environment, other pathogens like PRRS, PCV, App, Hps, etc…).

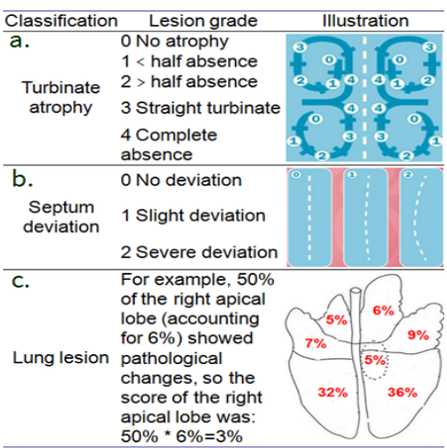
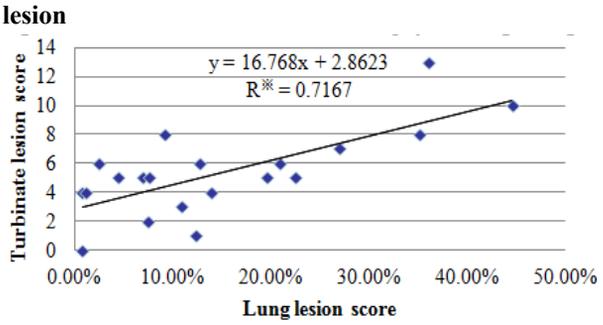


Fig 1. Scoring methods for turbinate atrophy and lung lesion



※ : |R|<0.4 Low linear correlation ; 0.4≤|R|<0.7 Significant correlation ; 0.7≤|R|<1 Highly linearly dependent （ Porter T M. Karl Pearson. 2005 ）

Fig 2. Linear relationship between turbinate atrophy and lung damage

Conclusions: A quite high linear correlation is observed between turbinate atrophy and lung lesion, strongly suggesting that AR may play an important role in respiratory diseases in pigs.

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Respi-005

Determination of minimal inhibitory concentration of tilmicosin against recent Thai isolates of *Actinobacillus pleuropneumoniae*

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Introduction: *Actinobacillus pleuropneumoniae* (App) is one of several important bacterial pathogens in the respiratory tract of pigs. The disease is associated with several risk factors including environment and management [1]. Carrier pigs tend to harbor the bacteria in their tonsils and nasal cavities [2, 3]. Tilmicosin is a member of macrolides used to prevent and control App. In Thailand, it has become a popular antimicrobial of choice to treat App, and other respiratory bacterial diseases in Thailand. Apart from field efficacy evaluation and monitoring, antimicrobial susceptibility testing is also important, Therefore, the MIC testing of tilmicosin against field Thai isolates of App was examined in this study with as objective to provide a valuable guideline for control and prevention of App outbreaks under field conditions in Thai pig farms.

Materials and Methods: A Total 50 field isolates of App recently collected during 2017-2018 from commercial pig farms and necropsy cases from Livestock Animal Hospital, Chulalongkorn University, Nakornpathom Province, Thailand, were used in this investigation. The isolates were selected from an outbreak/farm. The identification of App was based on bacterial culture and PCR subtyping methods of *apxICA, apxIICA, apxIIICA* and *apxIVA* genes. App serotyping was further examined using slide agglutination technique. The reference *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 type strains were used as test control. Tilmicosin (Pulmotil) was supplied from Elanco Co. Ltd. The MIC testing was determined by serial microbroth dilution assay according to CLSI approved standard VET01-A4 (2013). The MIC value was analyzed according to previous documentation [4, 5].

Results: The average MIC value for 50 App isolates was 13μg/ml with a range of 0.5-32μg/ml. With respect to MIC distribution data, the MIC50 value of tilmicosin against App was 16μg/ml. The MIC90 values was 32μg/ml. The number (percent) of App isolates displaying different MIC values at 0.5, 1, 2, 4, 8, 16 and 32μg/ml was 6 (12%), 7 (14%), 1 (2%), 2 (4%), 2 (4%), 25 (50%) and 7 (14%), respectively. Test control of tilmicosin using *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 type strains demonstrated MIC value at 4 and 8μg/ml, respectively. The serotypes of App isolates used in this investigation were serotypes 5 (n = 46, 92%), 2 (n =3, 6%) and 10 (n = 1, 2%), respectively.

Conclusions: The App isolates used in this study remain susceptible to Tilmicosin by MIC testing.

Acknowledgement: This work was supported by Elanco Co. Ltd.

References:

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Respi-006

Dynamics of Mycoplasma hyopneumoniae infection on pig farms of Vietnam

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Introduction: *Mycoplasma hyopneumoniae* (Mh) is the causative pathogen of enzootic pneumonia. Mh does not lead to high mortality like some emerging diseases recently, however, it is a major concern for the pig production around the world due to the economic damage [1]. To better control this pathogen, a better understanding of Mh infection dynamic is needed, and the result might be different from farm to farm [2]. The objective of the study was to investigate the dynamic of Mh infection of pigs at different ages in Vietnam swine farms using serological and molecular methods.

Materials and Methods: Ten commercial pig farms from different regions of Vietnam were included in the study. All farms used one shot Mh vaccines in piglets. Vaccination was performed at 1, 2 or 3 week of age (3, 3, and 4 farms, respectively) and blood samples as well as nasal swabs or oral fluid samples were collected from pigs at different ages. In each farm, 10 blood samples were taken from pigs at the age of 1 day and 1, 2, 3, 4, 8, 12, 16, 20 and 24 weeks, respectively. In addition, blood samples were collected from 10 gilts and 12 sows. From the same sampled sows, gilts and nursing piglets (1 days of age, 1-3 weeks of age), individual nasal swabs were also collected, whereas from the other age groups, pen based oral fluid samples were used. Nursing piglets were selected if their mothers have been sampled. Blood samples were investigated for antibodies using ELISA (IDEXX M. hyo. Ab test kit, USA) and nasal swabs/oral fluid samples were used to detect DNA of Mh using PCR test. Mixed linear model was used to analyze the Mh antibody titer change by week, age and vaccination program. Mixed logistic models were constructed to estimate the probability of PCR positive samples at every sampling. All analyses were performed in Stata 14.2 (StataCorp, College Station, Texas, USA).

Results: Results demonstrated that all investigated farms were infected with Mh. Sero-prevalence in gilts and sows was 95% and 68%, while their PCR-positive prevalence was 30% and 33%, respectively. The level of antibody had a trend to decline to negative after weaning and sero-conversion was observed by week 10-15. The linear model for this Mh antibody change is displayed in Fig 1. Serology also correlates with PCR results when the percentage of PCR positive increased slowly after weaning and reaches 40% - 60% at 12 weeks of age. The estimated probability of positive sample by week of age and vaccination program is displayed in Fig. 2 and revealed the persistant of Mh infection in a herd.

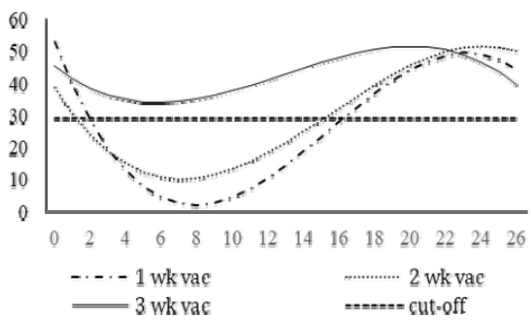

Fig. 1. Fitted Model of Mh antibody titer change with week of age and different Mh vaccination programs.

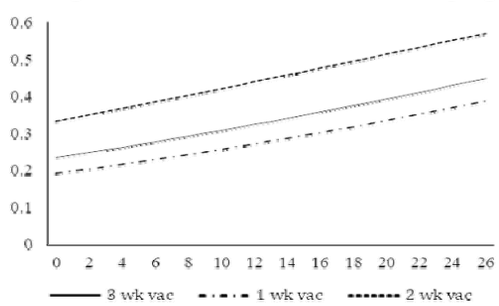

Fig. 2. Estimated probability of PCR positive sample by week of age and vaccination program.s

Conclusions: The finding of this study highlights the importance of Mh in pig farms of Vietnam and helps to understand the dynamic of Mhp infection in a herd.

Acknowledgement: This work was supported by Boehringer Ingelheim Animal Health Vietnam.

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Respi-007

Efficacy of the Porcilis® APP vaccine to induce antibodies against toxins APX Ⅰ, Ⅱ, Ⅲ and OMP

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Introduction: The etiologic agent of APP is *Actinobacillus pleuropneumoniae*. The organism is known to be involved in swine respiratory disease and often causes mortality in finisher swine. There are two biotypes distinguished by their dependency for NAD (nicotinamide adenine dinucleotide) in culture. Within the two biotypes, at least 15 serotypes are commonly recognized. The APP organism secretes 4 exotoxins, ApxI, ApxII, ApxIII and ApxIV, together called RTX toxins, and produces abundant endotoxin. The RTX toxins are cytotoxic and/or hemolytic. The toxins produced vary among the 15 serotypes. Antibodies to the exotoxins are important in generating protective immunity.

Materials and Methods: For this, APP negative piglets were vaccinated at 6 and 9 weeks of age through the intramuscular (IM) route with either Porcilis APP (G1),competitor toxoid vaccine (G2) or left unvaccinated as a negative control (G3). Serum was collected at 6,9,11,13,15,17,19 and 21 weeks of age and tested with the following ELISA tests: APX I, APX II, APX III, OMP (R&D Service Lab, MSD Animal Health) and APX IV (IDEXX).

Results: Pigs vaccinated with Porcilis APP displayed consistently higher APX I, APX II,APX III and OMP titres compared to pigs vaccinated with the competitor vaccine. Control pigs remained APX IV negative, indicating that all pigs were APP negative at the time of the study and that all antibodies induced were purely due to vaccination.

Conclusions: This study has shown that Porcilis® APP was able to generate and sustain higher levels of APX I, APX II, APX III and OMP antibodies in vaccinated pigs, throughout the monitoring period, compared to a competitor toxoid vaccine.

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Respi-008

Field Efficacy Comparison of Commercial Vaccines Against Progressive Atrophic Rhinitis in Taiwan

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Introduction

Pasteurella multocida (Pm) is the etiological agent of Progressive Atrophic Rhinitis (PAR) in swine and of considerable economic importance to the pig-rearing industry throughout the world. PAR is characterized by atrophy of the nasal turbinate bones, which in severe cases can lead to irreversible facial distortion, and may negatively affect growth rate and the efficiency of feed conversion.(1)

The objective of this study is to compare the field efficacy of commercial vaccines from different vaccination programs of pigs from the slaughterhouse.

Materials and Methods

The study, conducted from April 2018 to April 2019, included 2,500 sows from a commercial farrow-to-finish pig farm in middle southern Taiwan, which were divided in two groups: Group A (Vaccine A, AR vaccine with dl-alpha-tocopherol acetate as adjuvant) and Group B (RHINISENG®, HIPRA), vaccinated at 6 and 3 weeks, respectively, before parturition. Additionally, piglets from Group A were vaccinated at one week of age with commercial vaccine B (AR vaccine with aluminium hydroxide as adjuvant). The following was assessed:

Nasal score: 30 nose samples were randomly selected from slaughtered pigs (28 weeks old) from each group and scored based on the European Pharmacopoeia guideline.(2) Total score included turbinate atrophy score (0-4) and septum deviation score (0-2), maximum is 18 scores.

Results

Nasal scores from each group of pigs showed different distribution patterns (Fig. 1): 100% of the samples from Group A were affected, with a maximum and minimum grade of 15/18 and 1/18, respectively, whereas 90% of the samples from Group B were affected, with a maximum and minimum grade of 12/18 and 0/18, respectively.

Furthermore, regarding septum deviation scores, 56.7% (17/30) and 35.5% (11/31) of the samples from Group A and Group B, respectively, were affected.

Mean grade was 8.07 and 3.52 for Group A and Group B, respectively, with a statistical difference (t-test; $p < 0.001$) (Fig. 2).

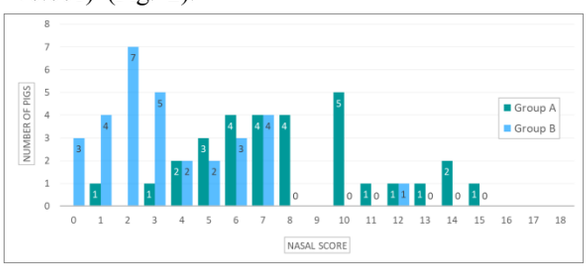

Fig. 1. Nasal score distribution of Group A and Group B; 100% of the samples from Group A were affected, with a maximum and minimum grade of 15/18 and 1/18; 90% of the samples from Group B were affected, with a maximum and minimum grade of 12/18 and 0/18.

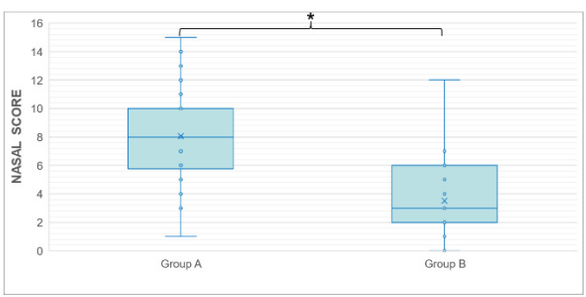

Fig. 2. Comparison between Group A and Group B; mean nasal scores for Group A and Group B were 8.07 and 3.52, respectively, statistically different (t-test; $p < 0.001$).

Conclusion

Under the conditions of this study, RHINISENG® reveals a better field efficacy to protect sows and piglets against progressive AR than Group A, with a statistically highly significant difference in nasal lesions.

References

[1] Robert L *et al*. Characterization and comparison of PM strains associated with porcine pneumonia and atrophic rhinitis. J. Med. Microbiol (2003).

[2] European Pharmarcopoeia 8.0. Monograph 04/2013: 1361.

Respi-009

Field Efficacy Comparison of Two Commercial Vaccines Against Non-Progressive Atrophic Rhinitis in Taiwan

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Introduction

Bordetella bronchiseptica (Bb) was the primary cause of atrophic rhinitis (AR) causing a mild-to-moderately severe reversible condition called non-progressive AR (1). However, in pigs infected at the age of 4 weeks, regeneration of the turbinates was noted 6 to 8 weeks after infection. When pigs were infected at 3 days of age, this process took five months (3). Thus, sow vaccination is useful to prevent piglet infection. The objective of this study is to compare the field efficacy of piglet protection during the nursery period produced from vaccinated sow herds.

Materials and Methods

The study, conducted from August to December 2018, included 1,600 sows from a commercial farrow-to-finish pig farm in middle southern Taiwan; these were divided in two groups: Group A (Vaccine A, AR vaccine with dl-alpha-tocopherol acetate as adjuvant)) and Group B (RHINISENG®, HIPRA), vaccinated at 6 and 3 weeks, respectively, before parturition. AR status was assessed using oral fluid samples (RHINICheck®, HIPRA, Spain) collected from nursery pigs, as well as the following:

- Nasal score: 30 nose samples were randomly selected from nursery pigs (5-12 weeks old) produced from each group, and scored based on the European Pharmacopoeia guidelines(2).
- Record of growth performance: Survival rate during nursery period.

Results showed BB positive (++), whereas toxigenic *Pasteurella multocida* was negative.

Results

After completing the vaccination program with two different vaccines, Group A and B showed different patterns of nasal lesion score distribution: 60% of Group B was concentrated below 2, whereas 60% of Group A was concentrated between 3-7 (Fig. 1). Mean nasal lesion scores for Group A and Group B were 6.6 and 2.9, respectively, statistically different (t-test; $p<0.001$) (Fig. 2). Survival rate of Group A during the nursery period was 83.5%, and Group B was 93%.

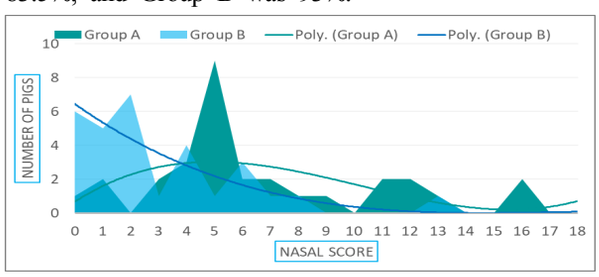

Fig. 1. Nasal lesion score distribution of Group A (Vaccine A) and Group B (RHINISENG®, HIPRA); the trendline reveals different patterns of score distribution, as 60% of Group B was concentrated below 2, whereas 60% of Group A was concentrated between 3-7.

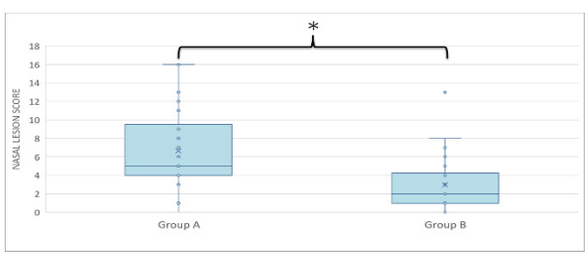

Fig. 2. Comparison between Group A and Group B; mean nasal scores for Group A and Group B were 6.6 and 2.9, respectively, statistically different (t-test; $p<0.001$).

Conclusion

Under the conditions of this study, Group B (RHINISENG®, HIPRA) reveals a better field efficacy to protect against non-progressive AR than Group A (Vaccine A), with a statistically highly significant difference in nasal lesions. Also, an improvement in growth performance was found.

References

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[2] European Pharmarcopoeia 8.0. Monograph 04/2013: 1361.
[3] Ross RF, Duncan JR, Switzer WP. Turbinate atrophy in swine produced by pure cultures of *Bordetella bronchiseptica*. Vet. Med. 1963(58): 566-570.

Respi-010

Genetic diversity of *pasteurella multocida* isolates from pigs with pneumonia

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Introduction: *Pasteurella multocida* is an opportunistic organism and is responsible significant economic losses with other respiratory pathogens in swine industry. *P. multocida* can be divided into three subspecies (*multocida, gallicida*, and *septica*), 5 capsular types (A, B, D, E, and F), and 13 biovars (1 - 10 and 12 - 14) [1]. Furthermore, *P. multocida* is classified into 8 lipopolysaccharide (LPS) genotypes (L1 - 8) which are required to identify potential vaccine candidates. In pigs, subspecies *multocida*, biovar 3, capsular type A and L6 are frequently characterized from pneumonic lungs [1, 2]. MLST has been used to study genetic relationship between *P. multocida* [3]. However, there is limited information on MLST data from swine *P. multocida* in Korea. The aim of this study is to genetically characterize *P. multocida* from pigs and elucidate genetic relatedness among isolates.

Materials and Methods: All *P. multocida* isolates were obtained from APQA and have previously been characterized [1]. The LPS genotypes were determined by multiplex PCR according to the previous study [2, 3]. Among the 166 *P. multocida* isolates, 151 isolates were characterized by MLST using seven housekeeping genes and sequence types (ST) were assigned by *P. multocida* RIRDC database [3].

Results: Among 151 *P. multocida* isolates, A:L6 (33.8%, n=51) was the most frequent Capsular:LPS type, followed by A:L3 (31.8%, n=48). However, eight isolates were not identified by LPS genotyping in this study [Table 1]. Of the 151 *P. multocida isolates* tested, eight different STs were identified by the MLST analysis. The most frequently detected STs in pigs were ST50 (31.8%, n=48), ST74 (28.5%, n=43), and ST13 (21.2%, n=32). ST286 (8.6%), ST27 (5.3%), ST9 (2.0%), ST347 (1.3%), and ST358 (new ST, 1.3%) were detected to a lesser extent [Table 2]. Interestingly, ST9, ST347, and ST358 have been identified in Korea only after 2014. All ST9, ST 13, and ST286 isolates comprised biovars 1, 2, and 13, respectively ($P < 0.001$). ST50, ST74, and ST27 were significantly associated with biovar 3 ($P < 0.05$) [Table 2].

Table 1. Distribution of capsular, LPS, and sequence types among 151 *Pasteurella multocida* isolates

No. of isolates within the following Capsular:LPS type and Sequence types										
A:L6 (33.8%)		A:L3 (31.8%)				D:L6	F:L3	D:L3	A:NT	D:NT
ST 74	ST 27	ST 13	ST 286	ST 347	ST 358	ST 50	ST 9	ST 286	ST 50	ST 50
43	8	32	12	2	2	41	3	1	6	1

[a]NT, Not determined.

Table 2. Distribution of sequence types and biovars among 151 *Pasteurella multocida* isolates

No. (%) of isolates within the following sequence types (STs) and biovars (B)							
ST50	ST74	ST13	ST286	ST27	ST9	ST347	ST358
B3	B3	B2	B13	B3	B1	B3	B3
48 (31.8)***	43 (28.5)***	32 (21.2)***	13 (8.6)***	8 (5.3)*	3 (2.0)***	2 (1.3)	2 (1.3)

*$P < 0.05$, ***$P < 0.001$

Conclusions: The most prevalent Capsular:LPS genotype of porcine *P. multocida* isolates was A:L6. And, MLST results of the present study appeared genetic population changes of P. *multocida* isolates in Korea since 2014 and relatively high correlation between biovars (1, 2, and 13) and STs (9, 13, and 286).

Acknowledgement: This work was supported by a grant from Animal and Plant Quarantine Agency, Ministry of Agriculture, Food and Rural Affairs of the Republic of Korea.

References:

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Respi-011

Investigation of Mycoplasma hyopneumoniae infection in gilts of different age

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Introduction: Mycoplasma hyopneumoniae (M. hyopneumoniae), which causes a chronic bronchopneumonia in pigs, plays an important role in the porcine respiratory disease complex (PRDC), and it is associated with large economic losses in pig production worldwide. M.hyopneumoniae transmission occurs mainly by direct contact (nose-to-nose) between infected to susceptible pigs as well as from infected dams to their offspring (sow-to-piglet) and gilts are considered the main bacterial shedders[1.2]. The objective of this study was to investigate the M.hyopneumoniae natural infection in replacement gilts

Materials and Methods:
In a large-scale pig farm in xin jiang, china, the replacement gilts were divided into 6 groups according to their age from 140doa to one day prior to weaning (350doa).30 pieces of Laryngeal swabs and serum were collected randomly from each group. Swabs were detected ofM. hyopneumoniae using PCR[3]. The sera were analyzed with the M. hyopneumoniae Antibody Test kit (IDEXX)

Results: Our results showed that it was common for replacement giltS to be infected with M.hyopneumoniae. The prevalence of M.hyopneumoniae positive gilts at all samplings is shown in fig.1. A significant increase in M. hyopneumoniae prevalence was observed from 140 doa (30% or 50%) to 170 doa (90%; $p < 0.05$), and a gradual decrease in prevalence was observed over-time thereafter by PCR and serum antibody detection, although the M. hyopneumoniae prevalence did not reach 0 in any of the subsequent samplings (140-350doa). Despite serology showed an higher prevalence of M. hyopneumoniae than that observed using PCR, the trend is the same

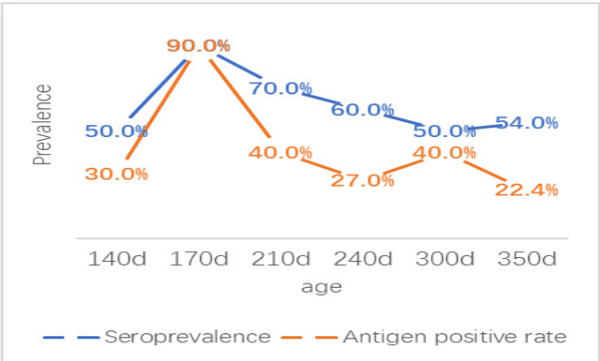

Fig.1. the number of positive detection of M. hyopneumoniae by PCR and Antibody at all samplings

Conclusions: It is very important to strengthen the detection and management of gilts to control M. hyopneumoniae infections in pig farm. The most frequently used method (ELISA and PCR) should be combined together.

References

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Respi-012

Investigation of *Mycoplasma hyopneumoniae*-like lung lesions at slaughterhouse in Taiwan

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[2]*Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan*
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Introduction:

Mycoplasma hyopneumoniae (M. hyo) is a chronic respiratory and costly disease, which is the primary pathogen of swine enzootic pneumonia in pig productive systems. Clinical signs of M. hyo includes dry cough. Anorexia and dyspnea. The level of severity of M.hyo infection could be evaluated by M. hyo-like lung lesion scoring at slaughterhouse. The controls of M. hyo are focus on the farm management and housing conditions combined with proper vaccination program. Up to present, several commercial M. hyo vaccines are available and have been reducing clinical signs. However, there is limited information of Mycoplasma-like lesions at slaughter in Taiwan. The aims of the present study were to evaluate the M. hyo-like lung lesion score at slaughterhouse and compare the M. hyo-like lung lesion score between different vaccines.

Materials and Methods:

During December, 2017 and March 2019, a total of 1,575 lung of fattened pigs were examined in Taiwanese slaughterhouse and the severity of the lung lesions was evaluated by the same swine veterinarian. The lungs were collected from pigs vaccinated with Porcilis M Hyo (group A, n = 198), M+PAC® (group B, n = 138), a commercial vaccine (group C, n = 880) and non-vaccination (group D, n = 359). Lung lesions were scored for M. hyo-like gross lesions following the method described by Madec et al. each of the seven lung lobes was judged individually and classified according to its individual macroscopic appearance using at 0-4-point system. The scoring of 0, 1, 2, 3 and 4 are represent no macroscopical lesions, superficial alterations less than 3×3 cm, large alterations up the half lobe, extensive lesion (up to 3/4 of lobe) and 3/4 to entire lobe is affected. The lung lesion score ranged from 0 to 28 at maximum. Non-parametric statistical analysis (Kruskal-Wallis test) was used to test difference in gross lung lesion scoring among pigs from different treatment groups.

Results:

At slaughter, the group B had the lowest lung lesion score (2.63) as compare to other groups (group A: 4.60; group C: 5.49; group D: 6.01). Group B (33%) had significantly more lungs with score 0 compare to group A (16%), group C (8%) and D (13%). Eighty-three percentage of pigs from group B had highest lung lesions scoring less than 5 as compared to group A (66%), group C (54%) and group D (52%).

Table 1. M. hyo-like lung lesion scoring at slaughter in pigs from different groups.

Group	N	Lung lesions scoring (%)					Mean	SD
		0	1-4	5-9	10-15	>15		
A	198	32 (16)	99 (50)	39 (20)	21 (11)	7 (4)	4.60	4.89
B	138	46 (33)	69 (50)	16 (12)	3 (2)	4 (3)	2.63	3.98
C	880	70 (8)	408 (46)	254 (29)	94 (11)	54 (6)	5.49	4.90
D	359	45 (13)	141 (39)	93 (26)	45 (13)	35 (10)	6.01	5.60

Conclusions:

Results of lung lesion score, Mycoplasma-like lesions were less severe in pigs from all of vaccination groups. The overall mean lung lesion score was the lowest in pigs from M+PAC® group.

References:

[1] Beilage et al., 2009. Prev. Vet. Med. 88, 255-263.
[2] Hillen et al., 2014. Prev. Vet. Med. 113, 580-588.
[3] Leneveu et al., 2005. Intern. J. Appl. Res. Vet. Med. 3, 295-265.
[4] Madec et al., 1982. Journ Rech Porc Fr 14: 405-412.
[5] Thacker, et al., 2006. Mycoplasmal disease. In: Diseases of Swine. Iowa State University Press, Ames, pp 701-717.

Respi-013

Molecular characterization of *Pasteurella multocida* isolated from pigs in Korea

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Introduction: *Pasteurella multocida* (*P. multocida*) is a pathogen causing in a wide range of host including porcine, avian, ovine, bovine and human. Multi-locus sequence typing (MLST) is a sequence based approach that fragments approximately 450-600 bp in length is sequenced from seven housekeeping genes. Furthermore, the sequence types (STs) achieved by MLST can be totally comparisons across laboratories and across time easy and routine in nature [2, 3]. This information can be used to develop disease control measures, epidemiological and evolutionary studies, targeted towards these pathogenic bacteria [1]. The aim of this study was to investigate MLST sequence types (STs) of *P. multocida* isolates from different host such as pig, wild avian and human.

Materials and Methods: In this study, a total of 30 *P. multocida* isolates from pigs (n=25), wild birds (n=2) and humans (n=3) were include. The allele sequences of the each seven genes were searched in the *P. multocida* multi-host database (http://pubmlst.org/pmultocida_multihost/) the appropriate allele number was assigned. Each unique allelic profile was assigned as a sequence types with a random number. The phylogenetic analyses were performed with Neighbor-Joining method implemented in MEGA5 program.

Results: The genetic relatedness amongst the *P. multocida* isolates investigated in this study was shown in the phylogenetic tree. Pig isolates were included ST3 (isolates 4), ST10 (isolates 6), ST11 (isolates 9) and novel STs (isolates 6). Wild bird isolates were included ST33 (isolates 1) and novel STs (isolates 1). All human isolates were novel STs (isolates 3). The isolate p04728 from human sputum was novel ST, is belong to ST10 group isolated from pigs. It is suggesting that p04728 strains might be particularly associated with host other than the pig. ST3, ST10 and ST11 had been identified previously in *P. multocida* isolates from porcine and in avian, bovine, and rabbit *P. multocida* isolates with different clinical backgrounds from diverse geographical areas.

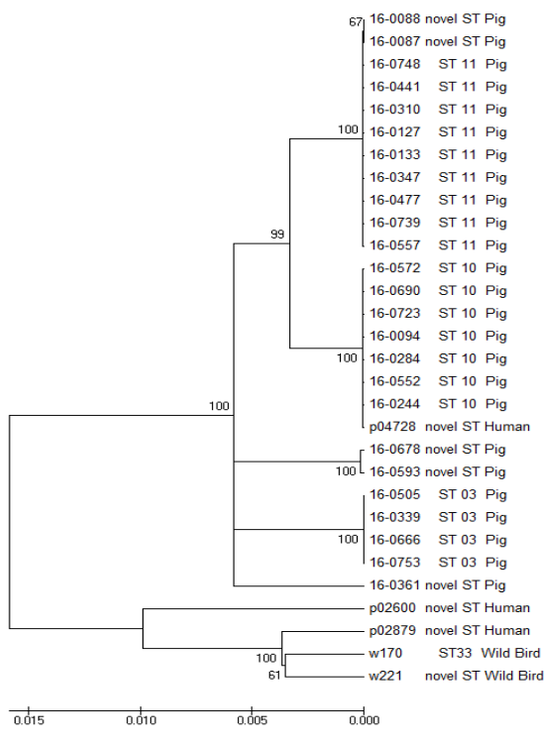

Fig. 1. Phylogenetic tree based on the neighbor- joining method of the concatenated sequences (*adk, aroA, deoD, gdhA, g6pd, mdh* and *pgi*; 3,990 bp) of ST included in the *P. multocida* Multi-host MLST Database.

Conclusions: *P. multocida* is reported as zoonotic pathogens, systemic spreading of the organism in pigs used for human consumption might represent a potential source of human infection.

References:

[1] Hotchkiss et al., 2011. BMC Microbiol. 25;11:115.
[2] Sarangi et al., 2016. Transbound Emerg Dis. 63(2): e286 92
[3] Subaaharan et al., 2010. Vet Microbiol. 24; 141 (3-4):354-61.

Respi-014

Observation of pleuritis and pericarditis in pigs at slaughterhouse in Taiwan

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Introduction:

Inflammation of the parietal or visceral pleura is called pleurisy or pleuritic. This is one of the most common pathological conditions observed in pigs at slaughterhouse. Pleuritis affects economics and efficiency of the entire pig production, such as reduced growth rate, decreased weight at slaughter, increased days to slaughter, medication costs and lost income. Pleurisy is most frequently caused by bacteria, which with polyserositis (Glasser's disease) caused by the gram negative bacteria *Haemophilus parasuis* (Hps). Pericarditis is one of the most lesion in Hps infected pigs. Overall, abattoirs suffer from increased production costs because pleuritis and pericarditis requires trimming of the carcass causing disruption, reducing line speed and increasing labour and wastage costs. The resolution of pleural lesion associated with pleuritis can take 3 months or more. Therefore, the status of pleurisy in slaughter pigs may be reflected the Glasser's disease in nursery pigs. Pleuritis and pericarditis prevalence in slaughter pigs has been evaluated in many studies in many different countries. However, there is limited information of pleuritis and pericarditis at slaughter pigs in Taiwan. The aim of the study was to assessment the pleuritis and pericarditis lesion in pigs at slaughterhouse.

Materials and Methods:

During January, 2018 and January 2019, a total of 1,474 carcasses from 12 pig herds were assessed in 3 Taiwanese slaughterhouses. The most common form of pleurisy found in slaughter pigs is fibrinous pleuritis. Diagnosis of pleuritis in pigs is identified post mortem. In case of inflammation, the pleura thickens and becomes opaque. The pleura can be covered with a thick, stringy, elastic, white-grey to yellow exudate. Lung fibrosis or abscess formation like in the case of chronic pleuropneumonia caused by *Actinobacillus pleuropneumoniae* will be rule out from this study. Define of pericarditis: heart is removed by veterinarian when pericardial thickening due to fibrinous inflammation.

Results:

Overall, pleuritic lesions were observed on 30.5% of lungs (450/1474). The minimum and maximum pleuritis prevalence across different farms were 4% to 53%. Abandoned hearts were observed on 9.0% of pigs (132/1474). The minimum and maximum pericarditis prevalence across different farms were 2% to 14.8%.

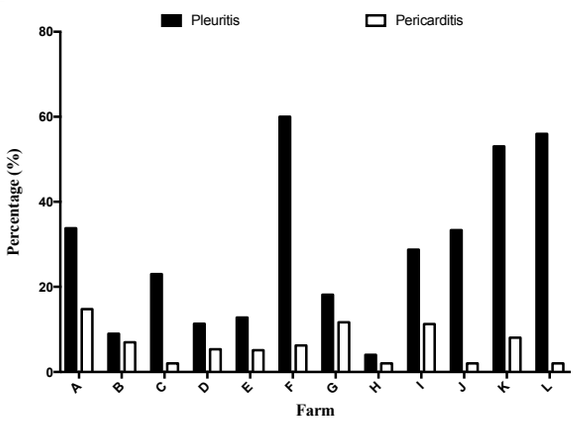

Figure 1. The prevalence of pleuritic and pericarditis were evaluated from 1,474 slaughter pigs in Taiwan.

Conclusions:

Overall, pleuritic lesions and abandoned hearts are the serious issue in Taiwanese pig production. This may be reflecting the severity of Glasser's disease in nursery pigs in Taiwan.

References:

[1] Lin et al., 2018. PeeJ 6: e6017.
[2] Merialdi et al., 2012. Vet. J. 193, 234-239.
[3] Jager et al., 2012. PLoS ONE. 7: e29655.

Respi-015

Prevalence and Intervention of Atrophic Rhinitis in Nursery Pigs in Taiwan

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[2]*Hipra Taiwan Co., Ltd.*

Introduction

Atrophic rhinitis (AR) is a widespread infectious disease of swine characterized by twisting or shortening of the snout. Two infectious agents are associated with the etiology of AR: toxigenic strains of *Bordetella bronchiseptica* (Bb) and *Pasteurella multocida* (Pm). Although Bb and Pm have the ability to cause turbinate atrophy in pigs, Bb was the primary cause of AR, causing a mild-to-moderate reversible condition called non-progressive AR.(1) In pigs infected at the age of 4 weeks, regeneration of the turbinates was noted 6 to 8 weeks after infection. When pigs were infected at 3 days of age, this process took 5 months,(3) which is often neglected but presents decreasing growth performance by retarding the time taken to reach slaughter weight.
The purpose of this study was to analysis AR status in nursery pigs and associated control strategies in Taiwan.

Materials and Methods

Between September and December 2018, 150 samples were collected from nursery pigs (5-12 weeks old) and sent to the Animal Disease Diagnosis Center in National Chiayi University to assess the following:

- Nasal lesion score based on the European Pharmacopoeia guideline.(2) Total score included turbinate atrophy score (0-4) and septum deviation score (0-2), with a maximum score of 18.
  AR lesions were further defined as: mild (0-4), moderate (5-8), and severe (9-18).
- Current vaccination program analysis for average nasal lesion scores.

Results

Average nasal lesion score was 6.53 with 14.67% involvement of the septum, distributed between 0-17 points. Most snouts included in this study had a normal appearance, without obvious lesions regarding twisting or shortening. After analyzing the proportion of AR severity, 67% of samples had a score over 5, defined as moderate to severe. However, 74.7% of these snouts were collected from pigs that had received commercial vaccines on a routine basis. Average nasal scores from different intervention strategies were as follows: 7.7 (no vaccination), 7.4 (vaccine A), 4.6 (vaccine B), 5.7 (vaccine C), 5.0 (other vaccines).

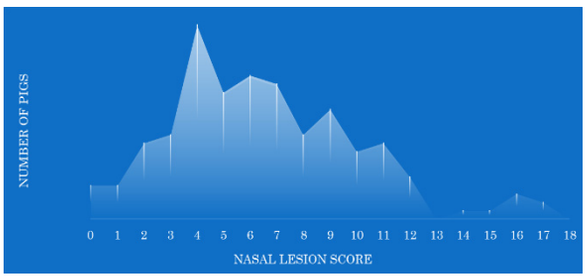

Figure 1. Nasal lesion score results and distribution for 150 samples collected from nursery pigs. Average nasal score was 6.53, distributed between 0-17 points.

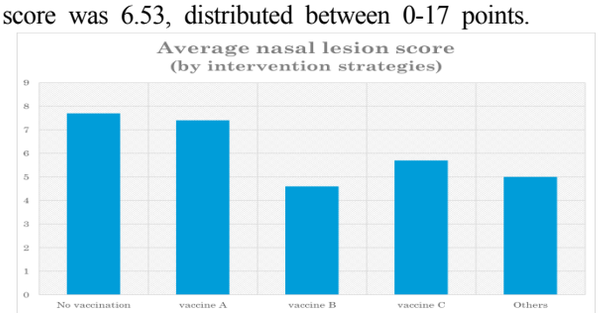

Figure 2. Nasal lesion score distribution by severity: mild (33%), moderate (39%) and severe (28%). Analysis of current vaccine: 74.7% of samples collected from pigs following a routine vaccination program. Mean nasal scores by vaccine: 7.7 (no vaccination); 7.4 (vaccine A); 4.6 (vaccine B); 5.7 (vaccine C); 5.0 (other vaccines).

Conclusion

Under the conditions of this study, the prevalence of AR in nursery pigs shows a certain proportion (67%) with moderate-to-severe lesions, although 74.7% of farms involved used a routine vaccination program with commercial vaccines, revealing a different protection efficacy against AR in nursery pigs receiving different commercial vaccines.

References

[1] Brogden KA, et al. Polymicrobial diseases. Washington (DC): ASM Press; 2002.
[2] European Pharmarcopoeia 8.0. Monograph 04/2013: 1361.
[3] Ross RF, Duncan JR, Switzer WP. Turbinate atrophy in swine produced by pure cultures of Bordetella bronchiseptica. Vet. Med. 1963(58): 566-570.

Respi-016

Serotyping and Development of Multilocus Sequence Typing (MLST) for *Actinobacillus pleuropneumoniae* Isolated from Pigs in Taiwan

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Introduction: *Actinobacillus pleuropneumoniae* (AP) caused pigs pleuropneumonia and result in huge economic losses. To date, 18 serovars and two biotypes of AP have been recognized in previously published, but the research to identify the serotype of AP isolated in Taiwan in recent years was rare. Also, the virulence of AP might be related to the serotype; therefore, to analyze of serotypes or related genotypes must be taken seriously. Besides, with the development of technology, there were many methods to classify bacterial genes, such as PFGE and AFLP; however, MLST for AP has not been reported previously. Therefore, the purpose of this study was to analyze the serotype of AP isolated from pigs in Taiwan, and tried to design a MLST method and to examine its potential use for AP sequence typing.

Materials and Methods: Clinical isolate strains were collected from pigs with typical hemorrhagic necrotizing pneumonia in the Animal Disease Diagnostic Center of National Chiayi University in Taiwan during June 2012 to August 2018. Finally, a total of 85 AP clinical isolate strains were collected. Serotyping was performed by PCR method according to Bossé et al. indicated in 2018 [1]. We selected seven housekeeping genes, including *recF, glyA, rhoAP, tpiA, pykA, recN* and *rpoA*, as potential target genes for the MLST assay for AP. Then, seven AP strains that complete genome sequences have been determined (hereafter, reference strains) were used to design PCR primers. Primers were designed through Primer-BLAST on NCBI website. Afterwards, PCR and sequencing work were performed, and we used BioNumerics version 7.6.3 software to analyze the data.

Table 1. Distribution of serotypes according to year

Serotype	Number of isolates							
	2012	2013	2014	2015	2016	2017	2018	Total
1			8	13	7	12	7	47
2				3		2		5
5	1	1	4	1	2	6	10	25
7				1				1
15						7		7

Results: Among the total of 85 AP clinical isolates, 47 strains belonged to serotype 1, followed by serotype 5, serotype 15, serotype 2, and serotype 7. The distribution of serotype according to year was shown in Table 1. After sequencing and analysis, 7 reference strains and 85 clinical isolate strains brought out 6 alleles for *recF*, 6 alleles for *gly*, 6 alleles for *rho*, 4 alleles for *tpi*, 5 alleles for *pyk*, 5 alleles for *recN*, and 7 alleles for *rpo*. The allelic profiles of seven housekeeping gene among all 92 strains yielded the 14 different sequence type, and we assigned them as the sequence type 1-14 (ST-1 to ST-14). After the minimum spanning tree constructed by the software (Fig. 1), 14 sequence type of 92 strains investigated in this study could form 3 different groups with 5 singletons.

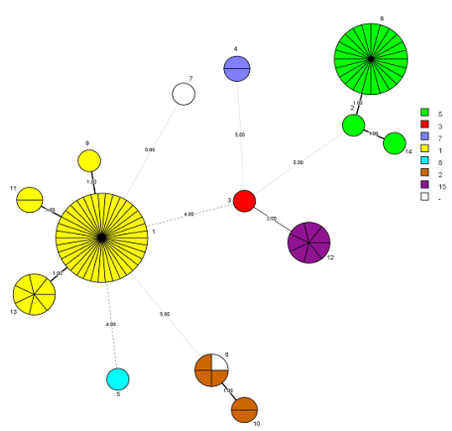

Fig. 1. MST graph constructed from the ST profiles of AP analysis by the BioNumerics version 7.6.3 software in this study.

Conclusions: Serotype 1 was the predominant isolate in this study, and we could isolate the serovar 7 and 15 that haven't been isolated in 2011 [2]. Besides, we could also find that serovar might have a relationship with the results of sequence types identified.

Acknowledgement: This work was supported by the Animal Disease Diagnostic Center of National Chiayi University, and we would like to express our thanks to all the members of the Bacteriology Laboratory in the department of Veterinary Medicine, National Chiayi University, Taiwan.

References:

[1] Bossé JT et al., 2018. Vet Microbiol 220: 83-89.
[2] Yang CY et al., 2011. J Vet Med Sci 73: 205-208.

Respi-017

Study of the Overview and Analysis on Swine Atrophic Rhinitis in Taiwan

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Introduction: Atrophic rhinitis (AR) is a contagious respiratory disease of pigs that is highly prevalent throughout the world. In addition, it is a difficult problem to deal with especially in nursery pigs. There are two forms of AR that have been recognized in terms of the causal agents. Nonprogressive form with mild to moderate turbinate atrophy is induced by *Bordetella bronchiseptica* (BB). On the other hand, diseased pigs infected with toxigenic isolates of *Pasteurella multocida* (PM) in combination with BB have more severe signs, such as epistaxis, head tilt and twisted snout. There are no reports regarding BB isolation in Taiwan and the last survey and investigation on AR was conducted more than twenty years ago. Therefore, the aim of this study is to provide an update on current conditions by AR investigating diagnostic samples from clinically diseased pigs in Taiwan.

Materials and Methods: 199 clinically diseased pigs from a total of 49 pig farms were studied. Visual scoring of the nasal turbinate bones was performed on vertically sectioned at the level of the first premolar teeth and was examined in a manner for turbinate bone atrophy (TA), and nasal septum deviation (NSD). Each of the four scrolls of the ventral turbinate bones was scored according to the European Pharmacopoeia guideline [1]. TA and NSD scores were summed for each individual to a maximum value of 18 (nasal lesion score, NLS). NLSs were divided into three intervals which could be defined as the patterns of AR, including 0-4, mild, 5-8, moderate, 9-18, severe. For this study, a total of 33 bacterial isolates were collected from diseased pigs as mentioned above. The bacteria examined were 10 isolates of BB, 24 isolates of PM. Furthermore, all 24 strains of PM were identified by polymerase chain reaction to analyze capsular serotype [2].

Results: Our results demonstrated that the average NLS was 6.83 with 14.57% septum affected. All visual scorings were distributed between 0-17 scores. And based on the data, further analysis were illustrated as Fig 1. On the other hand, PM capsular serotype analysis showed that 79 % were capsular type D and 21% were capsular type A. Furthermore, our results shown as Fig 2. indicated that the visual scoring was matched comparing with bacterial isolation.

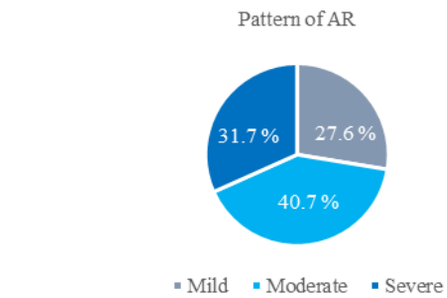

Fig 1. The proportion of patterns of AR from clinical samples in this study. Patterns of AR depends on the range of NLS.

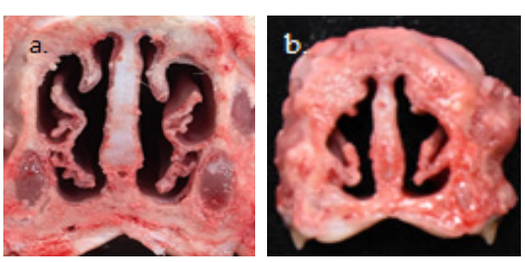

Fig 2. Illustration of cross sections of the snouts. (a) TA and NSD scores were 8 and 1 points respectively; (b) were 16 and 1 points respectively. According to bacterial isolation, (a) result showed that BB and PM capsular type D were isolated from lesions; (b) result showed that BB were isolated from lesions.

Conclusions: Based on the findings in this study, the situation of AR infection appeared to be common among swine industry in Taiwan, although diseased pigs show no clinical signs or obvious lesions such as head tilt and twisted snout.

Acknowledgement: This work was supported by Animal disease diagnostic center, National Chaiyi University, Taiwan. Sincerely, the author thanks colleagues in lab for technical assistance.

References:

[1] Bouin AS et al., 2014. European Pharmarcopoeia 8th, 1005-1006.
[2] Townsend KM et al., 2001. J Clin Micro 39: 924-929.

Corona-001

A comparative field trial evaluating the efficacy and growth performance of pigs vaccinated with Prime Pac® PRRS and Ingelvac® PRRS MLV

Sunit Mebumroong[1], *Adthakorn Madapong*[1], *Thitima Tripipat*[1], *Dachrit Nilubol*[1*]

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Introduction: Porcine reproductive and respiratory syndrome virus (PRRSV) modified-live vaccines (MLV) have been used widely to control the PRRSV infection [1]. Several MLVs are commercially available with different pros and cons in terms of virulence, viremia, protection, and etc [2]. In Thailand, Ingelvac® PRRS MLV has been commonly used in both sow and pigs. Although Ingelvac® PRRS MLV has been used intensively, nursery pigs still display clinical signs associated with PRRSV, and mortality is varied. The situation suggests that Ingelvac® PRRS MLV cannot completely overcome the genetically distinct field virus infection. Prime Pac® PRRS is a new MLV recently available in Thailand. The objectives of the study were to investigate the efficacy, mortality rate and growth performance in nursery pigs vaccinated with Prime Pac® PRRS MLV against field PRRSV infection in comparison to Ingelvac® PRRS MLV and another type 2 PRRSV MLVs (US2).

Materials and Methods: Seventy-six thousand and seventy-five (n=76,075) weaned pigs from a sow herd vaccinated quarterly with Ingelvac® PRRS MLV for more than 5 years, were allocated into 4 groups including non-vaccinated (n=1,112), Ingelvac® PRRS MLV (n=51,535), US2 (n=1,200) and Prime Pac® PRRS (n=22,228) groups, respectively. Pigs in vaccinated groups were vaccinated with either MLV at 10-14 days of age, in accordance with manufacturer's instructions. Average daily gain (ADG) was calculated at 70 days of age, and survival rate was recorded. Serum samples were collected at 0, 14, 28, 42 and 56 days post-vaccination (DPV), and assayed for PRRSV-specific antibodies by ELISA (IDEXX).

Results: The results demonstrated that the mortality rate in Prime Pac® PRRS MLV vaccinated group was significantly ($p<0.05$) lower than that of other 2 vaccinated groups (Figure 1). The mortality rate in the non-vaccinated group was not different from that of US2 group. Prime Pac® PRRS MLV vaccinated group had a significantly higher ADG compared to other groups (Figure 2). Moreover, the nursery pigs vaccinated with Prime Pac® PRRS MLV did not seroconverted to PRRSV during the nursery period.

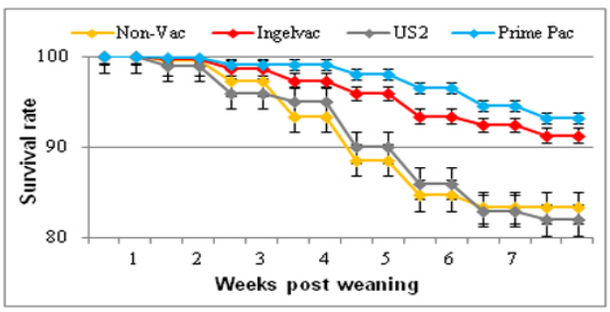

Figure 1. The survival rates of 4 treatment groups.

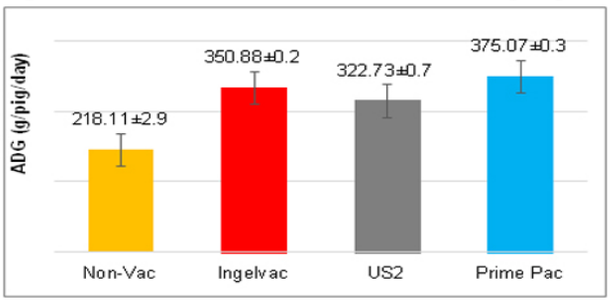

Figure 2. The mean ADG of 4 treatment groups.

Conclusions: Prime Pac® PRRS and Ingelvac® PRRS MLVs were able to reduce clinical losses associated with field PRRSV infection in nursery pigs in this herd, compared to non-vaccinated pigs. However, seroconversion to PRRSV did not appear in Prime Pac® PRRS MLV vaccinated pigs in the nursery. This suggests that Prime Pac® PRRS MLV was able to protect pigs against the field virus infection circulating in this herd. Additionally, the protection afforded to the Prime Pac® PRRS MLV vaccinated pigs, despite being vaccinated with a different vaccine to the sows, suggests that it is possible to use different MLVs at different stages of production and provide protection against field strains.

References:

[1] Nilubol D et al., 2014. Arch Virol. 158, 943-953.
[2] Madapong A et al., 2017. Arch Virol.162, 139-46.

Corona-002

Antiviral and adjuvant effects of lactic acid bacteria in suckling piglets against porcine epidemic diarrhea virus

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Introduction: Currently, porcine epidemic diarrhea (PED) vaccination is the major solution for PED virus infection, but the efficacy of the PED vaccination has been controversial among veterinarians due to its incomplete effectiveness [1]. The protective efficacy of PED vaccine is not able to reach to 100% in survival rate, and although the survival rate increases after vaccination compared to piglets farrowed by non-vaccinated sows, the clinical symptoms such as vomiting, watery diarrhea, and loss of body weight are not significantly reduced [2]. This is not only the continuous anxiety in swine farms, but also a serious challenge to vaccine companies and veterinarians. Herein, probiotics developed by CJ CheilJedang (Suwon, South Korea) is introduced as a sound solution to ameliorate the weak aspects of the current PED vaccination practice.

Material and Methods: Sows were treated with CJ probiotics (2 different *Lactobacillus plantarum* isolated from Korean fermented food, 10^{10} CFU/d) every day throughout the experiment and immunized twice with G2b type PED vaccines before farrowing. After farrowing, 4 day-old (enough time to consume colostrum) piglets were orally challenged with the highly pathogenic amount of PED virus (G2b type, 100 LD_{50}) and monitored for 14 d. The piglets were nursed by their mothers during the experiment. Clinical symptoms and survival rate were measured during the period, and afterwards, neutralization assay, virus detection, immunohistochemistry, and villus height-to-crypt depth (VC) ratio were conducted or measured using serum, colostrum, tissue, and fecal samples from sacrificed piglets. To assess the antiviral effect of the CJ probiotics, PED vaccine only group was compared. [Approved by IACUC of CAVAC: 180621-16]

Results: The piglets treated with CJ probiotics showed 73% survival on 7 d post infection (DPI), whereas those in the PED vaccine only (control group) exhibited 38%; the gap of the rates is more slightly increased on 14 DPI. The body temperature of piglets in the CJ probiotics group has been maintained in the normal range, while the vaccine-only group showed lower temperature. The diarrhea index score (0: normal, 1: pasty, 2: semi-liquid, 3: liquid) of the CJ probiotics group showed 1-2 score, whereas the vaccine-only group displayed over 2 index score. The VC ratio was almost 2-fold higher in CJ probiotics group (3.9) compared to vaccine-only group (2.2). Virus titer (measured by quantitative PCR) in the fecal samples of CJ probiotics-treated group was decreased over 90% compared to the vaccine-only group. Interestingly, neutralizing antibody (nAb) and vaccine-specific IgA of CJ probiotics-treated group (colostrum and sera from both sows and piglets) showed 43% and 44% increased level, respectively. Moreover, the body weight indicated over 1.3 kg differences between those two groups at the end of the experiment (18 d-old piglets).

Conclusion: The CJ probiotics as a feed additive to modulate sow immunity was able to increase PED vaccine efficacy, particularly nAb and vaccine-specific IgA, but decrease clinical symptoms significantly in piglets after the highly pathogenic PED virus challenge. As the first immune defensive line, more amount of nAb and vaccine-specific IgA transferred to piglets through lactogenic immunity reduced PED virus infection. In addition to the nAb and IgA, antiviral and anti-inflammatory cytokines induced by the probiotics were well transferred to the piglets via colostrum, and in turn the piglets were less damaged by PED virus infection compared to those of vaccine-only group. According to the results, the CJ probiotics can be a promising means to complement the current issues of PED vaccines. To make the CJ probiotics more effective and to be applied broadly, key components in immune modulation and potential usefulness for other enteric viral diseases are now explored.

Reference:

[1] Sun RQ, et al., 2012, *Emerg Infect Dis* 1:161-163.
[2] Makaduya N, et al. 2016, *Virol J* 13: 57.

Corona-003

Co-infection of porcine deltacoronavirus and porcine epidemic diarrhea virus prolongs virus shedding and IFN-α up-regulation

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Introduction: Swine enteric coronaviruses disease (SECD) is caused by swine enteric coronaviruses infection including porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) [1, 2]. PEDV and PDCoV are transmitted by fecal-oral route and mainly infect enterocytes of the small intestinal villi leading to indistinguishable abnormality signs such as villi atrophy, malabsorption, diarrhea, dehydration, vomiting, and high mortality, especially in neonatal piglets [3, 4]. Severity of PEDV in piglets (80-100% mortality rates) is higher than that of PDCoV (40-80% mortality rates) [1]. Understanding the co-habitation of these two viruses and outcomes following co-infection becomes interesting questions. The study, therefore, was conducted to investigate the severity of clinical diseases together with the antigen detection in epithelial cells in pigs experimentally co-infected with PEDV and/or PDCoV. Twenty-one genes associated with immunomodulation and expression of cytokine were additionally evaluated.

Materials and Methods: Twenty-four, 3-day-old pigs were divided into 4 groups of 6 pigs each including Neg, PDCoV, PEDV and co-infection groups. Pigs were orally inoculated with PDCoV and PEDV, either single or combination. Piglets were inoculated with 5 ml of each virus at a titer of 103 TCID50/ml. Neg was left as no-challenge. Clinical signs including diarrhea and fecal shedding were monitored daily. Three piglets in each group were euthanized at 3, and 5 days post inoculation (dpi) and five parts of small intestine including duodenum, proximal jejunum, middle jejunum, distal jejunum, and ileum were collected and kept in 10% formalin for hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC) investigation and RNAlater solution for gene expression evaluation.

Results: The results demonstrated that clinical diseases induced by PEDV, either single or co-infected with PDCoV, were significantly more severe than a single PDCoV infection. There was no difference in clinical diseases a single PEDV infection and co-infected with PDCoV. PEDV shedding in the co-infection group was significantly longer than the single infection group. However, there was no difference in PDCoV shedding between the single PDCoV infection and co-infected with PEDV groups. Immunohistochemistry (IHC) staining indicates that PEDV was more detected in villi of enterocytes while PDCoV was found in both villi and crypt enterocytes. PEDV infected cells were more density in the co-infection group than the single infection, especially in ileum. The up-regulation of IFN-α was observed until 5 dpi in the co-infection group, but not in either single infection group.

Conclusions: Co-infection of PEDV and PDCoV enhances the severity of clinical diseases. The co-infection prolongs PEDV shedding in fecal samples and up-regulation of IFN-α expression compared to either single infection.

Acknowledgement: This project was sponsored by Agricultural Research Development Agency (Public organization) (Grant number PRP6005020990), National Research Council of Thailand and Research and Researcher for Industry (Grant number PHD59I0094) for funding this research. Moreover, the partial funding was supported by Special Task Force for Activating Research (STAR), swine viral evolution and vaccine research (SVEVR), Chulalongkorn University.

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Corona-004

Evaluating the Immunogenicity of Porcine Epidemic Diarrhea (PED) Virus-Like Particles

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Introduction: Since late 2010, PEDV has causes profound economic losses in swine industry, signifying the crucial role of effective vaccines [1]. To develop a novel, efficacious, and economic vaccine for the PEDV, we constructed virus-like particles (VLPs) using a polycistronic baculovirus expression vector system (P-BEVS) and a baculovirus display system for S-Bac, M-Bac, and E-Bac generation. Both systems allow the co-expression of PEDV spike (S), membrane (M), and envelop (E) proteins in insect cells, which could be subsequently assembled into VLP. In the present study, the expression, characterization, and the immunogenicity of the PEDV VLPs was evaluated in mice.

Materials and Methods: The PEDV VLP (SME) and VLP (S+M+E) were prepared using the P-BEVS and baculovirus display system, respectively. Twenty-five, four-week-old mice were divided into five groups: 50 μg VLP (SME) with Freund's adjuvant (n=5), 50 μg VLP (S+M+E) with Freund's adjuvant (n=5), 108 TCID50/100 μ l S-Bac with Freund's adjuvant (n=5), foreign-antigen-free wild-type baculovirus (WT-Bac) with Freund's adjuvant (n=5), and Freund's adjuvant (n=5). The intramuscular injections with total volume of 100 μ l were performed two times with two-week intervals. The blood samples were collected at day 0 (prior to priming), day 14 (two weeks after priming), and day 28 (two weeks after the boosting). Oral and feces were collected every week. The in-house PEDV S-specific enzyme-linked immunosorbent assays (ELISA) were used to evaluates systemic IgG and mucosal IgA.

Results: A statistic method of generalized estimating equations (GEE) was used to assess the systemic IgG and revealed main effects of time [Wald Chi-Square=52.862, $p<0.001$], treatment [Wald Chi-Square=84.911, $p<0.001$], and time x treatment interaction [Wald Chi-Square= 111.805, $p<0.001$]. Pairwise comparisons identified the S/P ratio of S-Bac with Freund's adjuvant was significantly higher than wild-type baculovirus with Freund's adjuvant (MD=0.14, SE=0.05, p=0.008), and S/P ratio of VLP (SME), VLP (S+M+E), S-Bac, and WT-Bac were significantly higher than adjuvant (MD=0.32, SE=0.09, $p<0.001$; MD=0.28, SE=0.11, p=0.008; MD=0.29, SE=0.04, $p<0.001$; MD=0.14, SE=0.03, $p<0.001$). With respect to time, all groups displayed linear growth. Pairwise comparisons revealed significantly higher VLP (SME), VLP (S+M+E), S-Bac than adjuvant at day 14, and significantly higher VLP (SME), VLP (S+M+E), S-Bac, WT-Bac than adjuvant and significantly higher S-Bac than WT-Bac at day 28. On the other hand, OD value of the oral and fecal samples showed no significant differences.

Conclusions: In mouse models, intramuscular immunization of VLPs and S-Bac elicited systemic anti-PEDV S-specific IgG but limited oral and fecal mucosal IgA. The development of a vaccine regimen for inducing mucosal immunity is an important task for generating a successful VLP vaccine against PEDVs

Acknowledgement: This work was supported by the Ministry of Science and Technology, Taiwan, R.O.C. for grants MOST106-2311-B-002-028-MY3, MOST107-2321-B-033-002-, and 108L7842 from National Taiwan University, Taiwan, ROC.

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Corona-005

Evaluation of a novel PED vaccine in protection of newborn piglets against PEDV infection

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Introduction: Porcine epidemic diarrhea virus (PEDV) is a member of the *Coronaviridae* family. It primarily replicates in the villous enterocytes of the small intestine, which leads to atrophy of the intestinal epithelium and shortening of villi. Typical symptoms include watery diarrhea, vomiting, and anorexia. It infects pigs of all ages, but high mortality rate is often observed in newborn piglets. Passive immunity has been proven important for protection of newborn piglets against PEDV infection. In this study, the potential of a new injectable PED vaccine in inducing PEDV-specific antibodies in pregnant sows, which would pass on to the newborn piglets for protection against PEDV infection, was evaluated.

Materials and Methods: Pregnant sows were intramuscularly vaccinated with one dose of PED vaccine (vaccination) or adjuvant only (control) at 8, 5, and 3 weeks before farrowing. Blood samples and oral swab samples were collected before each vaccination and at parturition for antibody analysis. Newborn piglets from both vaccination and control groups were challenged with G2b Taiwan PEDV (strain Pingtung 52)[1] at 3 days old. Clinical signs and fecal scores of challenged piglets were recorded daily. Blood samples were collected at 0 and 10 days post challenge (dpc) for antibody analysis. In addition, fecal swab samples were collected daily up to 9 dpc to measure virus shedding. At the end of study, survival rate of piglets in both groups were calculated.

Results: At 3 weeks post first vaccination (5 weeks before farrowing), significantly higher serum IgG titers were detected in sows in vaccination group but not in control group. Higher serum IgG titers were sustained till farrowing in vaccination group as compared to control group. Similarly, significant higher oral IgA titers were detected in vaccination group after 2nd vaccination as compared to the control group (Table 1). On the day of PEDV challenge, 80% of piglets from vaccinated sows had higher serum IgG titer while all piglets from control group had low serum IgG titer. In addition, piglets from vaccinated sows had significantly lower clinical scores and fecal scores after PEDV challenge. Analysis of fecal samples showed that virus shedding reached peak at 1 x 10^7 PEDV genome copies at 2 dpc in control piglets while maximal virus shedding in vaccinated piglets was detected at 5 dpc with 1 x $10^{3.5}$ PEDV genome copies. At the end of study, challenged piglets from vaccinated sows exhibited 80% survival rate as opposed to 41% survival rate in challenged piglets from control group (Fig. 1).

Table 1. Antibody responses in experiment sows

	1st vaccination		2nd vacciation		Farrow	
	C	V	C	V	C	V
Serum IgG, S/P Ratio	0.05± 0.03	0.09 ± 0.03	0.04 ± 0.02	0.74 ± 0.23	0.02 ± 0.01	0.65 ± 0.22
Oral IgA, S/P Ratio	0.05± 0.03	0.45 ± 0.14	0.14 ± 0.08	1.57 ± 0.50	0.64 ± 0.37	1.07 ± 0.33

*C: control; V: vaccination

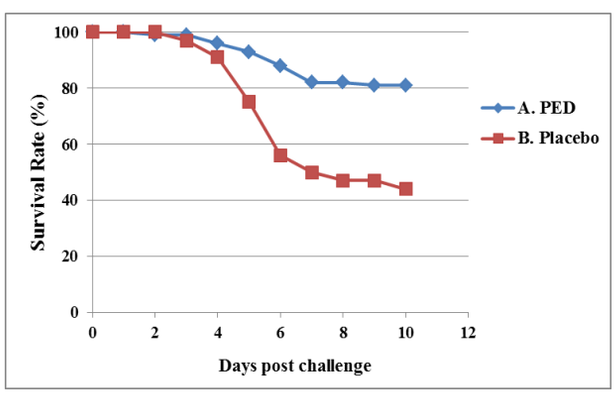

Figure 1. Survival rate of challenged piglets.

Conclusions: With a 3-shot vaccination program in pregnant sows, this novel PED vaccine stimulates PEDV-specific IgG and IgA secretion. These maternal antibodies were passed on to newborn piglets and provided sufficient immune protection for the piglets against PEDV infection.

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Corona-006

Genomic characterization and pathogenicity of a porcine deltacoronavirus strain CHN-HG-2017 in China

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Introduction: Porcine deltacoronavirus (PDCoV) was first detected in Hong Kong and has recently spread to many countries around the world [1]. PDCoV causes acute diarrhea and vomiting in pigs, resulting in significant economic losses in the global pork industry [2]. In this study, a Chinese PDCoV strain, designated CHN-HG-2017, was isolated from feces of a suckling piglet with severe watery diarrhea in a farm located in the central China. The full-length genome sequence of this strain was also sequenced and analyzed by MEGA software. Furthermore, the pathogenicity of this PDCoV strain was investigated in 5-day-old piglets by oral inoculation.

Materials and Methods: Feces (n = 17) from pigs experiencing diarrhea tested PDCoV-positive by N gene-based RT-PCRs were used for PDCoV isolation. Positive samples were suspended in DMEM, repeated freezing and thawing 3 times, and then centrifuged at 12000rpm for 10min at 4℃. The filtered supernatants were inoculated with LLC-PK1 cells. PDCoV N-protein-specific immuno-fluorescence was detected in most cells at 24 h post-infection. Total RNA was extracted and reverse transcription was performed with the Takara RNA PCR Kit. The genomic fragments were assembled with DNAStar Lasergene 7.0. Phylogenetic analysis was performed with the MEGA 7.0.14 software. Twelve 5-day-old piglets confirmed negative for PDCoV, PEDV, TGEV, and rotavirus were orally inoculated with CHN-HG-2017 at the titer of 1×10^6 $TCID_{50}$/ml (10 ml per pig). Clinical symptoms were recorded daily and tissues of intestines were fixed by 10% formalin for histopathology and immunohistochemistry analysis.

Results: As shown in Fig.1, cytopathic effect (CPE) in the form of enlarged, rounded and clustered LLC-PK1 cells after inoculation with positive samples. In addition, the PDCoV-specific immunofluorescence was detected in most cells at 24 HPI, and N protein was mainly localized in the cytoplasm. Typical crown-shaped coronavirus particles with spiky surface projections were observed by negative staining on the EM with the average of 80-160 nm in diameter. The full-length genome sequence of the PDCoV CHN-HG-2017 was 25,401 nucleotides in length. A homology analysis showed that CHN-HG-2017 shared 97.6%-99.1% nucleotide identity with the other 74 PDCoV strains in the database. PDCoV-challenged pigs showed diarrhea at 1 DPI and the intestines were clearly transparent, thin-walled, and gas-distended. The intestinal villus was blunted, fragmented along with the presentation of atrophy and vacuolation and PDCoV antigen was detected by immunohistochemical staining in the infected villous enterocytes.

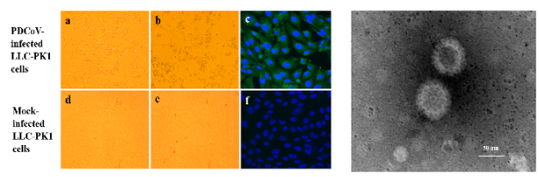

Figure 1. Cytopathic effect of LLC-PK1 cells infected with PDCoV strain CHN-HG-2017 and electron microscopic images of PDCoV particles. Bar, 50 nm.

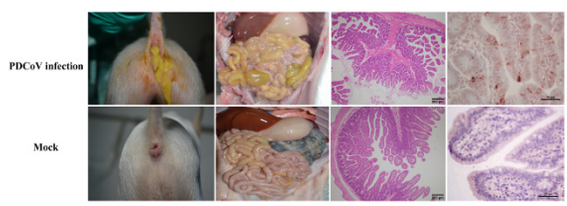

Figure 2. Clinical assessment and intestinal lesions of PDCoV-challenged pig.

Conclusions: we successfully isolated, characterized and obtained high-titer of CHN-HG-2017 in cultured cells and the strain is pathogenic in newborn piglets.

Acknowledgement: This study was supported by China Agriculture Research System (CARS-35), the National Key Research and Development Program of China (2018 YFD0501102), and Applied Basic Research Project of Wuhan (Grant No. 2017020201010227).

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Corona-007

Heat Shock Protein 70 positively regulates Porcine Epidemic Diarrhea Virus replication in Vero E6 cells

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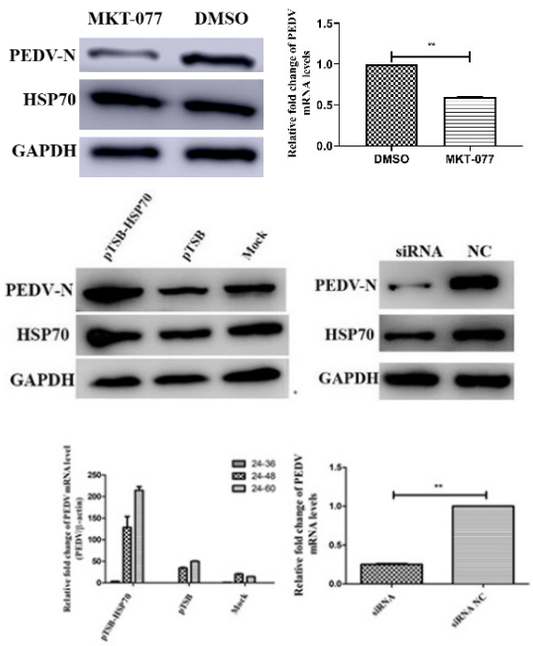
Introduction: Porcine epidemic diarrhea virus (PEDV), belonging to the order Nidovirales subfamily of Coronaviridae, which causes porcine epidemic diarrhea (PED), an enteric disease characterized by acute watery diarrhea, dehydration, vomiting, and high mortality in nursery piglets[1,2]. The incidence of new PEDV cases showed a seasonal pattern, peaking during winter, but the mechanism of "cold induced PEDV outbreak" still unclear[3]. HSP70 as a central component of the cellular chaperone are constantly recruited by virus and host stress responses[4]. However, HSP70 function in PEDV infection still unknown. We hypothesis that HSP70 was induced by cold stress and facilitate PEDV replication which cause morbidity of piglets. In this study, HSP70 inhibitors (MKT-077) was applied to reveal the role of HSP70 in PEDV protein synthesis. Depletion or over-expression of HSP70 were used to clarify the role of HSP70 in PEDV genome replication and viral protein maturation. We aim to uncover the role of HSP70 during PEDV replicaiton.

Materials and Methods: Vero E6 cells and CV777 strain were kept in our lab. Antibodies (HSP70, GAPDH) were purchased from Abcam (#ab2787 and #ab8245), goat anti-mouse IgG/HRP were purchased from Bioss (#bs-0296G-HRP). PEDV polyclonal anti-N antibody were obtained by repeat injection of purified recombinant protein produced in *E coli*. MKT-077 was purchased from Sigma (#M5449). siRNAs target HSP70 were synthesized by GenePharma (Suzhou, China). Real-time PCR and western blot were carried out measure the expression levels of HSP70 and PEDV N genes.

Results: Our results showed that (1) Cold exposure upregulate HSP70 expression and increase PEDV replication in Vero E6 cells (data not shown); (2) HSP70 inhibitor (MKT-077) significantly inhibit PEDV-N protein synthesis in Vero E6 cells; (3) Modulation of HSP70 expression levels positively correlated with PEDV-N mRNA and protein levels, suggesting HSP70 positively regulates PEDV replication in Vero E6 cells.

Figure 1. Effect of HSP70 activities and expression levels on PEDV replication in Vero E6 cells.



Conclusions: HSP70 positively regulates PEDV replication in Vero E6 cells.

Acknowledgement: This work was supported by the State Key Laboratory of Veterinary Biotechnology Foundation (SKLVBF201908), the Heilongjiang Province Postdoctoral Science Foundation (grant no. LBH-Q17139), the Talent Introduction Foundation of Heilongjiang Bayi Agricultural University (grant no. XYB2013-13), the Program for Young Scholars with Creative Talents in Heilongjiang Bayi Agricultural University (grant no. CXRC2017005).

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Corona-008

Isolation and evolutionary analyses of porcine epidemic diarrhea virus in Asia

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Introduction: Porcine epidemic diarrhea virus (PEDV) is a re-emerging coronavirus causing high morbidity and mortality since 2010 [1]. Although current data suggest that PEDV is undergoing frequent recombination and variation [2], the genetic and recombination characteristics of PEDV circulating in Asia remains unclear. For a better understanding of the molecular epidemiology of PEDV in Asia and provide more information for its prevention and control, it's necessary to analysis the evolutionary relationship in Asia.

Materials and Methods: Twelve nursing pigs experiencing severe watery diarrhea were obtained from a farm in Hubei province, China. The filtered supernatant of the intestine tissue was added to Vero cells for PEDV isolation. After 12 passages, the viral RNA was extracted for genome sequencing. The whole genome sequences of 207 strains PEDV from Asia were downloaded from NCBI. The alignment of all the 208 strains were conducted with MAFFT v7.4.02. The Neighbor-joining (NJ) phylogenetic tree was generated using Mega X and IQ-TREE.

Results: Eight PEDV strains were isolated from the nursing pigs and one isolate, designated as HB2018, was conducted with whole genome sequencing. The phylogenetic analysis of 131 PEDV strains from mainland China including HB2018 showed that all the strains isolated before 2008 belonged to genogroup GI, but genogroup GII appeared in mainland China and kept a high percentage since 2011. Until now, GII-c and GII-a (including HB2018) were the predominant subgroups in mainland China. S-indel variation belonging to subgroup GII-c has increased quickly in mainland China. In subgroup GII-c, the new emergence strains were not closely related, it remained that new variations may appeared in this subgroup. Analysis on 208 PEDV strains from Asia showed that GII-a was the predominant subgroup in Asia. The subgroup GII-c was only prevalent in China, while, all Japanese strains belonged to subgroup GII-a. The two strains from Taiwan/China had a very close relationship with Japanese strains. The comparison of S protein revealed that some marker nucleoid sites using to distinguish genotypes were not special among them. The N-glycosylation sites in 118, 127, 212, 1196 and 1249aa were not conserved.

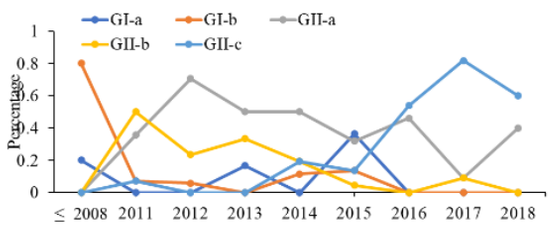

Figure 1. The percentage of PEDV strains obtained by subtype and year

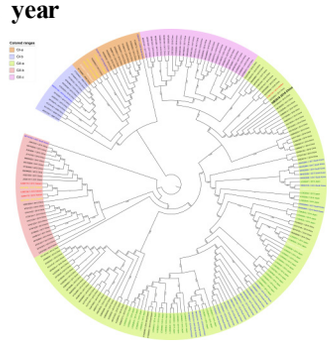

Figure 2. The NJ phylogenetic tree of 208 PEDV strains from Asia

Conclusions: The S-indel strains were increasing quickly in China. In addition, since subgroup GII-a had the most reported strains, more active actions are required for its prevention and control.

Acknowledgement: This work was supported by Key Laboratory of Prevention and Control Agents for Animal Bacteriosis (Ministry of Agriculture) (KLPCAAB-YTP-1801).

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Corona-009

Isolation and Identification of a Field Strain of transmissible gastroenteritis virus in Northeast of China

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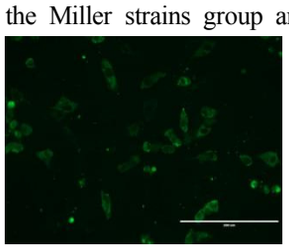
Introduction: Transmissible gastroenteritis virus of swine (TGEV) is an enveloped virus and belongs to a member of groups of coronaviruses, which is one of the most important causative agents of enteric infection [1]. The infection of TGEV is associated with high morbidity in animals of all ages and with high mortality in suckling piglets, which causes severe diarrhea in piglets and imposes a significant economic burden on pig farms [2]. Transmissible gastroenteritis (TGE) always break out in the spring and autumn in the northeast of china. In this study, a natural strain of TGEV-HQ2016 from the small intestine content of piglets in northeast of China was isolated and characterized.

Materials and Methods: In 2016, samples were collected from a pig farm in Heilongjiang province with diarrhea, and then identified by RT-PCR. The positive viral samples were treated with PBS, and cultured in blind passage for several times in PK15 cells until the appearance of the cytopathic effect. The collected cells were frozen and thawed for there times, and then were centrifuged for 5 min at 800×g, the supernatant was transferred into a new microfuge tube and centrifuged for 10 min at 13,400×g. Then, the pellet was negatively stained with 2% phospho-tungstic acid and analyzed on a transmission electron microscope. TGEV-HQ2016 infected PK15 cells which were cultured for 36h and then fixed with paraformaldehyde (4%), the mAb and FITC-labeled goat anti-mouse IgG were used to examine the immunogenicity of the isolated virus.

In order to confirm the virulence of TGEV-HQ2016 strain, pathogenicity analysis was tested on 3-day healthy newborn piglets. Ten TGEV, PORV, PEDV-free piglets from one farm were used and injected with 10^6 $TCID_{50}$ of TGEV-HQ2016 or DMEM. Clinical signs were observed per 3h. After 96h of inoculation, all of the piglets were killed, the pathological damage was examined by histopathology and immunohistochemistry.

Sequences of the TGEV reference strains were obtained from GenBank and nucleotide sequences and predicted amino acid sequences were analyzed with DNASTAR software. Phylogenetic trees were constructed with NJ method of MEGA6.

Results: The samples were positive for TGEV and successful isolated. The IFA result showed specific fluorescence in PK15 cells which injected with TGEV-HQ2016 (Fig 1). When analyzed via electron microscopy, typical coronavirus-like particles were observed (Fig 2). Pathological examination show that small intestines were filled with yellow intestinal fluids and thin, translucent walls were observed in test group. Histological changes show that many mucosal epithelial cells were degenerate, necrotic and lysed and severe villous atrophy were observed in jejunum and ileum compared to the negativecontrol. The homology results suggested that TGEV-HQ2016 showed higher identity to strains SC-Y, WH-1, HX and Purdue. The TGEV-HQ2016 strain had a close relationship with the Purdue strain and is more distant evolutionarily from the Miller strains group and strain ISU-1.



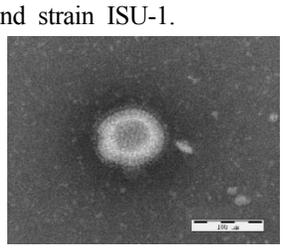


Fig. 1 IFA result of PK15 cell infected TGEV.
Fig. 2 Observation of TGEV by electron microscopy.

Conclusions: TGEV-HQ2016 strain was a vilent strain which can cause typical Clinical signs, pathological and histological changes of TGE disease. Isolation, genome property and pathogenicity analysis of TGEV strain will not only contribute to the basic research of TGEV, but also assist in developing biological products, the diagnosis and controlling of TGEV field strain.

Acknowledgement: This work was financially supported by a grant from Scientific research start-up plan of Heilongjiang Bayi Agricultural University

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Corona-010

Montanide™ gel 01 pr, an adjuvant for safe and efficacious porcine epidemic diarrhea and transmissible gastroenteritis bivalent inactivated vaccines

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Introduction: Porcine epidemic diarrhea (PED) and Transmissible Gastroenteritis (TGE) are highly contagious, intestinal infectious diseases caused respectively by porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV). These two diseases are characterized by severe diarrhea, vomiting and dehydration. PEDV and TGEV infections can occur in pigs of all ages, infections are most serious in piglets, with high morbidity and mortality. PED and TGEV severely impact the pig farming industry throughout the world. vaccination is an effective strategy to control PED and TGE diseases. Adjuvants are required to improve vaccines efficacy. Montanide™ GEL 01 PR (GEL 01) is an innovative adjuvant based on a dispersion of a high molecular weight polyacrylic polymer in water. In this study, we evaluated the immunopotentiating performance and safety of GEL 01 in a PED-TGEV bivalent inactivated vaccine.

Materials and Methods: Firstly, safety of vaccine based on GEL 01 used at 10% was compared to a water-in-oil-in-water (WOW) adjuvanted vaccine, a vaccine containing antigen with no adjuvant (non-adjuvanted group), and to a preparation containing no adjuvant and no antigen (control group). 3 pregnant sows per group were injected 4 mL intramuscularly (IM) at 6 weeks before delivery in the Houhai acupoint and 3 piglets of 3 days old were injected 1 mL IM. Pyrogenicity, general reactions, local reactions, and the effects of the vaccination on pregnancy and piglets health were assessed.

In a second trial, efficacy of the vaccines detailed above was determined on 3 pregnant sows per group and on their piglets. 3 pregnant sows per group were injected 4 mL IM each at 6 and at 3 weeks before delivery in the Houhai acupoint. The PED and TGEV viruses seroneutralization titers in sows blood were measured before injection, at 6 and 3 weeks pre-delivery and at delivery. IgA titers of PEDV were also measured in colostrum or in milk at farrowing, at 2 and 4 weeks post-farrowing.

Finally, a challenge trial was performed on piglets to define the protection rate after sows vaccination through maternal milk. 30 breastfed piglets were challenged orally 5 days after birth with 100 LD_{50} of either TGEV or PEDV, then duration of hyperthermia, intestinal lesions and viremia were measured.

Results: The safety trial results showed that the vaccine based on GEL 01 increased the body temperature in piglets and sows of less than 1°C. No other systemic reactions were observed in pregnant sows or piglets. Besides, GEL 01 vaccine did not induce any abortion, stillbirth or weak offspring.

The efficacy trial results showed that GEL 01 vaccine produced the highest TGEV and PEDV neutralizing antibody titers in sows among all the groups. Moreover, GEL 01 vaccine induced the highest IgA PEDV titers in sows milk post delivery, compared to WOW, non-adjuvanted and control groups, however, in all groups, the IgA titers decreased quickly. Finally, GEL 01 vaccine lead to the highest protections after oral challenge of piglets: 87% and 80% against respectively TGEV and PEDV challenges, followed by WOW vaccine with 73% and 60% against respectively TGEV and PEDV. Non-adjuvanted vaccine lead to low protection (less than 27%) and control group didn't protect against challenge at all.

Conclusion: GEL 01 allows the formulation of safe, efficient and protective PED-TGEV inactivated bivalent vaccines for both sows and piglets. GEL 01 is the most adapted adjuvant for pregnant sows, and is able to confer immunity to offspring through milk IgA after delivery.

Corona-011

The PEDV ORF3 protein is targeted to the Golgi area but is not incorporated into virions

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Accessory genes occurring between the S and E genes of coronaviruses have been studied quite intensively during the last decades. In porcine epidemic diarrhea virus (PEDV) the only gene at this location, ORF3, encodes a 224-residues protein shown to exhibit ion channel activity and to enhance virus production. However, little is known about its intracellular trafficking nor about its exact function during PEDV infection. In this study, two recombinant PEDVs were rescued by targeted RNA recombination, one carrying the full-length ORF3 gene and one from which the gene had been deleted entirely. These viruses as well as a PEDV encoding a naturally truncated ORF3 protein were employed to study the ORF3 protein's subcellular location and its incorporation into virions. In addition, ORF3 expression vectors were constructed to investigate the subcellular location of the individually expressed ORF3 protein. Our results show that the ORF3 protein uses the exocytic pathway to move to and accumulate in the Golgi area of the cell, similarly in infected and transfected cells. Also the C-terminally truncated ORF3 protein entered this pathway but it was unable to leave the early compartments and was retained in endoplasmic reticulum (ER) and ER-to-Golgi intermediate compartment (ERGIC). Despite the use of sensitive antibodies and assays no ORF3 protein could be detected in highly purified PEDV particles, indicating that the protein is not a structural virion component. Our results provide relevant new information about some important features of the PEDV ORF3 protein.

Corona-012

Screening of antiviral genes against porcine epidemic diarrhea virus

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Introduction: Porcine epidemic diarrhea virus (PEDV), the pathogen of porcine epidemic diarrhea (PED), causes vomit, watery diarrhea, dehydration and high mortality in suckling piglets[1]. Outbreaks of PED have resulted in extremely economic losses to the swine industry all over the world because of the emergence of highly pathogenic mutant strains[2]. The exploration of host restriction factors against PEDV will help to understand the interaction between host and virus. In the previous work we used high-throughput sequencing technology to screen the differentially expressed genes in PEDV-infected porcine intestinal epithelial cell clone J2 (IPEC-J2) cells. Here, based on the analysis of sequencing data, we selected ten potential antiviral gene candidates, DDX6, PABPC1, XPO1, DERLIN, UFD1, RNASEK, HSP40, TFPT, TRIM21 and TRIM38, to study whether they have anti-PEDV effect which can regulate the replication of virus on the Vero and IPEC-J2 cells.

Materials and Methods: After the validation of differentially expressed genes by qRT-PCR, the candidate genes were amplified from IPEC-J2 cells and cloned into the eukaryotic expression vector pcDNA3.1 with FLAG-tag. 24h after transfection with recombinant plasmids, cells were infected with PEDV GDS01 strain at MOI of 0.1 (Vero) or 1 (IPEC-J2) for different hours. At 3 and 6 hpi, qRT-PCR was used to detect mRNA of PEDV nucleocapsid gene. At 6 hpi, western blot was performed to detect PEDV nucleocapsid protein. At 9 hpi, plaque assay was used to determine the viral titers. Subsequently, gene silencing mediated by RNA interference method was used to confirm the results of overexpression. 24h after transfection with specific siRNA or negative control siRNA (siNC), cells were infected with PEDV GDS01 strain at MOI of 0.1 (Vero) or 1 (IPEC-J2) for the corresponding time, and harvested for analysis mentioned above.

Results: In this study, ten genes, DDX6, PABPC1, XPO1, DERLIN, UFD1, RNASEK, HSP40, TFPT, TRIM21 and TRIM38, were selected and their recombinant plasmids were successfully constructed. The protein overexpression results demonstrated that four proteins, PABPC1, XPO1, TRIM21 and TRIM38 could inhibit the replication of PEDV on both Vero and IPEC-J2 cells no matter on mRNA level, protein level or viral titers. Coupled with overexpression assay, gene silencing mediated by RNA interference method displayed the same results.

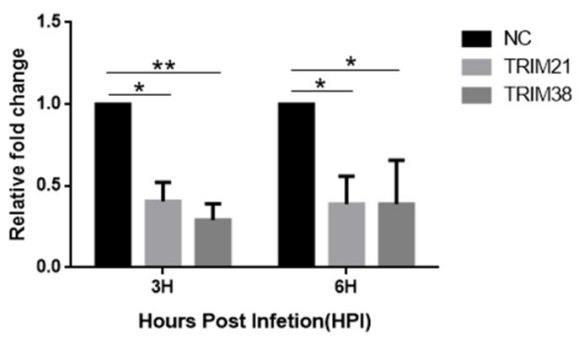

Figure 1. mRNA of PEDV nucleocapsid gene detected by qRT-PCR after overexpression on Vero cells.

Conclution: Taken together, our results demonstrated four genes, PABPC1, XPO1, TRIM21 and TRIM38, could inhibit the replication of PEDV on Vero and IPEC-J2 cells. Further research needs to be carried out to elucidate how the proteins restrict the replication of PEDV.

Acknowledgement: This work was supported by National Key Research and Development Program (2016YFD 0500101).

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[2] Song, D et al., 2012. Virus Genes. 44(2), 167-175.

Corona-013

The effect of PED vaccination frequency on IgA induction in colostrum

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Introduction: Colostral IgA antibody is a better indicator of protection against porcine epidemic diarrhea virus (PEDv) [1] due to its resistance to proteolytic degradation in the intestinal tract and higher virus neutralizing ability than IgG and IgM [2]. This study was conducted to analyze the effect of vaccination frequency against PEDv in sows on the levels of PEDv-specific IgA antibody in colostrum. In addition, the PEDv-specific IgA antibody levels in colostrum were also compared between vaccinated and non-vaccinated sows.

Materials and Methods: In the current study, a commercial killed vaccine (ISU460651A13 strain) against PEDV was used to vaccinate the pregnant sows. The sows were divided into 6 groups. Table 1 shows information of colostrum samples used in this study. Group A consisted of the non-vaccinated control sows. Group B, C and D comprised of the primiparous sows, sows undergoing 2nd farrowing and older sows with multiple farrowing records, respectively. The sows were vaccinated twice before each farrowing. The PED infected sows were placed in two groups, E and F. The sows in group F were later vaccinated. The colostrum within 24 hours after farrowing was collected in a conical tube. PEDv-specific IgA were detected using an in-house ELISA assay prepared with whole PEDv particles. The OD $\geq$ 0.6 was considered as a cut-off value based on ROC analysis. The PEDv-specific colostral IgA level were statistically analyzed using one-way ANOVA and Tukey's multiple comparison tests.

Results : On analyzing colostrum from all the sows for PEDv-specific IgA levels, groups with more than 3-time vaccination (groups C and D) revealed significantly higher levels of IgA compared to the control group A. In addition, the group F comprising PEDv infected sows which were later vaccinated, displayed significantly higher IgA levels as compared to group D ($\geq$5 vaccination) or group E (PEDv infection only) (Table 2). Based on ROC analysis of IgA antibody levels between control group and PEDv infected group, the sensitivity of the PEDv-specific IgA ELISA was determined as 94.6%, the specificity as 80.7%, and the AUC value was obtained as 0.94.

Table 1. Information of colostrum samples used in this study

Groups	Vaccination frequency	Sample size (N)
A	Non-vaccinated Control	83
B	Twice	42
C	3~4 times	30
D	More than 5 times	59
E	PED infected only	15
F	PED infected and vaccination	78

Table 2. Colostral IgA antibody levels in different groups.

Groups	Mean±SD	Groups	Mean±SD
A	0.43±0.31	D	1.10±0.62
B	0.53±0.28	E	1.16±0.43
C	0.82±0.43	F	1.75±0.94

Conclusions : The colostral IgA levels were determined to be directly proportional to the vaccination frequency. Interestingly, the colostral IgA level of group vaccinated more than 5 times was similar to the levels in PEDv-infected sows. In addition, significantly higher levels of colostral IgA level were induced when the vaccine was applied after PEDv infection. Therefore, to maintain the appropriate levels of IgA antibody in colostrum, regular vaccination of sows is recommended for both PEDv-free or PEDv infected farms.

Acknowledgement : This work was supported by the Technology Development Program for Bioindustry (Project No. 118093-03) of the Ministry for Food, Agriculture, Forestry and Fisheries of the Republic of Korea.

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[1] HA, G. W., et al. Korean Journal of Veterinary Public Health, 2010

[2] D.S. Song et al., 2007. Research in Veterinary Science 82, 134-140.

Trans-001

A direct reverse transcription loop-mediated isothermal amplification without nucleic acid extraction for on-site diagnosis of foot-and-mouth disease virus

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Introduction: Foot-and-mouth disease (FMD) is a highly infectious disease. Rapid and on-site diagnosis of FMD virus (FMDV) is the key factors in successful control of the disease [1] This requirements for prompt diagnosis during FMD outbreaks have motivated the development of on-site diagnosis. Several reverse transcription polymerase chain reaction (RT-PCR), real-time RT-PCR (qRT-PCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays have been developed for on-site detection of FMDV. However, a simple on-site nucleic acid extraction method from the clinical samples, and the integration of portable amplification equipment and the diagnostic assay are required for the diagnostic system to be used as a successful on-site diagnosis system. In this study, we developed a direct RT-LAMP (dRT-LAMP) combined with a simple nucleic acid extraction and evaluated whether it was useful as an on-site diagnostic method using clinical samples.

Materials and Methods: A set of six primers, including two outer primers (F3 and B3), two inner primers (FIP and BIP), and two loop primers (LF and LB), were adopted from our previous report [2] that can visually detect all seven serotypes of FMDVs. To allow more sensitive and rapid detection, a real time monitoring method using SYBR Green dye and Genie Ⅱ (Optigene, England) portable amplification equipment was additionally applied in the dRT-LAMP assay. For selecting on-site nucleic acid extraction, several simple nucleic acid extraction methods were tested and optimized for dRT-LAMP using virus-spiking samples (serum, OP fluid and epithelium) and the results were compared with those by a commercial nucleic acid extraction kit. Clinical evaluation of the dRT-LAMP was carried out with different serotypes of FMDVs and clinical samples and the results were compared with those of OIE-recommended RT-PCR or qRT-PCR.

Results: Among the direct nucleic acid extraction methods, a nuclease-free water (NFW) dilution method was confirmed to most suitable for the dRT-LAMP. The results of dRT-LAMP assay could be monitored visually by the naked eyes and real-time by Genie Ⅱ. The sensitivities of visual and real-time dRT-LAMP assays were equivalent to those of the qRT-PCR when using a nucleic acid extracted by commercial nucleic acid extraction kit as a template. On the other hand, when NFW-treated samples were used as a template, the sensitivities of visual and real-time dRT-LAMP assays (10^2 ~10^3 $TCID_{50}$/mL) were higher than those of qRT-PCR (10^3 ~10^4 $TCID_{50}$/mL). When the nucleic acids extracted by commercial kit from 37 FMDV-positive clinical samples were used as templates, the detection rates of all three methods were equal to 100%, but when NFW diluted samples were used as templates, the detection rates of visual and real-time dRT-LAMP assays or qRT-PCR were 89.2% (33/37) and 97.2% (36/37) or 70.3% (26/37), respectively (Table 1). These results indicate that the clinical sensitivities of developed dRT-LAMP assays are not significantly affected by using the crude nucleic acids as a template, unlike the qRT-PCR assay.

Table 1. Detection rates of FMDV RNAs from clinical samples by qRT-PCR, visual and real-time RT-LAMP

Nucleic acid extraction method	Detection rate (%) (No. of positive / tested)		
	qRT-PCR	Visual RT-LAMP	Real-time RT-LAMP
Commercial kit	100% (37/37)	100% (37/37)	100% (37/37)
NFW dilution method	70.3% (26/37)	89.2% (33/37)	97.2% (36/37)

Conclusions: The developed visual and real-time dRT-LAMP combined with NFW dilution extraction method (without nucleic acid extraction) and portable amplification equipment (Genie Ⅱ) proved to be useful as an on-site diagnostic method overcoming the obstacle of the nucleic acid extraction process. It is expected to be a valuable role in controlling FMD by enabling prompt diagnosis of FMDV at the site of FMD outbreaks.

References:

[1] OIE, 2016. Foot and Mouth Disease, OIE Terrestrial Mannual, pp. 1-31 (Chapter2.1.8).
[2] Lim et al., 2018, Korean J. Vet. Serv. 41, 29-39.

Trans-002

A Lateral Flow Assay for testing Nonstructural Protein Content in Foot-and-mouth disease Vaccine

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Introduction: According to OIE standard, foot-and-mouth disease (FMD) vaccine purity is currently evaluated through animal experiments for the detection of antibody against non-structural protein (NSP) in cattle after repeated immunization with the final vaccine product [1]. In case of failure, the manufacturing company should suffer severe economic loss. To prevent this unfortunate case in final vaccine product, a novel *in vitro* test is required to measure the amount of NSP remaining in vaccine antigen during the manufacturing process in advance. Thus, we developed a novel lateral flow assay device (LFD) to quantify NSP easily during FMD vaccine manufacturing process.

Materials and Methods: To determine the minimal amount of NSP to elicit antibody in livestock, goats were immunized repeatedly with various amounts of recombinant 3AB (rec.3AB; 2.6, 10.6, 42.5 or 170ng/doses) that was expressed in *E.coli* or FMD virus (FMDV) culture supernatants diluted serially by ten-fold. The detection of the immune response against NSP was assessed by commercial FMDV NSP ELISA. The LFD was constructed with the selected MAb that was conjugated with gold particles and was immobilized as a test line on cellulose membrane. NSP including 3B epitope present in a sample binds to the gold particles and results in a colored band (Fig. 1).

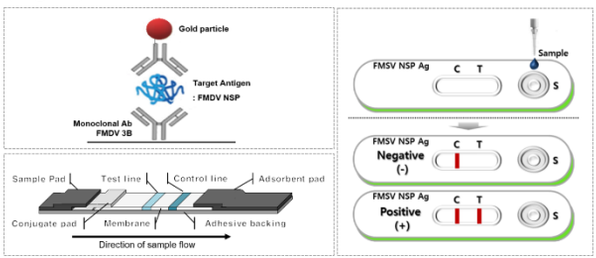
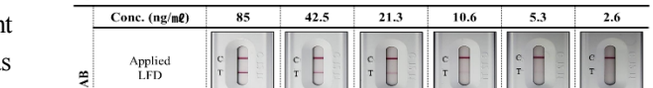
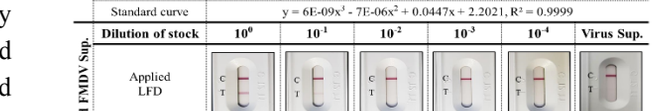


Figure 1. Construction of LFD

Results: Antibodies against 3AB were elicited after a second immunization with 10.6 ng to 42.5 ng of rec.3AB and a third immunization with a 10-fold diluted FMDV culture supernatant in goats. Even after the third immunization, goats inoculated with 2.6 ng rec.3AB remained negative for NSP antibodies. Taken together, our data suggest that the present LFD could be effectively employed for testing the purity of FMD vaccines with a cut-off value of 2.6 ng/ml. The LFD detected the minimal amount of rec.3AB and native NSP in FMDV culture supernatant required to induce NSP antibodies in goats. To quantify the LFD results, we generated a standard curve using 2-fold serial dilutions of rec.3AB (85-2.6 ng/ml) and calculated the amount of 3AB in the virus culture supernatant using the LFD reader (Table 1.).

Table 1. Quantification of 3AB in the virus supernatant by the LFD reader

	Conc. (ng/㎖)	85	42.5	21.3	10.6	5.3	2.6
Rec.3AB	Applied LFD						
	NSP Ab response in goats	N.A	post 2nd I.M	N.A	post 2nd I.M	N.A	None
	Peak value	1674.16	932.912	435.509	199.24	82.016	0
	Standard curve	$y = 6E\text{-}09x^4 - 7E\text{-}06x^2 + 0.0447x + 2.2021, R^2 = 0.9999$					
	Dilution of stock	**10^0**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**Virus Sup.**
Inactivated FMDV Sup.	Applied LFD						
	Peak value (n=3)	996.0±40.27	297.1±5.52	0	0	0	0
	3AB (ng/㎖)	45.73±1.95	15.03±0.24	N.D	N.D	N.D	N.D
	NSP Ab response in goats	post 2nd I.M	post 3rd I.M	None	None	N.A	N.A

Conclusions: The LFD enables simple, low-cost testing, provides rapid results, and should facilitate FMD vaccine evaluation by vaccine manufacturing companies before conducting animal experiments to test the vaccine purity.

Acknowledgement: This research was supported by the grant (B-1543386-2018-19-01) from the Animal and Plant Quarantine Agency, Republic of Korea.

References:

[1] OIE Terrestrial Manual. 2018. Foot and mouth disease.

Trans-003

A real time reverse transcription loop-mediated isothermal amplification assay with a target-specific fluorescent probe for specific detection of foot-and-mouth disease viruses

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious transboundary disease of domestic cloven-hoofed animals. Rapid and accurate diagnosis of FMD virus (FMDV) is a prerequisite for successful control of FMD. Currently, several reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR (qRT-PCR) assays have been developed to detect FMDV [1, 2]. Recently, several reverse transcription loop-mediated isothermal amplification (RT-LAMP) assays have been developed for the detection of FMDV as a valuable diagnostic tool with high sensitivity, specificity, rapidity, and simplicity. In this study, we developed a more advanced real time RT-LAMP (rRT-LAMP) assay with higher sensitivity, rapidity and reliability by using target gene-specific assimilating probe for rapid and real time detection of seven serotypes of FMDV.

Materials and Methods: For conventional RT-LAMP assay, a set of six primers, including two outer primers (F3 and B3), two inner primers (FIP and BIP), and two loop primers (LF and LB), were adopted from our previous report [3] that can detect all seven serotypes of FMDVs. Furthermore, to allow specific and real time monitoring of the assay results, an assimilating probe (LF probe) were designed against the forward loop regions of the amplicon. This fluorescent strand, labelled with FAM (6-carboxyfluorescein) at the 5' end, designed to be quenched by the same quench strand labelled with BHQ1 (black hole quencher-1) at the 3' end. A BLAST search was performed to check the specificity of primers. The reaction condition of rRT-LAMP was optimized and evaluated the specificity and sensitivity of the assay. Clinical evaluation of rRT-LAMP was carried out with different serotypes of FMDVs and compared with those of the RT-PCR and qRT-PCR each other.

Results: The rRT-LAMP could be completed in 30 min at 62°C, and the results could be detected in real time. The assay specifically amplified all 7 serotypes of FMDV RNAs but not amplified other viral and cellular nucleic acids. The limit of detection (LOD) of the rRT-LAMP was 102 RNA copies/μL of FMDV (O/Andong/KOR/2010), which was comparable to the previously reported qRT-PCR. Clinical evaluation of the rRT-LAMP using different serotypes of Korean and foreign FMDV strains showed that the results of the RT-LAMP was a 100% (51/51) agree with the results of the RT-PCR and qRT-PCR.

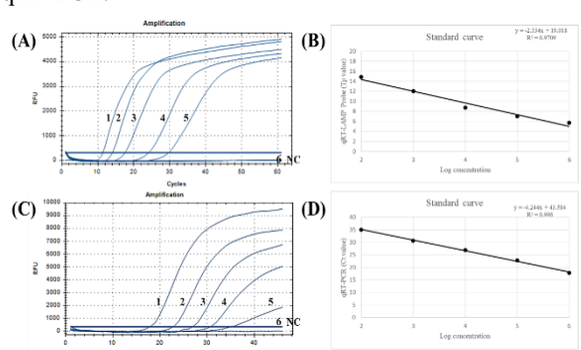

Fig. 1. Comparison of sensitivity of the rRT-LAMP and qRT-PCR assays. Detection limit of the rRT-LAMP (A and B) and qRT-PCR (C and D) for the detection of 3D gene of FMDV. Tubes and lines 1-6; 10-fold serial dilutions (from 10^6 to 10^1 copies/μL) of FMDV (O/Andong/KOR/2010); NC, negative control.

Conclusions: The newly developed rRT-LAMP assay using assimilating probe can rapidly and accurately diagnose all serotypes of FMDV. The results can be observed directly in real time, and it will be an alternative diagnostic tool for conventional RT-PCR and qRT-PCR.

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[1] Reid et al., 2000, J. Virol. Methods 89, 167-176.
[2] Callahan et al., 2002, J. Am. Vet. Med. Assoc. 220, 1636-1642.
[3] Lim et al., 2018, Korean J. Vet. Serv. 41, 29-39.

Trans-004

A serological surveillance of Seneca Valley virus in pigs in the Republic of Korea

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Introduction: Seneca Valley virus (SVV), which causes porcine idiopathic vesicular disease, has been reported in the United States of America (USA) and Canada [1]. Since 2015, it has also been identified in Canada, Brazil, USA, China and Thailand [2-4]. Because Korea has imported breeding pigs from USA and Canada, it cannot rule out the possibility of introduction of SVV through the pigs imported to Korea from these countries. However, there are no surveillance for SVV infection in South Korea. Therefore, we conducted a serological surveillance for SVV infections in pigs imported from USA or Canada during the period from 2015 to 2017 and cohabited pigs with imported ones.

Materials and Methods: The sera samples (n = 420) from 113 imported and 307 cohabited pigs were collected in 2018. For antibody test, the sera samples were tested by Enzyme Linked Immunosorbent Assay (ELISA) using RecombivirusTM Anti-Seneca Valley Virus IgG ELISA kit (Alpha Diagnostic Int., USA). ELISA-Positive samples were confirmed by virus neutralization test (VNT). The neutralizing activity of sera was determined by end-point dilution assay. The test sera were heat-inactivated at 56°C for 30 min and then two-fold serially diluted in the DMEM medium (25 μl/well). Each dilution was repeated in duplicate. An equal volume of 100 median tissue culture infective dose ($TCID_{50}$) SVV was added to each well of 96-well tissue culture microtiter plates (25 μl/well), with the exception of the wells for serum toxicity testing. The plates were incubated for 1 hr at 37°C. Then 100 μl of NCI-H1299 cells (2.3×10^4 cells) in DMEM medium were added to each well, and the plates were incubated at 37°C in a 5% CO_2 incubator [5]. The CPE was scored after 72 hr. The VNT titer was determined as the last dilution where CPE was inhibited (90-100% inhibition). A back titration of the challenge virus as well as positive and negative serum controls were employed to assess test performance. A titer of ≥ 1: 64 of the final dilution was regarded as positive.

Results: All tested sera were negative, suggesting that antibodies capable of neutralizing SVV in pigs is not present (Table 1).

Table 1. The serological surveillance results of imported and cohabited pigs for SVV in ROK

Area	Number of samples			Result
	Imported pigs	Cohabited pigs	Total	
Gyeonggi	3	30	33	Negative
Gangwon	-	40	40	-
Chungbuk	-	20	20	-
Chungnam	87	107	194	-
Jeonbuk	5	40	45	-
Jeonnam	-	10	10	-
Gyeongbuk	18	40	58	-
Gyeongnam	-	20	20	-
Subtotal	113	307	420	-

Conclusions: The results of this study could support that there has not been any infection by SVV in pigs in the Republic of Korea. Although no SVV has been reported in Korea, continuous monitoring of SVV is needed considering the occurrence of SVV in neighboring countries and the possible risk of introduction of SVV into Korea from pigs imported from the countries affected by the disease.

Acknowledgement: This work was supported by a grant (No. N-1543085-2018-18-01) from the Animal and Plant Quarantine Agency, the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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Trans-005

Antibody positive rates following national emergency vaccination in pig farm in 2019

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Introduction: Foot and Mouth disease virus (FMDV) is a highly transmissible virus that affects cloven-hoofed love stocks. Vaccination is being used to reduce the incidence of clinical FMD and eliminate the circulation of FMDV. National emergency vaccination against cattle and pigs was carried out following outbreak of FMD in 2019. One month later, we evaluated the immune response in pig for post-vaccination monitoring.

Materials and Methods: Nationally, a total of 1,051 farm (finisher 13,416, sow 2,385 pigs) were selected to monitor vaccine immunity in one month after national emergency vaccination following FMD outbreak in Anseong, Gyeonggi province, 2019. Selection of monitoring farm was based on the previous data including regions showing low antibody rate, densely populated and experiencing FMD area. Blood was collected 10 to 16 heads for finisher pig and 3 heads for sow per farm and subjected to Priocheck SPO ELISA (Thermo, USA) and NSP ELISA (Bionote and Median). Antibody positive rate was calculated by No. of positive/No. of tested pigs every farm. The difference of mean antibody rate of each province was compared with student t test comparing with those of ordinary monitoring data in 2018. $P < 0.05$ and $P > 0.95$ was determined as significant.

Results: Antibody positive rates following emergency vaccination were shown in Table 1. Mean positive rate of the farm tested was 81.1% and 94.1% in finisher pig and sow, respectively. The positive rate between provinces showed from 65.7% to 92.5% in finisher pig and from 89.8% to 100%. The significant increase of Jeonbuk and JeJu province was identified in finisher pigs rather than antibody rate in 2018. Mean antibody rate of sow from Chungbuk province was significantly low. No NSP positive pig was detected in this study.

Conclusions: Emergency vaccination following FMD outbreak increased overall antibody positive rate in the pig farm. However, one province (sow) was significantly lower than ordinary monitoring data

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Table 1.Comparison between antibody positive ratio of emergency vaccination and monitoring in 2018

	Ordinary monitoring(2018)				Emergency vaccination monitoring					
	Finisher		Sow		Finisher			Sow		
Province	No. of tested	Positive rate (%)	No. of tested	Positive rate (%)	No. of tested	Positive rate (%)	Cumulative distribution	No. of tested	Positive rate (%)	Cumulative distribution
GG	45,482	73.9	9,496	90.6	3,339	65.7	0.138	480	92.9	0.715
GW	10,924	81.0	2,608	94.9	819	90.8	0.914	172	100	0.897
CB	17,272	85.3	3,170	96.4	571	85.1	0.488	147	89.8	0.008
CN	50,845	82.0	10,561	94.0	2,121	83	0.551	492	93.5	0.415
JB	26,715	77.7	6,228	94.7	2,015	92.5	0.997	197	95.9	0.712
JN	24,269	76.7	6,372	91.1	915	85.6	0.831	239	95.4	0.838
GB	34,090	80.5	3,954	92.5	825	83.8	0.782	138	93.5	0.602
GN	27,072	80.7	6,329	91.5	1,832	84	0.689	357	94.4	0.795
JJ	11,545	61.9	3,014	84.2	864	86.6	0.982	148	91.9	0.899
SJ	1,816	74.2	128	100	115	69.6	0.407	15	100	0.5
Total	250,030	78.3	51,860	92.1	13,416	81.1	0.63	2,385	94.1	0.61

GG: Gyeonggi, GW: Gangwon, CB: Chungbuk, CN: Chungnam, JB: Jeonbuk, JN: Jeonnam, GB:Gyeongbuk, GN: Gyeongnam, JJ: Jeju, SJ: Sejong

Trans-006

Characterization of attenuated live classical swine fever vaccine (lom strain) within free region

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Introduction: Classical swine fever virus (CSFV) is a member of the genus *Pestivirus* of the family *Flaviviridae*. Classical swine fever (CSF) is a highly contagious, multisystemic, hemorrhagic, viral disease of pigs that may be manifested as an acute, subacute, chronic, or late-onset disease [1]. Modified live CSF vaccines are inexpensive and may induce complete protection against virulent CSFV, and thus are widely used in CSF endemic areas such as Asia, and Central and South America [2-4]. Since 1974, attenuated live vaccine (LOM strain) has been used to control CSF in South Korea. In December 2001, the Korean government declared the country to be CSF-free and CSFV vaccination was banned. In 2003, CSF outbreaks were reported throughout Korea, prompting a nationwide CSF vaccination campaign. Since then, anecdotal evidence from pig farmers and veterinarians has suggested that the LOM vaccine strain may cause serious side effects such as abortion, stillbirth, and reproductive failure in vaccinated pregnant sows. The OIE emphasizes the safety of attenuated live CSF vaccines against horizontal transmission of pig to pig and vertical transmission of pregnant sows. When considering the use of vaccines in CSF free region or country, the safety of these attenuated live vaccines should be considered carefully. We analyzed pigs infected by attenuated live CSF vaccine in free region (Jeju Island) that have not been vaccinated against CSF for 20 years.

Materials and Methods: We used samples from 2004 to 2018 in Jeju region, which is causing damage to pigs by attenuated live CSF vaccine (LOM strain). It sequenced the full genome of 21 CSF vaccine strains isolated from pigs for 14 years. In addition, commercially available CSF vaccines (LOM strain) and an original master seed strain (LOM-850) also sequenced the full genomes. We performed a histopathologic examination of pigs exposed with CSF vaccine strain for 5 years (2014-2018) within this region and also examined serum neutralization titres against 9 pig farms infected with CSF vaccine strains. To investigate epidemic transmission of CSF vaccine strain, vehicle inspection was conducted between a slaughterhouse and pig farms. Pigs cohabitation experiment were also conducted to confirm the horizontal transmission of CSF vaccine strain among pigs.

Results: The full genome mutation rate between the commercial attenuated live CSF vaccines in the market and the CSF vaccines (2014-2018) occurring in Jeju Island was not much different with the previous occurring CSF vaccines (2004-2007) in this region. Histopathological examination of suspicious organ samples of suckling piglets exposed with CSF should that most of them were co-infected with other pathogens such as PRRSV, PCV, Mycoplasma etc. Single infection with CSF vaccine strain were detected total 14 in suckling piglets (3 for 2016, 6 for 2017, and 5 for 2018). In the weaned piglets, single infection of CSF vaccine was not observed but all of them were co-infected. Immunochemical staining (ICS) examination of 8 fetuses showed positive in kidney of only one fetus and in the case of single infection (CSF vaccine) in suckling piglets were mainly found in tonsil, lymph node, spleen and lung. Serological neutralizing titer showed high antibody rates in the whole age groups from 9 pig farms. In the slaughterhouse vehicle test, 64.2% were found to be contaminated with CSF vaccine strains. In addition, it was confirmed that CSF vaccine strain may be possible of horizontal transmission among pigs.

Conclusions: In the future, the use of attenuated live CSF vaccine (LOM) should be considered carefully when reused in CSF free region, zone and countries.

Acknowledgement: This study was supported by a grant (Project Code No. B 1543083-2017-18-02) from the Animal and Plant Quarantine Agency (APQA), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea (2017).

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Trans-007

Classical swine fever virus in korean wild boar: 2010-2019

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Introduction: Classical swine fever (CSF), one of the most devastating diseases affecting the South Korean domestic pork industry, was first reported in 1947, with occasional outbreak occurring from domestic pigs until 2016. The major approaches used to eradicate CSF in domestic pigs in South Korea include the continuous programs; stamping out policies and strict quarantine measures during disease outbreaks. However, there is increasing concern that wild boars may act as an important reservoir for CSF, which may then spill over in the domestic pig population. Surveillance and monitoring of CSF in the wild boar population in South Korea are essential to achieve a CSF disease free status according to the Terrestrial Animal Health Code of the Office International des Epizooties (OIE). In this study, the prevalence of CSFV-specific antibodies and antigens in wild boar was examined from 2010 to 2019 using samples collected by the national surveillance program.

Materials and Methods: Wild boars were hunted in cooperation with the Korean Pork Producers Association and the Korean government from 2010. Blood samples were collected from 13,622 wild boars in eight provinces between November 2010 and April 2019. Serum were examined for antibodies against CSF E2 by using commercial ELISA Kit and Serum neutralizing test was performed according to protocols described in the OIE *manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (7th Edition, 2012). Whole blood samples were examined by reverse transcriptase PCR (RT-PCR) for the 5'UTR and E2 region of CSFV. The RT-PCR products were purified and then ligated into pGEM T-vectors. The plasmids were extracted, and the inserts were sequenced using an ABI Prism 3730xi DNA sequencer. Multiple sequence alignment and phylogenetic tree were carried out using BioEdit Sequence Alignment Editor Version 7.2.5 and Mega 6 program. Bootstrap values were calculated on 1,000 replicates of the alignments to access the confidence limits of the branching.

Results: CSF-antibodies were identified in 1.62% (220/13,622) of the wild boar samples using a virus neutralization test. The incidence of positive antibody to CSFV has increased since 2017; 20 in 2017, 47 in 2018 and 101 in 2019. Most the positive samples were from wild boars in the area close to the border with North Korea. The CSFV antigen detection rate was 0.14% (19/13,622) and 19 CSFV antigens were identified from wild boars captured in a nearby border area. A neighbor-joining tree of nucleotide sequences of E2 gene derived from 19 CSFV isolated in wild boars is confirmed as Subgroup 2.1d.

Conclusions: This study showed the circulation of CSFV in wild boar and the evidence of hidden infections in pig farms in South Korea. An epidemiological surveillance network should be established to continuously monitor the status of CSF in wild boar in the regions bordering North and South Korea, for CSF eradication in South Korea. Wild boar infected with CSFV can be source of transmission to domestic pigs. This should be taken into account when establishing CSF intensive monitoring program and wild boar vaccination.

Acknowledgement: This study was supported by a grant from the Animal and Plant Quarantine Agency (Project Code No. B-1543083-2019-21), Ministry of Agriculture, Food and Rural Affairs, South Korea.

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Trans-008

Combined DNA aptamer magnetic capture-RT-qPCR assay for detection of FMDV in oral fluid in pigs

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease of domestic cloven-hoofed animals as well as many wild species. It can cause significant economic losses due to reduced animal productivity and restrictions on international trade of susceptible animals and animal. Rapid and accurate diagnosis of FMDV is essential for surveillance and outbreak management. Currently, immunomagnetic separation (IMS) is the most commonly used method for capturing and concentrating pathogens from complex sample matrices [1]. Nucleic acid aptamers, which are single stranded oligonucleotides that naturally fold into three-dimensional structures demonstrating target-specific binding affinity, have been proposed as alternatives to antibodies. The purpose of this study was to develop a method to detect FMDV using a magnetic bead-bound DNA aptamer for target capture (aptamer magnetic capture or AMC) combined with quantitative real-time PCR (qPCR) for detection.

Materials and Methods:
FMDV vaccine were serially diluted and suspended in 10 ml pig oral fluid. qRT-PCR assays were performed according to previously described methods [2]. Capture efficiency was estimated according to the method previously reported by Joshi et al. (2009). Briefly, a standard curve was constructed using 10-fold serial dilutions of an overnight culture of PRRSV that were extracted for RNA isolation and subjected to qPCR. The data were plotted as log10 TCID (X axis) vs. Ct value (Y axis). The approximate TCID recovered after ligand-mediated magnetic pull-down was estimated from the standard curve based on resulting Ct values obtained from qPCR after capture. The percent capture efficiency (% CE) was calculated as the ratio of the estimated TCID (after capture and detection by qPCR) to the total input TCID per sample, multiplied by 100.

Results: The lower limit of detection of the combined AMC-qPCR was 1.0 $\log_{10}$ $TCID_{50}$/10 ml sample. Over this inoculum range, the capture efficiency of the combined pre-concentration-AMC-RT-qPCR assay ranged from 2.5-36%) (Fig. 1). Capture efficiency was significantly higher ($p<0.05$) than the negative controls which consisted of blocked beads in the absence of aptamer. Capture efficiency increased with decreasing virus concentration.

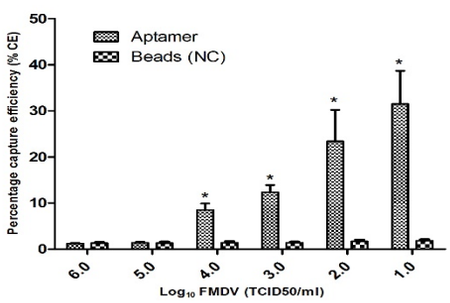

Fig. 1. Percent capture efficiency (%CE) of aptamer-conjugated magnetic beads as applied to serially diluted FMDV vaccine suspended in 10 ml pig oral fluid. Results are expressed as mean (n=3) ± S.D with Duncan's multiple range test used to determine statistical significance ($p < 0.01$) when comparing aptamer and control beads. The asterisk indicates statistically significant differences when comparing treatments at any one initial FMDV inoculum level.

Conclusions: Nucleic acid aptamers are promising alternatives to antibodies for magnetic bead-based capture followed by qPCR detection. Aptamers performed well in these assays and are hence promising alternatives to antibodies for use in magnetic separation approaches.

Acknowledgement: This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Animal Disease Management Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (116097-3)

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Trans-009

Comparative evaluation of Current Serological test of Foot and Mouth Disease Vaccine Immunity in Pigs

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Introduction: South Korea initiated vaccination based FMD control program in 2011. Since then cattle, goats and pigs have been systematically vaccinated, and mass sero-surveillance has been routinely carried out for estimation of herd immunity against FMDV. Commercial FMDV type-O antibody ELISA (PrioCHECK SP-O) kit has been used as a screening method for ensuring vaccine coverage. However, there can limit the guarantee for the assessment of national vaccination programs, because gold standard method, virus neutralization test (VNT), can be subjected to suit to measure protective antibody responses induced by vaccination. Also, it is important to evaluate whether screening PrioCHECK SP-O kit can detect FMD-O strains of different origin because various vaccines containing several vaccine strains are commercially available in Korea.
The objective of this experiment is to prove that PrioCHECK SP-O kit is fit for purpose to detect the antibody response introduced by the vaccination with ARRIAH FMD vaccine in pigs. Also, we investigated whether PrioCHECK SP-O ELISA is appropriate for determining to predict protective antibody level in herd comparing SP antibody responses obtained by VNT and SPCE.

Materials and Methods: In total 48 vaccinated pigs (8-10 weeks age) were divided into 2 groups of different vaccine strain. All pigs were vaccinated twice either FMD bivalent vaccine (n=30) containing Type O-Primorsky + A-Zabaikalsky(ARRIAH) or Monovalent (n=18) containing Type O-Primorsky. 194 serum samples were collected on 6 time points in a period of 10 weeks. PrioCHECK SP-O kit was used to measure the immune response after exposure to a vaccine at different time point. Also, VNT was performed in order to determine neutralization antibody (NA) titer against vaccine strains (homologous serotype) and heterologous serotype (FMDV/O/AS/2019, FMDV/A/GP/2018). The results obtained by these methods were compared with respect to correlation with each other and sensitivity at individual sampling point.

Results: Overall, PrioCHECK SP-O kits identified less positive samples than did the VNT against O-Primorskiy strain. On the other hand, the results of seropositivity tested by PrioCHECK SP-O kits yielded better agreement with those of VNT against vaccine strain following revaccination, showing significant correlation(ρ =0.42) between raw data by PrioCHECK SP-O kit and VNT. However, the correlation between NA-titers against hetero-strains (FMDV/O/AS/2019) and percentage inhibition (PI) value of PrioCHECK SP-O kits was fairly low. The pattern of the NA titer was similar in all serotypes, despite the lower NA titer against FMDV/O/AS/2019.

Conclusions: The better agreement of sensitivity between PrioCHECK SP-O ELISA and VNT against vaccine-strain results was produced by the booster vaccination, demonstrating that PrioCHECK SP-O kit can successfully detect the vaccine-induced antibody response after exposure of revaccination. These results showed that current PrioCHECK SP-O kit may reflect true vaccine coverage for ARRIAH in case of giving two-doses one month apart recommended when pigs are vaccinated against FMD.
Nevertheless, bivalent vaccination (O+A) in pigs is mandatory, vaccine coverage is routinely assessed by PrioCHECK SP-O kit. Therefore farmers are very doubtable how to determine the level of vaccine-induced antibodies against FMDV type-A if vaccine immunity is estimated only by serotype-O ELISA kit. Therefore, we observed the relationship of NA titer between serotypes (A, O) in case bivalent vaccine is applied. Similar NA titer patterns were observed between serotype A and O. Consequently screening of antibody level restricted to serotype-O was likely to be appropriate.
Considering that correlation with VNT results was substantially low, SP ELISA test was not able to predict cross-protection. Therefore, establishing validated and reproducible tests for serological assessment of national vaccination campaign requires considerable effort, particularly when a variety of vaccine and field strains are involved.

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Trans-010

Comparative genetic analysis of the recent foot-and-mouth disease O/ME-SA/PanAsia genotype from the South East Asia countries

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Introduction: Foot-and-Mouth disease (FMD) is a highly contagious viral disease in the cloven-hoofed animals such as pig and cattle. FMDV has been being circulating in the most of Southeast Asia countries including Vietnam, Lao, and Cambodia. O/ME-SA/PanAsia genotype was one of the indigenous genotypes in the South East Asia. In Korea, O/ME-SA/PanAsia genotype had been reported in 2000 and 2002. To collect and analyze the genetic information from South East Asia is very important to prepare FMD preventive measures. In this study, FMDV O/ME-SA/PanAsia genotype were collected from 2016 to 2018 in Vietnam (n=7), Laos (n=6), and Cambodia(n= 15) collected through the international collaborative research projects. The results were compared with the previous O/ME-SA/PanAsia from Korea(2000 and 2002) and available data of VP1 sequence in NCBI.

Materials and Methods: Viral RNAs were extracted from the clinical epithelium samples using a MagnaPure96 system (Roche). The VP1 regions were amplified using a one-step RT-PCR kit (Qiagen). PCR products were purified with ExoSAP-IT (USB) and directly sequenced on an ABI 3130 genetic analyzer (Applied Biosystems) using a Big Dye Terminator Kit v3.1 (Applied Biosystems). Phylogenetic tree estimated using the Maximum Likelihood method based on the Kimura-2 parameter model by MEGA6. Numbers at node indicate the confidence level in bootstrap analysis with 1,000 replications.

Results: Phylogenetic analysis based on the VP1 gene showed that FMDV from Vietnam clustered into three different genetic clusters within the O/ME-SA/PanAsia (Fig 1). All recent FMDV from Vietnam, Lao, Cambodia were quite different with the Korean FMDV(2000 and 2002). Only Vietnam FMDV were found within Group 1, 2 and 3. But, FMDV from Vietnam(2017), LAO(2017~2018) and Cambodia(2018) were clustered within the Group 4.

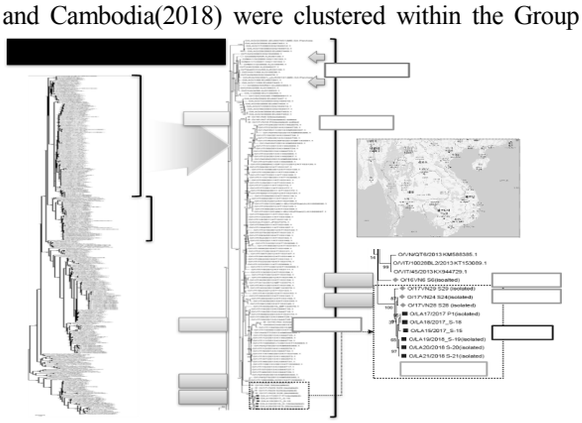

Figure 1. Phylogenetic analysis of FMDV O/ME-SA/PanAsia from Vietnam, LAO, and Cambodia

Conclusions: O/ME-SA/PanAsia genotype was one of the indigenous genotypes in the South East Asia, and also occurred in Korea in 2000 to 2002. Our finding confirmed that at least four genetically different O/ME-SA/PanAsia genotypes have been found in Vietnam. And also, one FMDV within Group 4 have been circulating in Vietnam, Lao and also Cambodia. Comparative genetic analysis of FMDV isolates circulating in the South East Asia countries could give valuable information for predicting the FMD risk in Korea and also Asia region.

Acknowledgement: This research was supported by the Research of Animal and Plant Quarantine Agency (Project No.I-1543084-2015-24-01), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Trans-011

Comparative genetic analysis of the recent foot-and-mouth disease virus O/SEA/Mya-98 in Vietnam, LAO, and South Korea

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Introduction: Foot-and-Mouth disease (FMD) is a highly contagious vesicular disease in the cloven-hoofed animals, such as swine and cattle. FMD has been occurred a total of 11th times in Korea, introduced from neighboring countries with unknown sources, since 2000. FMDV has been prevalent in the most of Southeast Asia countries including Vietnam and Lao. O/SEA/Mya-98 genotype was one of the indigenous genotypes, showing a high incidence rate in South East Asia. In Korea, O/SEA/Mya-98 genotype had been reported from 2010 to 2016. It is very important to collect and analyze their genetic information from South East Asia to predict the potential risk. In this study, FMDV O/SEA/Mya-98 genotype were collected from 2014 and 2017 in Vietnam (n=23) and Laos (n =5), collected through the international collaborative research projects. The results were compared with those of O/SEA/Mya-98 from Korea and publically available data of VP1 sequence.

Materials and Methods: Viral RNAs were extracted from the clinical epithelium samples using a MagnaPure96 system (Roche). The VP1 regions were amplified using a one-step RT-PCR kit (Qiagen). PCR products were purified with ExoSAP-IT (USB) and directly sequenced on an ABI 3130 genetic analyzer (Applied Biosystems) using a Big Dye Terminator Kit v3.1 (Applied Biosystems). Phylogenetic tree estimated using the Maximum-Likelihood method on the Kimura-2 parameter in MEGA6.

Results: Phylogenetic analysis based on the VP1 gene showed that FMDV from Vietnam and Lao belonged to three different genetic clusters within the O/SEA/Mya-98 (Fig 1). Only some FMDV from Vietnam (2014~2016) were found within Group 1. As of Group 2, FMDV from Vietnam(2016~2017) and LAO(2016~2017) were clustered within the same group. The other FMDV from Vietnam(2014~2015) were grouped with Korean FMDV(2014~2016) with high similarity at the Nucleotide level.

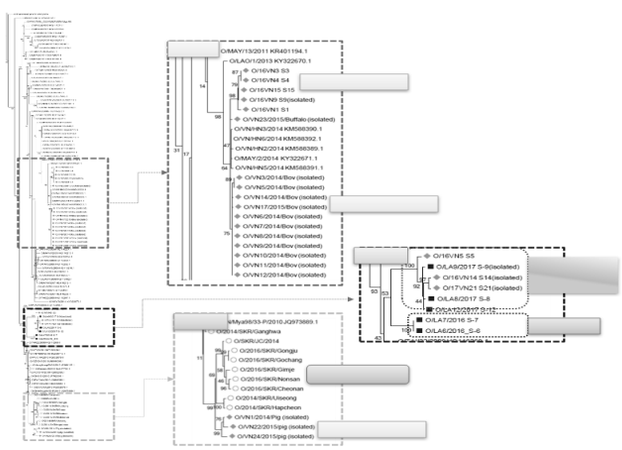

Figure 1. Phylogenetic analysis of FMDV O/SEA/Mya-98 from Vietnam, LAO, and South Korea.

Conclusions: O/SEA/Mya-98 genotype was one of the indigenous genotypes in the South East Asia, and also caused the massive economic loss in Korea from 2010 to 2016. Our finding confirmed that at least three genetically different O/SEA/Mya-98 genotypes, one closely related to the Korean FMDV in 2014, have been circulating in Vietnam. In LAO, FMDV closely related with the Group 2 from Vietnam has been circulating. Accumulation of the comparative genome analysis of FMDV isolates in South East Asia countries including Vietnam and Laos, could give valuable information about the genetic diversity and the antigenic relationship with FMDV in Korea.

Acknowledgement: This research was supported by the Research of Animal and Plant Quarantine Agency (Project No. I-1543082-2018-22-02), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Trans-012

Compare with quality control method between antigen and antibody of foot-and-mouth disease vaccine

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Introduction: Over the last decade, approximately 10 foot-and-mouth disease (FMD) outbreaks have occurred in South Korea. Vaccination is one of the most practical and effective measures to control or prevent these outbreaks. Recently, high-performance liquid chromatography (HPLC) measurement of FMD virus antigen has been reported to be simple, rapid and effective. In this study, we compared new quality control methods between antigen and antibody for FMD vaccines. HPLC enabled rapid and quantitative determination of 146S antigen content and is a promising testing method for national lot release in South Korea.

Materials and Methods: Several commercial FMD vaccine lots from other countries were used. During the initial evaluation of antigen content in vaccines, we used a monovalent vaccine. To measurement of vaccine antigen content by HPLC, pretreatment was done from final vaccine product. Each manufacturer provided separation methods to isolate antigen from the aqueous phase of oil emulsion vaccines after reagent treatment. Next, host cell DNA digestion was done and antigen content measured by HPLC. To measurement of vaccine antibody by virus neutralization tests (VNT), numerous pigs were vaccinated by several vaccines and antibody titers in the serum sample at three weeks post-vaccination are determined using VNT in biosafety level 3 facilities.

Results: We measured antigen content of each vaccine lots by HPLC. This measured values do not represent absolute 146S antigen content but the relative measurements produced by the HPLC in our laboratory. And we also measured antibody of each vaccine lots by VNT. We intend to evaluate correlation between the amount of FMD vaccine antigen in each monovalent vaccine strain and the results of VNT. The quantity of 146S antigen comparatively correlated with antibody value in each vaccine strains.

Conclusions: The vaccine quality can be accurately assessed by simply measuring antigen content of the FMD vaccine. The measurement of antigen content using HPLC may be an alternative way to improve quality control of FMD vaccines. This method also resolves animal welfare concerns by reducing animal experiments, and saves money and time in evaluation of vaccine potency.

Acknowledgement: This study was supported by grants from the Animal and Plant Quarantine Agency (I-1543073-2018-20-02).

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Trans-013

Comparison of Classical swine fever virus neutralizing antibody level between two classical swine fever vaccines in sows

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Introduction:

CSFV has been controlled effectively by vaccination with C-strain in China[1]. As an eradication tool, a baculovirus-expressed E2 subunit vaccine was licensed in 2018 with the ability to differentiate infected animals from vaccinated animals (DIVA). Recently, it has been demonstrated repeatedly that E2 can induce much higher and more uniformly E2 antibody than C strain in sows as a boost vaccine as shown by ELISA kit from IDEXX[2]. However, whether the difference shown by ELISA antibody also represents a similar difference in terms of the neutralizing antibody against a prevalent CSFV genotype 2.1b in the field is unknown. Therefore, this assay is designed to compare the NA titer induced by E2 vaccine and C-strain vaccine in sows

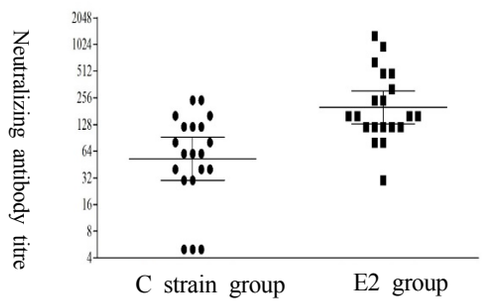
Materials and Methods:

Two hundred sows of mid-gestation stage were confirmed of CSFV free and allocated into two groups with 100 sows each. One group was vaccinated with a ST cell line adapted C strain once while the other group was vaccinated with an E2 vaccine from TECON once. All the sows were housed in the same building and subjected to the same management.Blood samples were collected from 20 sows randomly from each group at 4 weeks post vaccination for detection of NA against CSFV genotype 2.1b by Changchun Veterinary Research Institute of Chinese Academy of Agricultural Sciences following a standard protocol recommended by OIE[3].

Results:

The geometric average of NA titer in sows was 53, with the lowest being 5 and highest being 240 in the C strain vaccine boosted sows whereas the geometric average NA titer of sows was 199, with the lowest being 30 and highest being 1280 in the E2 vaccine boosted sows.

Fig1. NA against CSFV 2.1b in sows boosted with C strain or E2 vaccine



Conclusions:

E2 vaccine has the advantage of not being interfered by the presence of pre-existing antibody at the timing of vaccination and can induce much higher and more uniformly E2 NA titer than C strain in sows as a boost vaccine.

References

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[3] Anonymous,2007. EU Diagnostic Manual for Classical Swine Fever(CSF) Diagnosis: Technical Part (Third Draft June 2007).

Trans-014

Comparison the immune response induced by live attenuated classical swine fever (CSF) vaccine and CSF-E2 subunit vaccine in field farms

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Introduction: CSF is one of the most devastating swine diseases affecting pigs worldwide. Besides stamping out of infected herds, vaccination with live attenuated CSF vaccine (Lapinized Philippines Coronel, LPC vaccine) or CSF-E2 subunit vaccine can be utilized for disease control in endemically affected regions. However, the maternal derived antibody (MDA) may impact the efficacy of LPC vaccine. Therefore, an appropriate vaccination program is crucial for LPC vaccine to induce protective efficacy. In this study, the immune responses induced by LPC vaccine and CSF-E2 subunit vaccine in sows and piglets were monitored to illustrate the efficacy of two types of CSF vaccines in field farms.

Materials and Methods: Sixty sows were divided into two groups. The group A (n=25) sows received two shots of CSF-E2 subunit vaccine at 3 and 5 weeks before parturition, while group B (n=35) sows were immunized with the LPC vaccine before breeding. Serum samples were collected at 3 days after parturition to examine the induced immune response. Piglets from each group were randomly selected and divided into 3 groups with receiving CSF-E2, LPC and PBS vaccination, respectively. Serum samples were analyzed with IDEXX CSFV Ab test kit to monitor vaccine-induced immune response and results were expressed as blocking percentage. The blocking percentage greater than 40% is considered as positive. The decreasing of MDA was monitored to determine the vaccination time point of LPC vaccine on piglets. The dynamic of serum antibody positive ratio was calculated to compare the coverage vaccine-induced immune response in different MDA levels.

Results: The group A sows receiving CSF-E2 subunit vaccine induced significantly higher CSF-specific antibody level (88.75±2.54) than those of group B (71.66±24.28). Besides, lower coefficient of variance (CV) was noted in group A (2.86%) than group B (33.88%) indicating that immunization with CSF-E2 subunit vaccine could induce more consistent immune response in sows. Moreover, the MDA from group A piglets (62.68±5.88 at 8 weeks old) showed higher and longer lasting than piglets from group B (29.33±2.57 at 8 weeks old). The dynamic of CSF antibody positive percentage in piglets with different vaccination programs was summarized as Table 1.

Table 1. Dynamic of CSF antibody positive percentage after vaccination.

Vaccination		Weeks after vaccination	CSF antibody positive %
Sows	Piglets		
CSF-E2 (Group A, n=25)	CSF-E2 (n=20)	0	100.0
		4	90.0
		8	85.0
		12	95.0
	LPC (n=10)	-4	80.0
		0	80.0
		4	100.0
		8	100.0
	PBS (n=10)	0	90.0
		4	90.0
		8	80.0
		12	0.0
LPC (Group B, n=35)	CSF-E2 (n=10)	0	50.0
		4	100.0
		8	100.0
		12	100.0
	LPC (n=10)	-4	100.0
		0	90.0
		4	0.0
		8	70.0
	PBS (n=10)	0	50.0
		4	0.0
		8	0.0
		12	0.0

Conclusions: Both CSF-E2 subunit vaccine and LPC vaccine could induce good immune response in sows and piglets. However, CSF-E2 subunit vaccine induce higher and more consistent immune response in sows and piglets, even under the interference of high MDA titers. These results reveal the flexibility of CSF-E2 subunit vaccine in CSF prevention.

Trans-015

Concentration of foot-and-mouth disease virus (FMDV) by tangential-flow ultrafiltration to produce FMD vaccine antigen

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Introduction: Foot-and-mouth disease (FMD) vaccines are mostly made up of inactivated foot-and-mouth disease virus (FMDV), which induce sufficient neutralizing antibodies. Whereas precipitation by polyethylene glycol (PEG) is commonly used for concentration of inactivated virus supernatant to produce the FMD vaccine antigen, ultrafiltration is newly described as a major method for concentration in the World Animal Health Organization (OIE) manual. The type of tangential-flow filtration (TFF) has an advantage of preventing the fouling on the surface of membrane compared with normal-flow filtration (NFF). Therefore, TFF is suitable for continuous operation of high-concentration samples in the industrial facilities for vaccine production [1, 2].

Materials and Methods: BHK-21 suspension cells were maintained in a shaking incubator at 37℃ with 5% CO2 and growth medium were changed to inoculate the FMD virus. $6x10^6$ cells/ml of BHK-21 were inoculated with O/Jincheon/SKR/2014 virus at a multiplicity of infection of 0.001 for 16 hours. FMDV was inactivated with 3mM of binary ethylenimine at 26 ℃ for 28 hours. Tangential flow filtration (TFF) was performed using Labscale® TFF device with Biomax® 100K, 300K (Millipore) and Minimate™ 500K (PALL) membrane cassettes. The inactivated crude FMDV was added into the reservoir and concentrated 10 times. The quantity of 146S antigen in concentrate was estimated by the sucrose density gradient ultracentrifugation and optical density analysis by using the UA-6 absorbance detectors (Teledyne Isco, USA). The removal of protein impurities were confirmed by BCA method and Western blotting with anti-FMDV 3B antibodies.

Results: Optimal conditions for membrane materials, molecular weight cut-off (MWCO), feed pressures and flow rates, were explored to increase the recovery of 146S and decrease the impurities, especially FMDV non-structural proteins (NSPs). Polyethersulfone (PES) material was selected through testing of 146S recovery. The transmembrane pressure (TMP) was optimized at 10 psi. In the 500KDa of MWCO, NSPs were significantly removed, but antigen recovery was only 37%. The 300KDa of MWCO showed high antigen recovery, but NSPs were not removed.

Table 1. The concentration efficiency of FMDV supernatant using TFF system. The crude FMDV was concentrated with the membrane of 300KDa MWCO under different values of TMP. 146S antigen was quantified and protein concentration was measured. (a) 146S recovery is the ratio between retentate and feed loaded. (b) Protein removal is the ratio between filtrate and feed loaded. (c) TMP= $(Pressure_{Feed} + Pressure_{Retentate})/2 - Pressure_{Filtrate}$

Parameter	TMP (psi) [c]						
		1.8		5		10	
	Feed	Retentate	Filtrate	Retentate	Filtrate	Retentate	Filtrate
146S (μg/ml)	3.7	2.7	-	3.05	-	3.5	-
146S recovery (%) [a]	100	73	-	82.4	-	94.6	-
Total protein (mg)	5,480	-	3,258	-	2,682	-	3,499
Protein removal (%) [b]	100	-	59.45	-	48.94	-	63.85

Conclusions: Although ultrafiltration alone was not able to remove NSPs, optimal concentration parameters for improving the recovery of FMD vaccine antigen were established. Scale-up studies are being performed for application in vaccine manufacturing facilities. It would be useful for the downstream process of domestic FMD vaccine production.

Acknowledgement: This research was supported by the grant (B-1543386-2018-19-02) from the Animal and Plant Quarantine Agency, Republic of Korea.

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Trans-016

Continuous transmission of classical swine fever virus LOM with genetic evolution in Jeju island

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Introduction: Classical swine fever (CSF) is a highly contagious viral disease in pigs that severely affects the swine industry. As an effective control measure against CSFV, vaccination strategy based on live attenuated vaccines has been used successfully in many countries including Korea. However, recently, safety issues of a vaccine strain LOM in immunologically naïve pigs, which has been a sole vaccine strain in Korea for several decades, were raised by our research group [1]. Since 2014, when the LOM strain was introduced by accident into Jeju island, previously free of CSFV, the vaccine strain continues to circulate within and between farms without a downward trend (Table 1), causing health issues economic damage on farms. The aim of this study is to understand epidemiological situation of LOM infection in Jeju island and to investigate genetic alterations occurred over time.

Materials and Methods: Clinical specimens from commercial pig farms suffered from LOM outbreaks were collected during 2016-2018. The samples were pre-treated properly and tested for CSFV infection using RT-PCR technique. Positive samples were subjected to whole genome sequencing following the method as described in [2]. One representative sequence from each year was selected and aligned using BioEdit software and analyzed by comparison with a reference LOM sequence (GenBank accession no. EU789580).

Results: Through sequence comparison of polyprotein coding region with a reference LOM strain at nucleotide (nt) and amino acid (aa) level, it was identified that the 2018 isolate showed the highest genetic distance (98.4%/98.8%), while the lowest is observed in 2016 isolate (99.3%/99.3%) (Table 2). Among protein coding regions, capsid and Npro protein shared the relatively low similarity with the reference strain in 2017 isolate, while capsid and p7 protein in 2018 isolate (data not shown). Interestingly, despite the absence of multi T insertions observed in other isolates, 3' UTR of 2018 isolate showed genetic identity of 96.8% with a reference sequence.

Table 1. Summary of the number of farms infected with LOM strain in Jeju island from 2014 to 2018*

Farm type	Year				
	2014	2015	2016	2017	2018
New infection	20	22	26	over18	NA
Recurrent infection	0	3	13	NA	NA
Total	20	25	39	26	33 (late April)

*NA, not available

Table 2. Comparison of nucleotide and amino acid sequence identity between reference LOM strain and LOM variants from 2016 to 2018*.

Isolated year	% of nt/aa sequence identity with a reference LOM strain		
	5' UTR	Polyprotein	3' UTR
2016	99.7/-	99.3 / 99.3	98.2/-
2017	98.9/-	98.9 / 99.1	99.6/-
2018	98.4/-	98.4 / 98.8	96.8/-

*nt, nucleotide; aa, amino acid

Conclusion: In this study, it was revealed that genetic changes of CSFV LOM have been made under evolutionary process since its introduction in Jeju island. To prevent further genetic changes related to reversion to virulence, proper control measures should be adopted in the near future.

Acknowledgement: This work was supported by grant from the Department of Homeland Security Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) at Kansas State University, USA.

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Trans-017

Correlation of Serum Neutralization Test (SNT) and ELISA Tests as Diagnostic Tool for Classical Swine Fever (CSF)

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Introduction:

Classifcal Swine Fever (CSF) is an endemic disease in Malaysia, which can lead to immunotolerance, immunosuppression and subsequently huge mortality loses in pig farms. Control of the disease in Malaysia had mainly relied on vaccination using a live attenuated C-strain vaccine, as cell mediated immunity (CMI) plays a vital role in providing protection against CSF for sows and piglets. Serum Neutralization Test (SNT) is acknowledged globally as the gold standard serology test for CSF (Brown, 2018). Nevertheless, due to lack of access to SNT services, commercial Enzyme-Linked Immunosorbent Assay (ELISA) test kit remained as the main serology diagnostics for CSF. The objective of this trial is to investigate the correlation between the SNT and ELISA titer for vaccinated pigs.

Materials and Methods:

Serology samples from 7 farms across Peninsular Malaysia were collected as part of routine monitoring diagnostic tests. A total of 101 serum samples from the breeding herd and 168 serum samples from the porker herd were collected. The breeding herd is consisted of sow and gilts while the porker herd is consisted of pigs from 4 weeks old up to 20 weeks old. All the samples were equally split for ELISA test using IDEXX CSFV Ab Test in the Faculty of Veterinary Medicine, Universiti Putra Malaysia and then sent for SNT test in the Faculty of Veterinary Science, Chulalongkorn University in Thailand. Correlation test was then conducted, comparing the blocking % results from the ELISA test, and the SNT value from the SNT test. The Spearman correlation test was done using Minitab version 18.

Results:

Results are summarized in table 1.

Table 1: Spearman correlation coefficient for both samples

Serum samples ID	Number of samples	Spearman correlation, rho value
Sow	101	0.396
Porkers	168	0.329

The rho value for both groups of samples are lesser than 0.4, which is suggestive of a weak correlation. Thus, from this trial it is indicated that the CSF ELISA titers has a weak correlation with the CSF SNT titers. With that, SNT would still be the preferred serology diagnostic for CSF as compared to the ELISA in circumstances requiring an accurate diagnosis. Nevertheless, SNT is more laborious and costly than ELISA, as porcine cell line culture is required.

Using serology antibody titer as an evaluation tool of live attenuated CSF vaccine efficacy has its limitations as only the humoral immunity is assessed, whereas CMI is more vital in providing protection against this disease. It was also proven that complete clinical protection against CSF can be achieved as early as 5 days post vaccination with live attenuated C-strain CSF vaccine, despite significant increment in SNT titers were only detected about 10 days post vaccination (Graham, 2012).

Conclusions:

ELISA for CSF has a weak correlation with the CSF SNT test.

References:

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[2] Graham SP, et al., 2012. PLoS ONE 7(1): e29310

Trans-018

Epidemiological and genetic characteristics of swine pseudorabies virus in mainland China between 2017 and 2018

Ying Sun[1], *Wan Liang*[1,2], *Xueying Wang*[1], *Sisi Xie*[1], *Hechao Zhu*[1], *Qingyun Liu*[1], *Zhong Peng*[1], *Bin Wu*[*1]

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Introduction: Pseudorabies virus (PRV) mainly causes reproductive failure in sows as well as respiratory and neurological symptoms in piglets [1]. The first report of a PRV outbreak in China occurred in the 1950s. Due to the widespread use of Bartha-K61 vaccine, the outbreak of pseudorabies in China was controlled well between 1990 and 2011. However, since late 2011, a pseudorabies (PR)-like disease has occurred in many Chinese pig farms that had been vaccinating pigs with the Bartha-K61 vaccine. PRV has been finally confirmed to be responsible for those outbreaks, and a number of studies have noted that the Bartha-K61 vaccine appears to be unable to provide full protection against PRV strains isolated from those outbreaks [2]. These findings suggest that there may be important changes in the PRVs currently circulating in China. In this study, we report the detection/genetic analysis of PRVs recovered from pigs in China between 2017 and 2018.

Materials and Methods: A total of 6,867 samples including tissue from lungs, lymph nodes, brains, serums, stillbirths, kidneys, and spleens were collected from pigs of different ages with signs suspected of PRV infection in farms (no. of sows $\geq$100) in 29 Provinces in mainland China between 2017 and 2018. PCR assays for the detection of the presence of the clinical samples were designed by PRV gE gene. Mutations in gB, gC, and gE were analyzed by sequence comparisons. Phylogenetic analysis was performed according to gC gene.

Results: Of the 6,867 samples detected, 474 samples (6.90%) were positive for the detection of PRV-gE. Monthly, higher positivity rates of PRV were detected in May, April, March, January, February and June, while no positive samples were detected in December. When the seasonal rates were compared, it showed that spring (March, April, and May; 10.77%) and summer (June, July and August; 9.11%) were the seasons with the higher positivity rate of PRV detection during 2017 and 2018. The positive rates in autumn and winter were only 2.33% and 4.66%, respectively. Among the graphic regions, the positive rates of PRV detection in Northwest and Southeast of China were higher than 10.00%, while the positive rates in other parts of China all ranged between 6.35%~8.90%. According to the positive rate of each province, it showed that there were seven province negative for PRV, they were Heilongjiang (0/2), Tianjin (0/6), Shanxi (0/60), Inner Mongolia (0/19), Hainan (0/24), Yunnan (0/44), Qinghai (0/24) which were not all adjacent. Phylogenetic analysis based on gC showed that PRV strains prevalent in China belonged to genotype II which had a remarkably distinct evolutionary relationship with PRVs from other countries, most of the PRV strains circulating in our country.

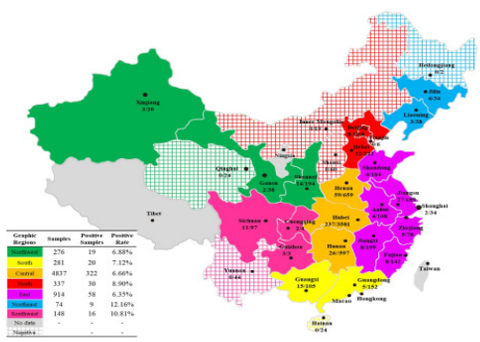

Figure 1. The positive rate of PRV detection in mainland China in 2017-2018

Conclusions: Our data revealed an average positive rate of 6.90% for PRV detection between 2017 and 2018. The evolutionary relationship between the PRV isolates circulating in China and those from the other countries were remarkably distinct.

Acknowledgement: This work was supported by the National Key R&D Program of China (Grant number: 2018YFD0500800), and the National Key Technology Support Program of China (Grant number: 2015BAD 12B04).

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Trans-019

Evaluation of disinfectants efficacy against FMD virus depends on temperature and contact time

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Introduction: Foot-and-mouth disease (FMD) caused by virus infection of a small non-enveloped ribonucleic acid (RNA) virus belongs to family Picornaviridae, genus Aphthovirus. FMD is an economically important disease because it is highly contagious and infects many cloven-hoofed animals, including cattle, pig, sheep, goat, deer, boar, etc. and once animals have been infected, culling or stamping out policies are implemented in most countries [1]. For environmental control of FMD viruses, disinfectants are used mainly for the decontamination of environmental surfaces in animal farm. Virucidal activity of disinfectants is tested either in suspension or carrier test. Since pathogens in nature are normally found adsorbed to surfaces and/or embedded in organic or cellular debris, the results of carrier tests are more relevant to predicting the activity of disinfectants under field situations [2]. In this study, we evaluated the changes in the efficacies of seven commercial disinfectants at different temperature and contact times against FMD virus using carrier-based test.

Materials and Methods: The FMD virus O/Jincheon/SKR/2014 strain and LFBK cell line were used. Viral stocks with a titer of $>10^7$ $TCID_{50}$ mL^{-1} were stored in aliquots at -70℃ until use. Seven disinfectants were selected from active ingredient such as acids, quaternary ammonium, oxidizing agents, and complex compounds. To evaluate the efficacy of the disinfectants against FMD virus, we used a modification of the ASTM (American Society for Testing and Materials) International E2197 standard quantitative disk carrier test method to determine the log reductions [3]. The infectivity of the virus before and after the treatment of disinfectants was measured by titration in the LFBK cells on the 96-well microtiter plate, where the virus-induced cytopathic effect(CPE) was checked for 72 h and a $TCID_{50}$ was determined.

Results: Since the dried virus on stainless steel was shown lower titer compared to checked in suspension method, virucial effects against FMDV in this study were judged as follows: great, 3.0 log_{10} reduction; moderate, 1.5-3.0 log10 reduction; little, <1.5 log_{10} reduction. Disinfectants efficacy against FMD virus were investigated at 2 different temperatures (0℃ and room temperature) and 2 contact time (10 and 20 min). Among the seven disinfectants, the three appeared to be little effect in the reduction of virus titer after treatment. And the temperatures (0℃ and room temperature) and contact times(10 and 20 min) seem to influence the results a little. Other four disinfectants showed moderate and great virucidal activity at 0℃ and room temperature. We found that disinfectant was more effective at inactivating FMD viruses when incubated with virus for 20 min than for 10 min.

Conclusions: In this study, we evaluated the virucidal effect of seven commercially available disinfectants, and got the following results: Among seven disinfectants, the four had great virucidal effect against FMDV on disk carrier test method, and showed higher effect at long contact time. And temperatures seem to influence the result a little.

Acknowledgement: This research was supported by a grant from the Animal and Plant Quarantine Agency, the Ministry of Agriculture, Food and Rural Affairs of the Republic of Korea.

References:

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Trans-020

Foot-and-mouth disease vaccine development using A/SKR/YC/2017 classified to A/ASIA/Sea-97 topotype

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Introduction: Foot-and-mouth disease (FMD), an acute contagious disease, affects cloven-hoofed animals like cows, pigs, and sheep. Currently, inactivated vaccines are used worldwide (including Asia) to prevent the transmission of FMD virus (FMDV). Seven serotypes of the virus: A, O, C, Asia1, and South African Territories (SAT) 1, 2, and 3 exist. Among them, FMDV, A serotype outbreaks are frequent in Asia. Recently, the outbreaks of A/ASIA/Sea-97 topotype have occurred in east and south-east Asia such as Republic of Korea, China, Vietnam and Mongolia. The present study outlines FMD vaccine development using A/ASIA/Sea-97 topotype, G2 lineage, A/SKR/YC/2017 virus (isolated in the Republic of Korea) which induced protective, neutralizing antibodies in pigs.

Materials and Methods: A/SKR/YC/2017 was serially passaged in porcine kidney (LFPK) cells, adherent baby hamster kidney (BHK)-21 cells, and suspension BHK-21 cells. cell-adaptation of the virus was tested with Chinese hamster kidney (CHO)-K1 cells with heparan sulfate and without integrin as a receptor of FMDV. cell-adapted A/SKR/YC/2017 was cultured in suspension BHK-21 cells and inactivated using 3mM binary ethylenimine (BEI). The inactivated antigen was concentrated and purified by polyethylene glycol (PEG) precipitation. 15-45% sucrose density gradients (SDG) method was used to quantify 146S antigen. intact particle(s) were scanned by a transmission-electron microscope (TEM). An experimental vaccine containing 15ug of 146S antigen and Montanide ISA 206 (Seppic, France) adjuvant was formulated to W/O/W emulsion and was injected into pigs. Virus neutralizing antibody test (VNT) performed on pig serums (0-6 weeks post-vaccination). Furthermore, we performed VNT using heterologous viruses.

Results: The results revealed that A/SKR/YC/2017 (serial-passaged) used heparan sulfate as a receptor and intact 146S particle(s) could be produced in BHK-21 suspension cells. We demonstrated that experimental vaccine injected in pigs induced >2 Log 10 virus neutralizing (VN) titer in 3 weeks post vaccination. Moreover, it was observed that the vaccine induced >1.5 Log10 VN titer against heterologous viruses

Conclusions: We developed an A/ASIA/Sea-97 topotype G2 lineage vaccine using the virus isolated from the Republic of Korea in 2017 and verified antigen-productivity and immunogenicity. We suggest the use of the constructed vaccine against FMDV A serotype in Asia.

Acknowledgement: This work was supported by the Animal and Plant Quarantine Agency, Republic of Korea.

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Trans-021

Genetic characterization of porcine parvovirus 7 from domestic pigs in Korea

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Introduction: Until now, seven genotypes of porcine parvoviruses (PPV) have been identified in pig populations, which are taxonomically divided into four genera based on amino acid similarity in the NS1 protein: PPV1 in Protoparvovirus; PPV2 and PPV3 in Tetraparvovirus; PPV4, PPV5, and PPV6 in Copiparvovirus; and PPV7 in Chapparvovirus. Although PPV1 is a well-known infectious agent that causes reproductive failure in swine herds worldwide, the clinical significance of other genotypes of PPV infections remains uncertain. PPV7 is the most recently identified PPV genotype and was first identified by mutagenomic sequencing from healthy adult pigs in the US in 2016.

Materials and Methods: The lung tissues of the finishing pigs and fetuses were homogenized with PBS (pH 7.2) and stored at -80ºC until use. Total DNA was extracted from the homogenized samples using the DNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. PPV7 DNA was amplified with primers targeting the VP gene of PPV7 (PPV7-3434-F and PPV7-3654-R) using Hotstart PCR premix (Bioneer, Daejeon, Korea) as previously described. The expected 241-bp amplicons for PPV7 were confirmed. To further characterize Korean PPV7 strains, the REP or VP genes of PPV7 were amplified using four sets of primers (PPV7-380-F and PPV7-1336-R, PPV7-1270-F and PPV7-2262-R, PPV7-2158-F and PPV7-3203-R or PPV7-3022-F and PPV7-4033-R) as previously described. The amplicons were ligated into the pDrive vector (Qiagen, Hilden, Germany) and sent to a commercial sequencing company (Macrogen, Seoul, Korea) for sequencing of the REP or VP gene. The sequences were assembled using the SeqMan program in Lasergene 12.0 software (DNASTAR, Inc., Madison, Wisconsin, US) and aligned with other PPV sequences downloaded from GenBank by Clustal Omega (http://www.ebi.ac.uk/). Phylogenetic trees were inferred from amino acid sequences of the VP protein by the maximum-likelihood method using the Le and Gascuel with gamma distributed rate variation and frequency of each amini acid (LG+F+I) model implemented in MEGA v6.06. Support for individual nodes was determined by 1,000 bootstrap replicates

Results: PPV7 DNA was detected in 30 of the 125 tested fetal samples and 262 of the 350 tested finishing pig samples (Table 1). The prevalence of PPV7 in aborted pig fetal samples (24.0%, 30/125) was higher than the prevalence observed in the US (8.6%) but lower than that detected in China (32.8%). In addition, the prevalence of PPV7 in finishing pig lung tissue samples (74.9%, 262/350) was higher than the prevalence observed in the US (8.6%) and China (32.8%).

Conclusions: To the best our knowledge, this is the first report for PPV7 detection from aborted pig fetuses and finishing pigs in the Republic of Korea. The results of this study contribute to the understanding of the molecular epidemiology of PPV7, and further studies using PPV7 isolates will be needed to elucidate the pathogenesis of this virus in pigs.

Acknowledgement: This study was supported by the Research of Animal and Plant Quarantine Agency.

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Trans-022

Genetic diversity of atypical porcine pestivirus in Korean swine population

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Introduction: Atypical porcine pestivirus (APPV) is a newly identified swine pathogen involved in genus Pestivirus (family Flaviviridae) [1]. Since the first identification of APPV in North America via metagenomic sequencing [2], many countries have reported its presence and high relevance with congenital tremors in suckling piglets. However, despite the growing concern, the epidemiology of APPV in Korea has not been investigated until now. Accordingly, oral fluid surveillance was performed to reveal the prevalence and genetic diversity of APPV in Korea.

Materials and Methods: Pen-based oral fluid samples (approximately 20 pigs per pen) were collected from 64 different pig farms nationwide in 2016. Nucleic acids were extracted and tested using a pan-APPV specific primer set [3] and a newly designed universal APPV primer set, yielding 254-bp PCR fragments of the 5′ untranslated region (5′ UTR). Amplified DNA products were purified and subjected for sequencing.

Dataset based on 202-nt sequences of 5′ UTR was constructed, which was composed of 19 global APPV sequences (Austria, n=1; China. n=6; Germany, n=1; Netherland, n=9; Spain, n=1; USA, n=1) and 15 Korean APPV sequences obtained in this study. The phylogenetic tree was inferred by neighbor-joining (NJ) using MEGA7 software with the Kimura 2-parameter nucleotide substitution model. Branch support in both trees was estimated by bootstrap analysis of 1,000 replicates.

Results: A total of 15 Korean APPV sequences were obtained from oral fluid samples. The Korean strains shared mean nucleotide identity percentage of 94.3% for 5′ UTR region. Most farms were infected with single strains of APPV. However, co-circulation of two different strains (KU16-14 and KU16-15) showing approximately 10% nucleotide disparity was also confirmed in one farm. As shown in Fig. 1, Korean APPVs were scattered throughout the phylogenetic tree based on the 5′ UTR sequences. Out of 15 Korean strains, 8 were classified as Clade 1.1, 5 as Clade 1.2, and the remaining two as U.I. Further, Korean APPV strains of Clade 1.1 (n=8) were sub-classified into 1.1.1 (n=2), 1.1.5 (n=3), and 1.1.6 (n=3).

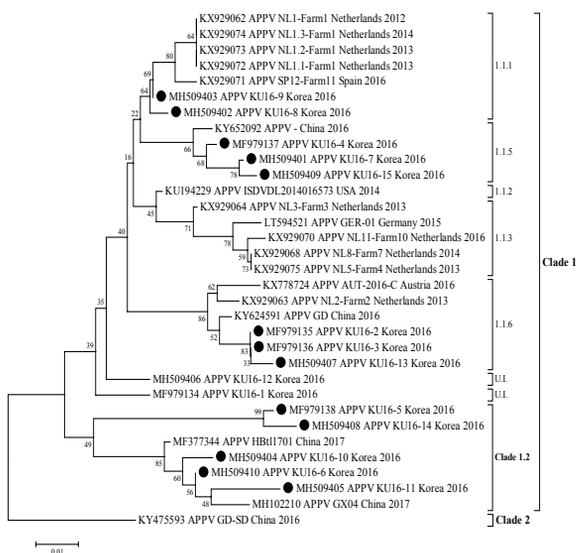

Fig. 1. Phylogenetic tree based on the 202-nt 5′ untranslated region. The tree was constructed by neighbor-joining methods with the Kimura 2-parameter nucleotide substitution model and assessed by bootstrapping with 1,000 replicates. The number at each branch represents the bootstrap percentage. The black circle indicates Korean APPV strains detected in this study.

Conclusion: Our results indicated that APPV infection is prevalent in Korean pig population with high genetic diversity. Further, phylogenetic analysis revealed that global APPVs were genetically highly variable with no significant bias in the genetic distribution of APPVs between countries.

Acknowledgement: None to declare

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Trans-023

Genome sequence of pandemic H1N1 swine influenza virus from pig in the Republic of Korea

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Introduction: Swine influenza A virus (H1N1) was derived from several viruses circulating in swine, and the initial transmission to humans occurred several months before recognition of the outbreak. Genetic reassortment in pigs allows for the generation of novel influenza viruses and further demonstrates that pigs can serve as intermediate hosts and act as "mixing vessels" for human, swine, and avian influenza viruses. To date, mortality associated with pandemic A/H1N1 2009 influenza has been reported with variable completeness worldwide and in particular subgroups, including inpatients, patients in critical care, (Kumar, #2;, 2009 #7) pregnant women, and children.

Materials and Methods: We report on a complete sequence of H1N1 influenza virus that was identified in nasal swabs taken from domestic pigs on farms located in Gyeongbuk Province in Korea in 2016. H1N1 Swine influenza virus (SIV) was isolated in specific pathogen free (SPF) embryonated chicken eggs with supernatants of the homogenized swine nasal swab collected. RNAs were extracted from lung tissue samples using an RNA extraction kit (Qiagen, Inc., Germany). PCR amplifications were generated by reverse transcription PCR using influenza-specific primers as protocols described. The sequences in plasmids were confirmed by DNA sequencing (Macrogen, Korea). The sequences were analyzed and assembled using DNASTAR version 5.0.

Results: The genome of A/swine/Korea/61/2016 (H1N1) strain consisted of the following eight gene segments: polymerase (PB2, PB1, and PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix protein (M), and nonstructural protein (NS). The sequence of the PB2 gene consisted of 2,341 nucleotides (nt), the PB1 gene 2,341 nt, the PA gene 2,233nt, the HA gene 1,776 nt, the NP gene 1,564 nt, the NA gene 1,428 nt, the M gene 1,027 nt, and the NS gene 890 nt. Phylogenetic analyses showed relatedness of the HA gene to those of the 2009 pandemic strains (up to 98% nt identity with reference strains), including the Sao Gabriel human strain (98%), whereas the NA gene was more similar to that of a Guangdong human strain from 2009 (97%). It is therefore imperative that human-to-animal, or possibly animal-to-human, transmissions of the pandemic 2009 influenza virus (especially events involving pig herds) are closely monitored (10). Phylogenetic analyses showed relatedness of the HA gene to those of the 2009 strains (up to 96.5% nucleotide identity with the LACENRS-1626 strain) and human isolates, while the NA gene was found to be more similar to the Guangdong human-derived H1N1 SIV strains (96.4% nucleotide identity).

Conclusions: These findings suggest that the H1N1 strain evolved from human-to-swine through reassortment. Thus, the A/swine/Korea/61/2016 influenza virus strain is a previously unregistered circulating reassortant variant of the human-to-swine H1N1 subtype in the Korean domestic pig population.

Acknowledgement: This study was supported by the Research of Animal and Plant Quarantine Agency.

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Trans-024

Genome Sequencing and Analysis of Pseudorabies Virus Isolated from 2012 to 2017 Highlights Genomic Diversity and Widespread Recombination

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Introduction: Pseudorabies virus (PRV) is a highly contagious and economically significant porcine infectious agent, which re-emerged in China in 2011 with stronger virulence leading to substantial economic losses to pig industry. The association of viral genomic variability with pathogenicity has been suspected. Previous researches focusing on specific ORFs or genomes of limited strains have suggested the recombination between wild strain and vaccine strain [1-2], the phylogenetic relationship and selection pressure of gB, gC, and gE [3]. However, the knowledge concerning a comprehensive profile of genomic diversity and evolution of PRV re-emerged strains are still unclarified.

Materials and Methods: 54 PRV strains, isolated from clinical pig samples in China from 2012 to 2017, were sequenced and analyzed with the whole genomes, along with 19 previously published strains. Assembly, annotation, multiple sequence alignment, phylogenetic analysis, ORFs alignment and divergence, and selection pressure of each ORF were performed after the high throughput genomic sequencing.

Results: Alignment and phylogenetic analysis found that China strains and US/Europe strains were separated into two genotypes: phenotype I and phenotype II. Re-emerged PRV strains are highly homologous and phylogenetically close to China classic strain Ea and Fa. Several strains formed unique genetic clusters such as SC, ADV3275 and HuB1/CHN2017. BootScan analysis showed frequent recombination between re-emerged strains and classic strains like Bartha-K61 and Becker. The selection pressure analysis confirmed strong purifying selection on most ORFs, but evidence of a number of amino acid residues under diversifying selection was found in 33 ORFs. In addition, several distinguishing mutations were recognized exclusively in 13 ORFs of re-emerged PRV strains.

Conclusions: In the study, we comprehensively analyzed PRV genomic diversity and evolution with 73 PRV genomes, of which 54 genomes were newly sequenced, thus giving an unbiased and comprehensive genetic overview of PRV re-emerged strains.

Acknowledgement: This work was supported by National Key R&D Program of China (Grant number: 2018YFD 0500802), the National Key Technology Support Program of China (Grant number: 2015BAD12B04).

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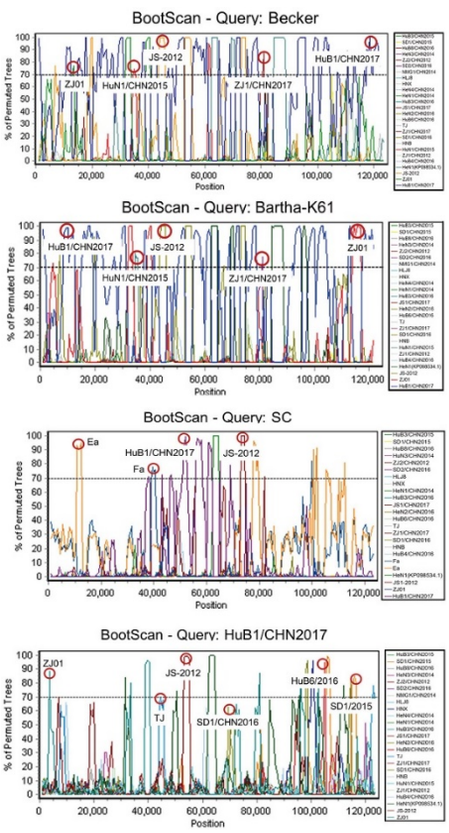

Figure 1. Recombination happened pervasively in PRV re-emerged strains

Trans-025

Identification of a novel variant posavirus 3 in Korean domestic farm, 2016 - 2018

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Introduction: Posaviruses were found in the feces of healthy pigs and water collected in domestic swine farms (1). Posaviruses occurrence has been reported in some countries such as USA, China, and Vietnam (2). To date, some studies have been conducted topology of posaviruses with their molecular genetics analysis (2). It is suggested that the posaviruses were divided into genotype 1 to 3 (2), and lineages 1 to 9 is the most recently identified posaviruses genotype (3), which are belong to different genera, and proposed in a novel family (unassigned) of the order *piconavirales*. In South Korea, there were no reported studies focused on the detection of posaviruses. This study attempted to investigate the prevalence of posaviruses by molecular-based method with performed complete sequencing for genetic characterization of posavirus viral genome sequence, and phylogenetic tree studies.

Materials and Methods: Stool samples of pigs showing signs of diarrhea (n = 1,242) randomly collected January 2016 to August 2018 were screened for the presence of posavirues lineages 1, 2, and 3 from 68 commercial farms in 9 provinces. Age groups ranged from suckling to sow pigs. The total RNA was extracted using a Viral DNA/RNA The RNA was then converted into cDNA with the use of random hexamers and commercial RNA to cDNA EcoDry Premix kit (Clontech, Otsu, Japan) following the manufacturer's protocol. The presence of posaviruses 1 to 3 were detected by RT- PCR specific primers (2). To phylogenetic study, the best nucleotide substitution model, the complete genome sequence model was selected automatically by specifying the '-m TEST' option in IQ-TREE version 1.3.8. In this study, the best plot model (HKY+G4) was used for phylogenetic analysis. Overall, the final dataset contained 110 complete sequences originating from Asia (China, Korea, Japan, and Vietnam), America (USA and Venezuela), and Germany, over a sampling period from 2010 to 2018, with complete gene aligned by BioEdit version 7.2.5. For alignment, all nucleotide stop coding sequences were removed. Reference sequences from GenBank with information on collection date and country of origin were included in the analysis.

Results: In screen result, although posaviruses lineages 1 and 2 showed all negative results, posavirus lineage 3 was detected at a very low rate of 0.08% (1/1,241 samples). The posavirus lineage 3 was positive in 1 out of 5 sow (200-days-old) stool samples from NP farm on Chungnam domestic Korean swine farm. The full-length genome of NP1 strain was registered in GenBank (Accession number. MK250903). The complete 8,829 bp nucleotide encoding of polyprotein 2,942 amino acid BLASTp searches identified 4 conserved protein domains, which were including an RdRp domain from amino acids1606-1939 (E: 1.58e-26) and two picornavirus capsid domains both 2046-2206 and 2296-2475 (E= 3.93e-08 and 7.94e-10, respectively). Another domain, putative immunoglobulin-blocking virulence protein domain of *Mycoplasma* and *Ureaplasma* species were identified (a.a. 118-307, E = 8.38e-04). The phylogenetic tree inferred based on the complete genome clearly divided posaviruses into 9 lineages with the highest posterior probability. Of the NP1 strain belonging to posavirus 3 lineage and were close to the USA_958-4 strain. Surprisingly, except for lineages 2 and 8, the Vietnam strains were all located in lineages as well as fisaviruses showed close to basaviruses, and posaviruse 8 and posaviruse 9 were located close to husaviruses and husa-like viruses unlike the previous reports (2).

Conclusions: In spite of a low prevalence rate, this study (NP1) first identified posavirus complete genome allocated in 3 lineage. Using bioinformatics genetic analysis tools, posavirus variant and diversity were characterized. Taken together, our findings demonstrate need to monitor infection status.

Acknowledgement: This study was supported by a grant (No. PJ011184) from BioGreen 21 Program, Rural Development Administration

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Trans-026

Improvement of serological diagnosis for the detection of antibody to the Non-Structural Proteins of Foot and Mouth Disease Virus In cattle

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Introduction: Foot and Mouth disease (FMD) is highly contagious viral disease which targets cloven-hoofed animals such as cattle, pigs, sheep and goats. Korea adopted 'vaccination-to-live' policy for the control of FMD since 2011. Therefore, it is mandatory to perform serological surveillance to detect antibodies resulting from infection using NSP test to estimate the prevalence or substantiate freedom from FMD infection or transmission. Paired serology using combined ELISA kits commercially available has been applied to measure antibodies to the NSP in Korea. However, OIE prescribed that enzyme-linked immunoelectrotransfer blot assay (EITB, NCPanaftosa) for antibodies against NSPs (3A, 3B, 2C, 3D and 3ABC) has proven to be used as a confirmatory test for the 3ABC-based ELISA screening method. In the present study was designed to compare the specificity and sensitivity of the 3 NSP ELISA kits used in Korea and EITB assay with sera from cattle to identify the reliability of EITB as a better NSP marker tests. Also, this study was examined the most suitable combination tests for sero-surveillance where intensive vaccination policy is practiced.

Materials and Methods: Fifteen sera from cattle with experimentally induced FMDV infection with 2 types of virus strain isolated in Korea were tested sequentially at various day-post-infection (DPI). 123 serum samples were obtained from outbreak farm and post-outbreak epidemiologically linked farms. 223 samples were collected from seropositive reactors to NSP either with or without any evidence of previous exposure to FMDV but positive by one more ELISA test during routine sero-surveillance. 4 NSP ELISA kit were used to compare their performance to detect antibodies to NSP with those of EITB assay using same serum panel simultaneously. There were; (1) NCPanaftosa-screening from PANAFTOSA (2) PrioCHECK FMDV-NS (Thermo-Fisher, USA) (3) Bionote (Bionote, South Korea) (4) Median Diagnostics (VDPro NSP, South Korea).

Results: The results of EITB in 15 experimentally infected sera showed detecting positive response at 8~10 dpi, equivalent to the other 3 kinds of ELISA. The sensitivity of PrioCHECK ELISA in sera from field farm was most highly correlated with the results of EITB assay. Bionote kit showed most highly correlated in relative performance of the 3 NSP-ELISAs compared with PrioCHECK. 55.3% of total 347 sera gave concordant positive results in all of the tests except EITB-screening assay. EITB showed 81.7%, the highest concordance rate in sensitivity. In addition, PrioCHECK-EITB combined test was proved most highly correlated with the NCPanaftosa kit (paired system) showed 0.7921 in AUC. Specificity of the EITB test was indicated by negative reaction to one or more bands for sera observed as positive results by one more ELISA test.

Discussion and Conclusions: This study showed a major advantage of the EITB procedure lies in the elimination of the number of false positive results yielded by other single-NSP based ELISA. In addition, the paired system with EITB and other NSP ELISA screening was found to provide a highly sensitive, specific, and consistent system, suitable for detecting FMDV. Although EITB test shows high sensitivity and specificity, yielding a visibly definite result of immunoblot technique, it is not well-suited screening large number of samples. Therefore, newly developed domestic diagnostic kit for DIVA of FMD, multiplex-LFD strip based on multiplex alternatives to western blotting is ongoing in Korea. This rapid kit developed can be a simple and reliable tool for further confirmation in a short time as an adjunct to other NSP-based screening kits.

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Trans-027

In vitro anti-viral effect of ribavirin against Japanese encephalitis virus

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Introduction: The Japanese encephalitis virus (JEV), belongs to the *Flavivirus* genus within *Flaviridae* family, is one of the most important zoonotic agents in the human and animal. Five genotypes (G1-G5) have been identified based on the phylogenetic analysis of the 'E' gene of viral envelope protein. JEV can cause fatal encephalitis or subclinical infection in infected humans and horses, and also cause reproductive failure in sows, resulting into abortion, stillbirths or fetal mummification. JEV is naturally circulated in mosquitoes, wild birds and pigs. The main vector of JEV is *Culex tritaeniorhynchus* in most parts of Asia. Among them, pigs act as important amplifiers of JEV. Wild birds can also be related to amplification and spread of JEV. Human and horse are dead-end hosts and do not transmit viruses because of low titer and short duration of viremia [1]. JEV is widespread in Asian countries and has recently spread to western India and the western Pacific region. JEV G3 has been identified in Asia including Korea and Japan, but the JEV G1 has been mostly replaced since 1990. Vaccination is most important preventive measure for protective immunity against JEV infection. Anti-viral drugs could be used as an alternative treatment strategy to control virus infection against JEV. Ribavirin (1-β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a broad-spectrum antiviral ribonucleoside [2]. It was previously reported that several antiviral compounds including ribavirin have antiviral activity against pathogenic flaviviruses [3]. This study was conducted to investigate the *in vitro* anti-viral effect in proliferation of JEV G1 KV1899 virus isolated from swine blood in 1999 in Korea according to concentration of ribavirin.

Materials and Methods: Virus titration-KV1899 virus was diluted with 10-fold in medium containing ribavirin at concentrations of 0 μM (negative control), 200 μM, 400 μM and 800 μM in each 96-well plate. Vero cells were added and incubated at 37℃ under 5% CO_2. Cells of each well were observed for detection of cytopathic effect (CPE) and the virus titers were expressed as the reciprocal of the final virus dilution that showed CPE. Fluorescence assay (FA) test-The microplates were fixed in cold acetone for 20 min. After 3 washings with PBS (pH 7.2), the infected cells were reacted with monoclonal antibody against JEV and then with fluorescence isothiocyanate (FITC) conjugated goat-anti mouse IgG+IgM (KPL, Gaithersburg, MD, USA). After rinsing with PBS, the fluorescence was observed under ultraviolet (UV) light illumination using a fluorescent microscope (Nikon, Tokyo, Japan).

Results: The titers of JEV KV1899 at the each plate containing 0 μM, 200 μM, 400 μM, 800 μM of ribavirin were $10^{5.75}$, $10^{5.75}$, $10^{5.5}$, and $10^{4.5}$ $TCID_{50}$/mL, respectively (Table 1). In FA test, ribavirin inhibited the proliferation of JEV KV1899 and decreased the fluorescence of the infected cells in the wells treated with 800 μM of ribavirin.

Table 1. The titers of genotype 1 Japanese encephalitis virus KV1899 strain according to the concentration of treated ribavirin

Ribavirin con. (μM)	0	200	400	800
Virus titer (Log $TCID_{50}$/mL)	5.75	5.75	5.5	4.5

Conclusions: These results suggested that ribavirin inhibited the proliferation of JEV G1 KV1899 and decreased the fluorescence of the infected cells in the high concentration of ribavirin.

Acknowledgement: This work was supported by a grant (N-1549085-2017-36-01) from the Animal and Plant Quarantine Agency, Ministry of Agriculture, Food, and Rural Affairs (MAFRA), Republic of Korea.

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Trans-028

Intradermal immunization by inactivated Foot-and-Mouth disease virus using mice against lethal FMDV challenge

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Introduction: Foot-and-mouth disease (FMD) is a highly infectious disease that causes serious economic losses in terms of animal products as well as controlling disease prevention [1]. Due to the circumstances FMD virus vaccine is a major purpose in countries. FMD vaccines used in worldwide are based on inactivated virus particles in an adjuvant and are usually administered intramuscularly (IM) [1]. Recently, vaccine could be commercialized with development of mass production of antigen. However, it was also reported various side effects by IM such as edema, inflammation, short-term hyperthermia, reduction in milk yield, and neurologic problems [2]. Especially, abnormal tissue reaction like granuloma, pyogranuloma, abscess, necrosis, and fibrosis are recognized as main problem. It is already known that water in oil type component added as adjuvant could induce those reactions [3]. And it is emerged that location of injection site and kinds of injector affected to outbreak abnormal tissue. For these reason, intradermal inoculation was suggested as alternative way on decrease of abnormal tissue regarding immunity study of dendritic cell, side effect of syringe needle, and prevention of bacterial contamination. Thus, in this study, we compared effect between IM and intradermal (ID) vaccination by several experiment. And we investigated the prevention of abnormal tissue formation after vaccination and immune specific antibody.

Materials and methods: The mice were purchased from KOSA BIO, 6weeks of age. All of the mice are housed in an environmentally controlled system during experimental period. The mice divided into three groups as control, IM (n=5) and ID (n=5) group. Control group are not vaccinated and mice in IM group were inoculated with dose of vaccine contained 1.5ug/dose of O PA2 and A22 antigen. The mice in ID group were vaccinated with the same vaccine by intra-dermally method with 1.5ug/dose. Serum samples were obtained after 7 days. To evaluate antibodies level against non-structural proteins of FMDV, NS-ELISA was performed using commercially available ELISA (CeditestFMDV-NS). The test was followed by instructions of the manufacturer. Immune specific antibody factor was determined using real-time PCR. Neutralizing antibody titer (VNT) against FMDV O PA2 and A22in serum samples were measured using the neutralization assay as described previously.

Results: All vaccinated mice differ from non-vaccinated group. According to the NS-ELISA, some of mice represented positive result in the post-vaccination.

Table 1. Overview experimental groups.

Group	Application	Antigen dose (ug)	Administered volume (ml)	Vaccine serotype
1	-	-	-	-
2	IM	1.5	0.1	O
3	IM	1.5	0.1	A
4	IM	3	0.1	O+A
5	ID	1.5	0.1	O
6	ID	1.5	0.1	A
7	ID	3	0.1	O+A

Real time PCR analysis of transcript related ID immunization such as IL1a, CX3CL1, CXCL12, TNFa, GM-CSF, CCL8, IL1b, CCL20, CCL2, CCL3, IL6, IL13, and CXCL8 genes were measured as mRNA expression level. O PA2 antigen was prepared on a suspension BHK21 cell culture. Virus was obtained from concentrated supernatant. For inactivation of virus 0.003N Binary Ethyleneimine (BEI) was treated. And sodium thiosulfate was treated to neutralize and remove residues of BEI. VNT was proceeded with $1.6*10^4$ $100TCID_{50}$/0.1ml value. $TCID_{50}$ inactivated FMD virus type O PA2 was used as the antigen payload per dose.

Conclusion: As a results, ID injection seems to react efficiently with higher formation of antibody against FMDV. And it showed the possibility that reduce of abnormal meat formation. Therefore ID inoculation might be good alternative suggestion for IM injection.

Acknowledgement: This study was supported by a grant (B-1543386-2019-21-04) from the Animal and Plant Quarantine Agency's National Animal Disease Research Project.

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Trans-029

Investigation of vaccine-induced effects in pigs vaccinated and subsequently challenged with foot and mouth disease virus

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Introduction: FMD is economically important, highly contagious disease of cloven-hoofed animals. It can spread rapidly by a multitude of routes and affect both domestic and wild livestock. Korea adopted 'vaccinate-to-live' policy as a control strategy of FMD in 2011, and has established post-vaccination monitoring(PVM) system to verify the vaccination regimen and programs since 2017. Depending on the control policy, investigation of vaccine-induced effects in various crucial parameters (virus replication, excretion, clinical signs and serological immune responses) of infected pigs with FMDV is necessary for optimizing current diagnostic methods and surveillance system. In addition, building FMDV diagnostic windows of detection by vaccine-dependent is important to identify the infectious status and estimate the timing of entry of infection where vaccination is practiced. The aim of this study was to provide a quantitative description of the infectious process in pigs vaccinated and subsequently infected with FMDV, obtained by time course studies of viral load and antibody response in saliva and serum. The other objective was to verify the existence of sub-clinical infection in vaccinated pigs, to evaluate the correlation between clinical sign and seroconversion against NSP of FMDV.

Materials and Methods: Pigs (n=33) were divided into two experiments which were vaccinated (either O_1 campos or O1Manisa+O3039+A22Iraq) and subsequently challenged with 2 serotypes (O/MESA/Ind2001d or A/ASIA/Sea-97) of FMDV respectively. Two randomly chosen pigs from each experiment were inoculated with FMD virus either O/BE/SKR/2017 or A/YC/SKR/2017, while the other pigs remained were exposed to the seeder pigs by direct contact. They were monitored for 28 days post-challenge(DPC) to obtain clinical, virological and serological results in saliva and serum. We also studied in comparison with those of unvaccinated infected animals.

Results: Virus transmission and clinical signs were observed in almost all contacted animals affecting both vaccinated and non-vaccinated group, however, virus excretion was significantly decrease in vaccinated group. Also, the severity of the disease to be correlated with seroconversion to NSP was revealed in vaccinated group, showing 40% of the pigs challenged with serotype-O and 89% with serotype-A in seropositivity to NSP until 28 DPC. Also, the induction of NSP antibodies in the vaccinated pigs after infection was lower and shorter duration as compared to the non-vaccinated infected pigs.

Conclusions: Relationship between clinical disease and virus replication was observed by evaluating seroconversion to NSP. However, six pigs in vaccinated group remained negative in the NSP-ELISA up to 28 DPC, whereas viral RNAs were detected from saliva by RT-PCR in these pigs. This result suggested that the use of NSP-ELISAs for serosurveillance might be useful but results of NSP testing should be interpreted with caution in country practicing intensive vaccination policy. Due to low sensitivity of this assay, especially early in infection, and low specificity by multiple vaccination, the use of this NSP-ELISA assay would only be recommended for screening herds of animals, not on an individual animal basis. Also, these results demonstrated that vaccination can reduce the clinical signs after infection, virus excretion and production of NSP antibodies although pigs were not completely protected. Moreover, viral strain, infectious dose and species might have influence on those parameters, which needs further investigation.

Acknowledgement: This work was supported by a grant from Animal and Plant Quarantine Agency, Ministry of Agriculture, food and Rural Affairs (MAFRA), Republic of Korea.

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Trans-030

Isolation, characterization and neutralizing activity of porcine epidemic diarrhea viruses isolated from Vietnam

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Introduction: Porcine epidemic diarrhea virus (PEDV), the etiological agent of porcine epidemic diarrhea (PED), is a large-enveloped RNA virus, which is a member of the genus Alphacoronavirus within the Coronaviridae family placed with the Arteriviridae family in the order Nidovirales. Porcine epidemic diarrhea (PED) is characterized by acute enteritis, watery diarrhea, weight loss, dehydration, and death with high mortality in neonatal piglets. Especially, the spike (S) protein of PEDV is the major envelope type I glycoprotein of the virion, which interacts with the cellular receptor during virus entry and stimulates induction of neutralizing antibodies in pigs. The S protein is considered a primary target antigen for induction of neutralizing antibodies and developing an effective vaccine against PEDV. The pathogenicity and antigenicity of PEDV could be changed regarding to mutations, deletions and/or insertions of amino acids in the S protein.

Materials and Methods: PEDV HID9047 and HID9048 isolated from infected samples collected in Ha Nam province and Thai Binh province, Vietnam in 2016, and PEDV HID9049 isolated in Hung Yen province, Vietnam in 2017 were used in this study. Isolation and propagation of PEDV in Vero cells was adapted from Chen Qi et al., 2014 and Park et al., 2018 with modifications. To confirm virus growth in Vero cells, immunofluorescence assay was performed using the monoclonal mouse anti-PEDV and fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. The neutralizing activity of antisera collected from guinea pigs immunized with a PEDV vaccine strain SM98 against PEDV isolates HID9047, HID9048 and HID9049 was evaluated.

Results: The viral titer of HID9047, HID9048 and HID9049 were 10^6, $1^{06.5}$, $10^{6.3}$ TCID50/mL at passage 10, respectively. As shown in table 1, the nucleotide identities of S gene between Vietnam isolates and reference strains ranged from 91.80% to 92.71%, and the deduced amino acid sequences showed 96.74% to 97.61% similarity. Furthermore, compared with Vietnam isolates (HUA-PED45, HUA-PED111, VN97 and VN-K28), three isolates shared 96.78% to 97.52% nucleotide homology and 98.19% to 99.13% amino acid homology. In addition, sequence alignment of amino acids in the S protein showed that, compared with reference strains, mutations in amino acid sequence including substitution, insertion and deletion was observed in Vietnam isolates.

Table 1. Pairwise comparisons of the nucleotide and deduced amino acid sequences of the full-length S gene of PEDV isolates with reference strain

	CVV 7	DR 13	SM 98	HUA -PED 45	HUA -PED 111	VN9 7	VN- K28	HID 9047	HID 9048	HID 9049
CVV7		99.88	96.14	92.04	91.75	92.84	92.31	92.64	92.50	91.97
DR13	99.71		96.17	92.11	91.82	92.91	92.33	92.71	92.57	92.04
SM98	97.90	97.97		91.99	91.77	92.69	92.12	92.23	92.09	91.80
HUA-PED45	96.88	97.39	97.59		97.28	97.24	97.14	97.79	97.69	97.79
HUA-PED111	96.81	97.31	97.23	98.70		96.54	96.25	97.16	97.07	97.23
VN97	97.17	97.75	97.67	98.41	98.19		98.29	97.52	97.48	97.26
VN-K28	97.32	97.97	97.52	98.27	98.12	98.63		97.28	97.24	96.78
HID 9047	96.81	97.61	97.30	98.63	98.70	98.12	98.70		99.76	98.24
HID 9048	96.74	97.31	97.01	98.56	98.70	98.19	98.41	99.64		98.15
HID 9049	97.03	97.61	97.45	98.92	99.13	98.70	98.63	99.06	99.06	

Conclusions: Three novel PEDVs HID9047, HID9048 and HID9049 in Vietnam were isolated and characterized which had significant variations on the S gene. Cross neutralizing activity of vaccine strain against three isolates was assessed in guinea pigs.

Acknowledgement: We would like to acknowledge veterinarians who collected the infected samples and the contribution of all authors for this study.

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Trans-031

Japanese Encephalitis Virus infection induces inflammation of swine testis through RIG-I-NF-κB signaling pathway

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Introduction: JEV is a mosquito-borne flavivirus causes severe central nervous system diseases in humans, resulting in 10,000 to 15,000 deaths annually (Ghosh and Basu, 2009). Pigs are considered to be amplifing hosts which play an important role in JEV transmission cycle. JEV infection of swine can cause reproductive failure in sows and orchitis and infertility in boars, which threaten the agricultural economy and food security (Mansfield et al., 2017). However, the mechanism for JEV causing orchitis is still unclear. In this study, JEV infection models in swine testis, primary swine testis cells, and swine testis cell line were established, and the mechanism of JEV-induced orchitis was investigated.

Materials and Methods: Swine testis samples isolated from JEV infected swine were fixed in buffered formalin and processed for routine H&E and IHC. Swine testis cell line (ST) cells were maintained in Dulbecco modified Eagle medium (DMEM; Sigma) supplemented with 10% fetal bovine serum (SUE), 100μ g/ml streptomycin, and 100 U/ml penicillin at 37°C in a 5% CO_2 atmosphere.

Results: To characterize JEV infection in swine testis, histopathological analysis and IHC was performed on testis of JEV-infected boars (Fig. 1). Inflammatory cell infiltration and haemorrhages can be seen in the JEV-infected testis compared to uninfected testis, and JEV antigen was detected. These indicate that JEV can infect and cause inflammation and damage on testis. The in vitro assay revealed that JEV can also infect and propagate in both primary swine testis cells and ST cell line. Furthermore, the expression of inflammatory cytokines, such as TNF-α , IL-1β , IL-6, IL-8, and RANTES in JEV-infected swine testis, primary swine testicular cells, and ST cells were increased significantly during JEV infection. To investigate the mechanism for JEV-induced inflammation in testis cells, the expression of pattern recognition receptors (PRRs) and activation of key transcriptional factors, NF-κB and AP-1, were detected. Our results showed that expression of RIG-I and the phosphorylation of NF-κB subunit p65 were significantly up-regulated after JEV infection. To elucidate the function of RIG-I in JEV-induced inflammatory response, RIG-I knock-down (RIG-I KD) ST cells was generated and RIG-I knock-down was found to down-regulate p65 phosphorylation and inflammatory cytokine production after JEV infection (Fig. 2). In addition, treatment of ST cells with QNZ, the specific inhibitor of NF-κB, also reduces the expression of inflammatory cytokines induced by JEV.

Conclusions: JEV could infect swine testis cells and cause inflammatory response both in vivo and in vitro. RIG-I-NF-κB signaling pathway was involved in JEV-induced inflammation in swine testis cells. This study may provide new insight into JEV pathogenesis in pigs.

Acknowledgement: This work was supported by National Natural Science Foundation of China (31825025) National Key Research and Development Program of China (2016YFD0500407)

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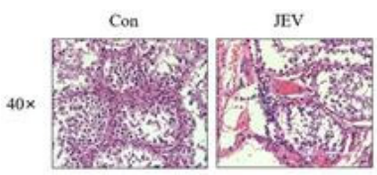

Fig. 1 Histopathology of the testis in male swine infected with JEV.

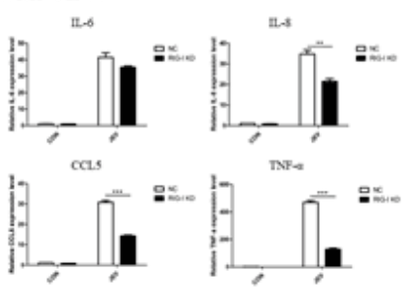

Fig. 2 RIG-I knock-down reduces JEV-mediated inflammation responses expression of inflammatory cytokines during JEV infection.

Trans-032

Methodological comparison for concentration and purification of foot-and-mouth disease vaccine antigen

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Introduction: After the severe outbreak of foot-and-mouth disease (FMD) in South Korea in 2010, the Korean government implemented a vaccination policy and set out to develop an FMD vaccine using a local FMD virus (FMDV) strain. For production of FMD vaccines, it is most important to concentrate the FMDV particles (146S) and eliminate non-structural proteins (NSPs) according to Office International des Epizooties (OIE) standards [1]. Therefore, the objective of this study was to determine the most efficient method to recover intact 146S FMDV antigens among three well-known methods.

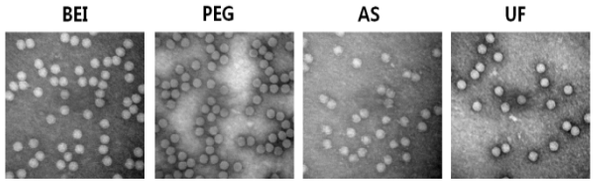
Materials and Methods: Baby hamster kidney-21 suspension cells (6×10^6 cells/ml) were inoculated with the FMDV O Jincheon/SKR/2014 (O-JC) strain. The 146S particles of FMDV were concentrated through 7.5 % polyethylene glycol (PEG), 50 % ammonium sulfate (AS) treatment or ultrafiltration (UF) using a polyethersulfone membrane cassette with a nominal molecular weight limit of 300 kDa. To measure the amount of the purified 146S particles (㎍/ml), 146S particles were evaluated by spectrophotometer using a continuous density gradient fractionator connected to an UA-6 monitor. Also, the purified FMDV 146S particles were confirmed by transmission electron microscopy (TEM) and SDS-PAGE. The microplate BCA protein assay reagent kit and FMD IPC 3ABC ELISA kit were used to measure the total protein and NSP content.

Results: Inactivated FMDV supernatants were concentrated 50 times with 7.5% PEG or 50% AS. On the other hand, the supernatants were concentrated 10 times by UF. PEG treated samples (3.70 ± 0.17 ㎍/ml) yielded more 146S particles than either AS treated samples (2.82 ± 0.11 ㎍/ml) or UF samples (1.49 ± 0.04 ㎍/ml). On the basis of these results, respective recovery was calculated as follows: PEG treated samples, 83.36 ± 1.81%; AS treated samples, 63.59 ± 2.72%; and UF samples, 33.50 ± 0.39% (Table 1). Concentrated and purified 146S particles of FMDV from each test group were confirmed to be intact based on a spherical shape and diameter of 25-30 nm by TEM (Fig. 1). In this study, inactivated FMDV concentrated by 7.5% PEG showed significantly higher antigen recovery than either 50% AS or UF.

Table 1. Methodological comparison for 146S particles and the contaminant (total proteins and NSPs) content

	BEI-inactivated	7.5% PEG	50% AS	UF
146S particles (㎍/ml)	4.44 ± 0.14	3.70 ± 0.17	2.82 ± 0.11	1.49 ± 0.0
Recovery of 146S particles (%)	100	83.36 ±1.81	63.59 ± 2.72	33.50 ± 0.39
Total protein (㎍/ml)	3251 ± 234.3	15.7 ± 1.547	55.58 ± 7.594	856.8 ± 87.79
NSP (ng/ml)	219.1 ± 16.80	2.4 ± 0.545	4.33 ± 0.384	24.17 ± 7.427

Figure 1. Morphological intactness of viral particles verified by TME



Conclusions: 7.5% PEG precipitation is the best method of concentrating and purifying FMDV antigen for vaccine production with O-JC strain. These findings may provide important insights for the development of new FMD vaccines using a local FMDV strain in the near future.

Acknowledgement: This research was supported by the grant (B-1543386-2018-19-01) from the Animal and Plant Quarantine Agency, Republic of Korea.

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Trans-033

Molecular characterization of *Streptococcus suis* isolated from pigs in Korea

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Introduction: *Streptococcus suis* (*S. suis*) is causing meningitis in pigs and a wide range of disease to include such as sepsis, septicaemia, endocarditis, arthritis and pneumonia [1]. Multi-locus sequence typing (MLST) is a highly discriminatory method of characterizing bacterial isolates on the basis of the sequences seven house-keeping genes [2]. All MLST sequence types (STs) can be shared and compared by MLST database (https://pubmlst.org/ssuis/). STs allows gathering further information about the genetic diversity of the S. suis strains within the same or different serotypes. The objective of this study was investigated STs of S. suis isolated from diseased pigs in domestic.

Materials and Methods: A total of 18 strains isolated from pigs (13 from lung, 2 from brain and 2 from swab from in South Korea) were used in this study. Cultured bacteria genomic DNA was extracted using the DNeasy Blood & Tissue kit (QIAGEN, USA). Seven housekeeping genes were amplified by PCR, and amplification fragments were sequenced.

Results: A total of 7 sequence types (STs) were found in currently study, and 5 isolate were novel STs at the time of this study. A number of profiles 1,096 STs currently are available in the *S. suis* MLST database (https://pubmlst.org/ssuis/). Serotype 2 or 1/2 was belonged to ST25 (n=6) and ST28 (n=1), respectively. Serotype2 (ST25 and ST28) was already associated various disease such as meningitis, septicaemia and pneumonia in pig (https://pubmlst.org/ssuis/). ST27 and ST108 were identified serotype3. Of the 3 serotype 4 strains, 2 were ST485 and 1 was ST977. ST485 (Serotype 4) was from lung and brain in diseased pig, there are reported in pig with septicaemia in Thailand (https://pubmlst.org/ssuis/). Serotype 7 strain 17-0509 was untypeable, whereas other strain was ST29. Serotype 8, 9, 12 and 19 strains were identified as novel STs, respectively. The 17-0509 from a pig lung was new STs, it was different from ST29 by the dpr allele only. The novel STs (17-0461) was single locus variant from ST87 dpr allele only. Previous report, both the ST87 and ST27 was found a significantly higher number of isolates from lung disease than the ST1 [2].

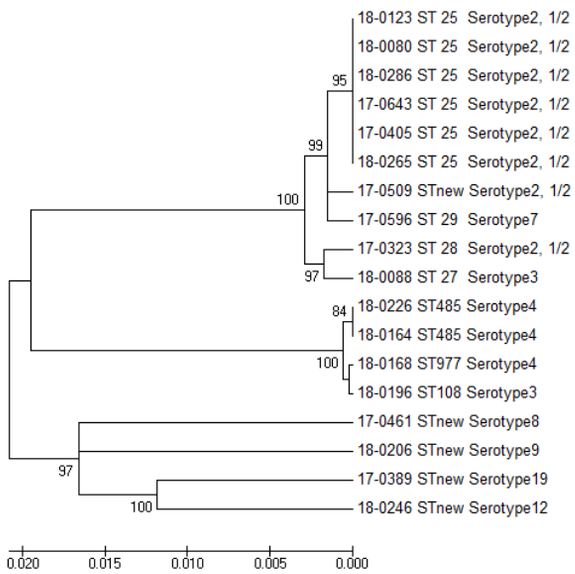

Fig. 1. Phylogenetic tree was constructed based on neighbor-joining method by different MLST STs and serotypes. Alignments were imported into Mega 5 to construct a phylogenetic tree using neighbor-joining method with 1,000 bootstrap replicas. Numbers at the node are bootstrap values.

Conclusions: MLST methods are helpful in characterization the infectious agent and will can help people take quick measures to control outbreaks of *S. suis* infection in both swine and humans beings.

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Trans-034

Monitoring of the mutation in 3D and IRES genomes for foot-and-mouth disease virus diagnosis

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Introduction: FMD (Foot-and-mouth disease) viruses produce variants through persistent genetic mutations, making it difficult to diagnose foot-and-mouth disease and to use vaccines, so researching virus genetic mutations is crucial for the prevention of foot-and-mouth disease. After the first outbreak of FMD on December 3, 2014, the FMDV continued to circulate in Korea for more than one year until April 2016. Thus, for this mutation study, we investigated whether there might be a problem in the diagnosis by observing the variation of 3D and IRES regions which is widely used for diagnosis of FMDV using the viruses which were circulated for more than 1year. Materials and Methods: FMD Viruses were isolated from suspected cases of foot-and-mouth disease (FMD) in cattle and pigs collected in Korea between 2014 and 2015. The total RNA of Viruses originated from these periods were extracted according to the manufactures's instruction and tested to generate 3D and IRES sequence. The 3D and IRSES sequences were generated using the standard method and primers of APQA by 3730 DNA Analyzer (ABI), according to the manufacturer's instructions and assembled using SeqMan Pro 13 Software (DNAStar Inc., USA).

Results: RNA samples derived from total of 90 FMD viruses were assayed designed in this study to recognize the coding sequences for the 3D and IRES of FMDV circulating between 2014and 2015 in Korea. The primer/probe binding sites within the virus 3D genome were compared to the respective primer and probe sequences to identify nucleotide substitutions at these sites. One nucleotide mismatch to the forward primer in each of the two samples was observed in the corresponding binding sites within the 3D coding region and one nucleotide mismatches to the reverse primer of all samples were noted in the corresponding binding sites within the 3D coding region. It was confirmed that all viruses were mutated at position 7782. Similarly, Comparison of the sequences of the primers binding sites within IRES genome of 5'UTR revealed one nucleotide mismatch in the forward primer of one sample and in the reverse primer of one sample. The same samples were assayed using the pan-FMDV assays, targeting part of the 3D coding region and the 5'-UTR within the FMDV genome. The pan-FMDV assays confirmed the presence of FMDV RNA in all the viruses tested. Mutations were observed at the primer binding site in several viruses but did not affect the pan-FMDV assays.

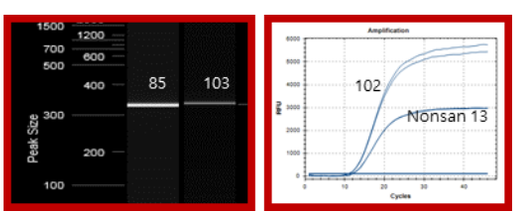

Fig 1. The two samples (samples No. 85 and 103) were amplified in RT-PCR(Left). The two samples (samples No. 102 and Nonsan13) were amplified in rRT-PCR(Right).

Conclusions: We investigated whether there might be a problem in the diagnosis by observing the variation of 3D and IRES regions which is widely used for pan-specific FDMV assay using the viruses which were circulated for more than 1year. The primer binding sites within the virus 3D and IRES genome were compared to the respective primer sequences to identify nucleotide substitutions at these sites. The mutations were observed at the primer binding site in several viruses but did not affect the pan-FMDV assays.

Acknowledgement: This research was supported by the Research of Animal and Plant Quarantine Agency (Project No. B-1543082-2018-19), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

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Trans-035

Optimal conditions for foot-and-mouth disease virus production in pilot-scale

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Introduction: Foot-and-mouth disease (FMD) is the highly contagious disease (FMD) which could infect cloven-hoofed animals. The causative agent, FMD virus, is a small (25-30 nm), without an envelope, positive-sense, and single strand RNA virus belonging to the genus *Aphthovirus* within the family *Piconaviridae* [1]. Outbreaks of FMD in South Korea 2011 caused unprecedented damage to livestock industry. Since then, the Korean government has implemented the vaccination policy and started to develop its own FMD vaccine. The goal of this study was to find out optimal conditions for FMD virus production in pilot-scale (100L) using type O vaccine seed virus developed in South Korea.

Materials and Methods:
Virus infection. FMDV O PAN2 was propagated in BHK21 suspension cells at 37 ℃ in CO2 shaking incubator, collected 16 - 48 hours after inoculation and clarified by centrifugation. When the cell number reached 6 x 10^6 cells/ml, FMD virus at 0.005 MOI was inoculated and viral supernatant was harvested at 16 h to 48 h. The ratio of media replacement before virus infection was from 0% to 100%. Pilot scale (100L) of virus production was conducted with the optimal harvest time and the media exchange ratio.

Virus titration. Viral titers for each aliquot were determined by 96-well plate virus titration method. End-point titers were calculated as the reciprocal of the final virus dilution that exhibited cytopathic effect on monolayered cells in 50% of the wells by Karber equation.

Quantification of 146S antigen. The harvested virus culture medium was treated with chloroform 1:1 (v/v) and 100U benzonase. Then, the virus medium was transferred onto the sucrose gradient tube (15 ~ 45 %) and then was ultracentrifued followed by scanning at 259 nm using UA-6 (ISCO) continuous spectrophotometer.

Results: As the exchange ratio of media increased, the viral titer and 146S antigen showed correlative increment. In addition, virus harvest time was critical to the yield of 146S antigen because the 146S antigen yield was low until 16 hours after virus inoculation. In pilot scale (100L) test, based on the optimized conditions set up in lab scale tests, the yield of live FMD vaccine antigen reached more than 2 ug/ml.

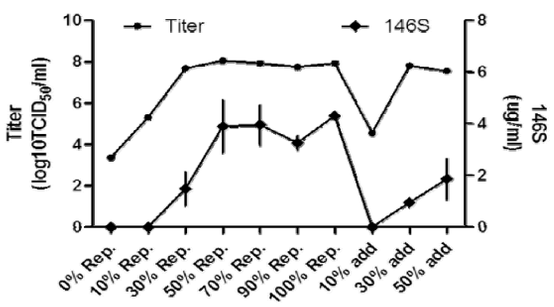


Fig 1. Exchange ratio of media before virus inoculation.

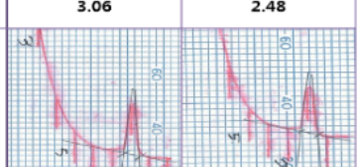
	Harvested medium	After continuous centrifuged
146S (ug/ml)	3.06	2.48
UA-6		
Titer ($TCID_{50}$/ml)	7.18	7.18

Fig 2. The amount of FMD vaccine antigen (146S) produced in pilot-scale production.

Conclusions: The optimal conditions for FMD virus production using type O local vaccine seed virus was successfully established up to pilot-scale.

Acknowledgement: This work was supported by the grant (N-1543386-2018-19-01) from the Animal and Plant Quarantine Agency, Republic of Korea.

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[1] Chia-Ying Wu, et al., 2015. Journal.pone.0136420

Trans-036

Overexpression of porcine beta-defensin 2 in transgenic pigs inhibits swine influenza virus infection

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Introduction: Swine influenza virus (SIV) is the causative pathogen strongly associated with an acute respiratory disease in pigs, which brings about great economic losses on the global pig industry [1]. Porcine beta-defensin 2 (PBD-2), an antimicrobial cysteine-rich cationic peptide, is found in different tissues in pigs and is utilized by host cells to kill various microorganisms including enveloped viruses [2]. The interspecies transmission of SIV has great significance in public health and enhancing the resistance of pigs to SIV is of importance to the prevention of SIV. Given that our lab has generated transgenic (TG) pigs overexpressing PBD-2 [3], the effect of PBD-2 on the infectivity of SIV is investigated. The TG pigs are expected to exhibit enhanced resistance to SIV infection compared with wild-type (WT) ones.

Materials and Methods: The influenza isolate A/Swine/Nanchang/F9/2010(H1N1) was used for the in-contact challenge trial to mimic the natural infection of SIV. Briefly, at 40 days of age, TG pigs (n=6) and the littermate WT pigs (n=6) were cohabited with four pigs intratracheally injected with 2 mL of SIV (10^7 EID_{50}/200 μ L). All pigs were sacrificed on the 5th day post-infection (dpi). Lung tissues were collected for lung lesion scoring using Consolidation Lung Lesion Score [4]. Besides, viral titers (EID_{50}) in the lung homogenates collected from TG and WT pigs were determined in embryonated chicken eggs and were calculated using Reed-Muench method [5].

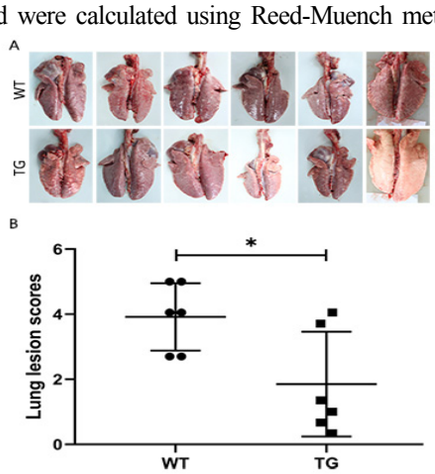

Figure 1. Lung lesions of WT and TG pigs. (A) Gross injury of lungs from WT and TG pigs. (B) Lung lesion scores of WT and TG pigs. *P < .05 unpaired one-tail Student's *t-test*.

Results: In the in-contact challenge trial, there were no apparent lesions in the lungs of two TG pigs while minor lung lesions were present in the other four TG pigs. Lung lesions of all WT pigs were severe, manifesting as dark red lungs with scattered consolidation. The lung lesion scoring demonstrated that TG pigs exhibited significantly milder lung lesions compared with WT pigs (Fig. 1). In addition, the inhibitory effect of PBD-2 was obvious, with a significantly lower viral titer in the lung homogenates of TG pigs than that of WT pigs (Fig. 2).

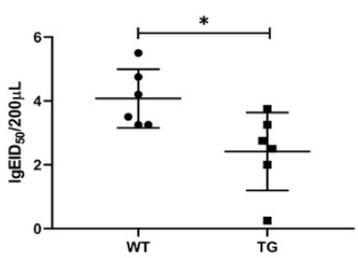

Figure 2. Viral titers in the lung homogenates of WT and TG pigs. *P < .05 unpaired one-tail Student's t-test.

Conclusions: Overexpression of PBD-2 is effective in inhibiting SIV infection in transgenic pigs, which will offer more insights regarding SIV control.

Acknowledgement: This study was founded by the National Transgenic Project of China (2016ZX08006003-004), National Key R & D Program of China (2017YFD0500201), Hubei Province Natural Science Foundation for Innovative Research Groups (2016CFA015).

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[1] Brown, 2000. Vet Microbiol, 74, 29-46.
[2] Veldhuizen *et al.*, 2008. Mol Immunol, 45, 386-394.
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[5] Yen *et al.*, 2007. J Virol, 81(13), 6890-6898.

Trans-037

Phylogenetic analysis of the foot-and-mouth disease virus O/ME-SA/Ind2001 in LAO, Vietnam, Myanmar and Republic of Korea.

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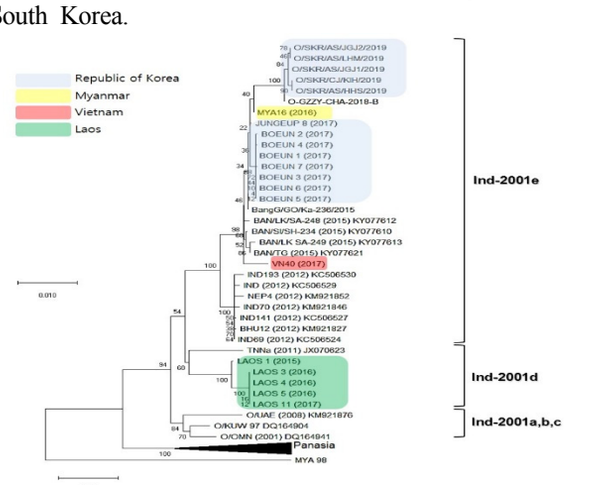
Introduction: Foot-and-mouth disease virus (FMDV) remains one of the world's most widespread epizootic and a highly contagious animal disease that affects a wide host range of cloven-hoofed farm animals. The O/ME-SA/Ind-2001 lineage has recently caused increasing concerns due to it multiple trans-regional movements. Currently, O/ME-SA/Ind-2001 genotype was rapidly spreading from Pool 2 region to Pool 4 region including Pool 1 region[1]. The O/ME-SA/Ind-2001 are continuously occurring in Myanmar, Laos and Vietnam, which are neighboring countries. In Republic of Korea, the genotype occurred in 2017 and 2019. Therefore, it is very important to collect gene information and understand its characteristics. In this study, FMDV O/ME-SA/Ind-2001 isolated from 2015 to 2018 in South East Asia such as Myanmar (n =1), Laos (n =5) and Vietnam (n =1) which were collected through collaborative research were investigated using the VP1 region. The results were compared with those of O/ME-SA/Ind-2001 isolated from Korea (n=13) and publically available data of VP1 sequence.

Materials and Methods: Viral RNAs were extracted from clinical epithelium or vesicular fluid samples (n=20) using a MagnaPure96 system (Roche). The VP1 regions were amplified using a one-step RT-PCR kit (Qiagen). PCR products were purified with ExoSAP-IT (USB) and directly sequenced on an ABI 3130 genetic analyzer (Applied Biosystems) using a Big Dye Terminator Kit v3.1 (Applied Biosystems). Phylogenetic tree estimated using the Maximum-Likelihood method in MEGA-7

Results: Phylogenetic analysis of VP1 gene showed that viruses recovered from the above outbreaks belonged to two different genetic clusters within the O/ME-SA/Ind-2001d/e(Fig 1). Sequence analysis revealed that viruses from Laos (n=5) belonged to the O/ME-SA/Ind-2001d. The viruses isolated in Myanmar was found to belong to the O/ME-SA/Ind-2001e, which was the same genotype as the isolated virus in Republic of Korea in 2017 and 2019.

Figure 1. The phylogenetic tree on VP1 gene of FMDV O/ME-SA/ind2001 from Vietnam, LAO, Myanmar and South Korea.



Conclusions: The O/ME-SA/Ind-2001 lineage had not been detected in South-East Asia(SEA) before 2015. However, the lineage has recently caused increasing concerns due to it multiple transregional movements. The FMDV O/ME-SA/Ind-2001e is occurring not only in Korea, but also in neighboring countries such as China, Vietnam and Myanmar, so continuous monitoring and genetic information collection are very important[2]. This genetic characterization will provide basic and essential information for epidemiology and control of FMD and support to describe risks of future outbreaks in Korea.

Acknowledgement: This research was supported by the Research of Animal and Plant Quarantine Agency (Project No. I-1543082-2018-22-02), Ministry of Agriculture, Food and Rural Affairs (MAFRA), Republic of Korea.

References:

[1] Y.Qiu et al., 2015. Transbound. Emerg. Dis. 65(1), e104-e112.

[2] Z.Zhu et al., 2018. Transbound. Emerg. Dis. 65(6), 2027-2031

Trans-038

Phylogenetic Analysis of the Spike Gene and Identification of a Novel Recombinant Porcine Epidemic Diarrhoea Virus Strain in Taiwan

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Introduction: Porcine epidemic diarrhoea virus (PEDV) causes a highly contagious intestinal disease, porcine epidemic diarrhoea (PED), characterized by vomiting, severe diarrhea, and dehydration in seronegative pigs at all ages with high mortality in suckling pigs. Before 2010, the PED was endemic and caused mild diarrhea majorly in post weaning pigs. However, the emerging new variants of group 2 (G2) PEDV since 2010 in China and G2b in late 2013 in North America and Asia caused up to 100% mortality in piglets less than 7 days old and severe economic losses highlighting the importance of continuously monitoring the genetic alteration of the virus for controlling the outbreaks of the disease. To better understand the genetic diversity of PEDVs recently circulating in Taiwan, full-length spike (S) genes of thirty-one PEDV strains from twenty-eight pig farms collected during 2016-2018 were sequenced.

Materials and Methods: A total of 31 intestinal and/or fecal specimens from 1-day to 6-week old piglets or sows with watery diarrhea from 28 different herds in Taiwan during 2016-2018 were confirmed PEDV positive by real-time reverse transcription polymerase chain reaction. For full-length S gene sequencing, cDNA samples were first amplified by PCR using the GDP-HiFi DNA polymerase (GeneDireX) with PEDV specific primers, 2F and 2R under following conditions: initial denaturation at 98℃ for 2 min, 40 cycles of denaturation at 94℃ for 15 sec, annealing at 60℃ for 30 sec, and extension at 68℃ for 4min 20sec, and a final extension at 68℃ for 5 min. The potential recombinant PEDV variant was performed for next generation sequencing (NGS).

Results: The majority (27/28 farms) of S gene sequences were closely related to the previous genogroup 2b (G2b) PEDV strains, increased genetic diversities leading to several nonsynonymous mutations scattering in the neutralizing epitopes of the S gene were detected in PEDVs recently circulating in Taiwan. Furthermore, a novel recombinant, the PEDV TW/Yunlin550/2018 strain, exhibiting recombinant events between the previous Taiwan PEDV G2b strain and a wild-type PEDV G1a strain was identified and further classified into a new genogroup, G1c.

Conclusions: The results provide updated information and the genetic diversity of current circulating PEDVs in the field and could help to develop the most suitable strategies for controlling the disease.

Acknowledgement: The NGS and analysis were supported by Center for Biotechnology and Bioinformatics and Biostatistics Core Lab, Center of Genomic and Precision Medicine, National Taiwan University. This work was supported by the Ministry of Science and Technology, Taiwan, R.O.C. for grants MOST106-2311-B-002-028-MY3 and MOST107-2321-B-033-002-, and 108L7842 and 107L7842 from National Taiwan University, Taiwan, ROC.

References:

[1] Chiou HY et al, 2017. *Transbound. Emerg.* Dis. 64, 157-166

Trans-039

Practical field experience of how to control and eradicate pseudorabies on a commercial farm in China

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Introduction: Pseudorabies (PR) is a widespread disease in China[1]. PR virus (PRV) infection in fattening pigs leads to slow growth and can trigger co-infections or secondary infections with other pathogens. To eradicate the disease, we must keep animals free from the field virus, therefore serologically negative to gE antibodies in fattening pigs and gilts. The aim of the present study was to determine whether, by using a commercial vaccine combination, with an optimized vaccination program, we were able to obtain gE-negative animals on an infected commercial farm in China.

Materials and Methods: One PRV-positive commercial farm with 3000 sows in Henan Province was selected for this study. At the beginning of the study, boars and sows were vaccinated with AUSKIPRA® GN (modified live virus vaccine) + A3 diluent every 3 months. Newborn piglets were intranasally sprayed with AUSKIPRA® GN + red diluent on the first day of life and vaccinated intramuscularly (IM) with AUSKIPRA® GN + A3 diluent at 6 and 13 weeks of age. The immunization procedure was changed 5 months later. Sows were vaccinated 4 times a year, with a combination of AUSKIPRA® GN (one shot every 6 months) and AUSKIPRA® BK (inactivated vaccine; one shot 4 weeks before farrowing). The first IM injection in piglets was delayed to 8 weeks of age. Gilts were vaccinated with AUSKIPRA® GN + A3 diluent 4 weeks before mating.

Results: Serum samples were collected from boars, gilts and growing pigs at different stages (10,12 or 13,15 or 16, 20, 24 weeks of age) and sows of different parities (1-2, 3-4, 5-6 parities) and gE antibody titers were analyzed by ELISA (Civtest, HIPRA, Spain). The results are shown in Figs 1, 2 and 3.

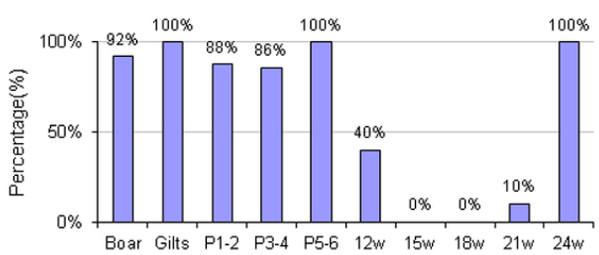

Fig 1. PRV gE antibody rate in May 2016

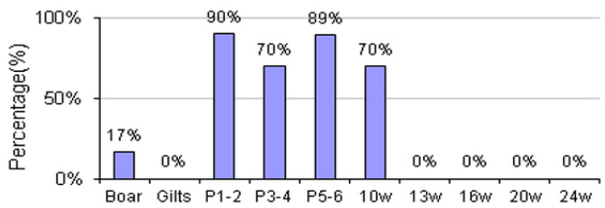

Fig 2. PRV gE antibody rate in Dec 2016

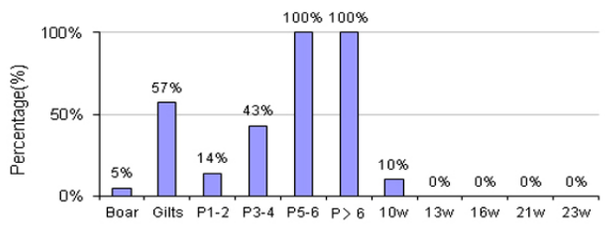

Fig 3. PRV gE antibody rate in Aug 2017

Conclusions: Five months after starting the program there was a reduction in gE-positive animals but there were still positive animals in the late fattening period (Fig 1). This was due to the fact that the positive pigs were not culled. A year after starting the new vaccination program, gE antibodies in fattening pigs became negative and the positive rate of gE antibodies in boars and sows of 1-4 parities decreased in varying degrees (Fig 2 and Fig 3). Finally, gilts became gE-positive at the end of the study owing to the introduction of positive animals on to the farm (Fig 3).

Field cases have challenging situations that put pressure on vaccine efficacy, such as increased infection pressure when positive animals are not eliminated, interference from maternal antibodies, and the introduction of positive animals.

Nevertheless, the present study demonstrates how a combined program with AUSKIPRA® GN and AUSKIPRA® BK was able to control the PRV on a farm and can be potentially used as a good tool for eradication of the disease.

Acknowledgement: This work was supported by HIPRA China.

References:

[1] Sun et al. (2018), PeerJ, DOI 10.7717/peerj.5785

Trans-040

Production of pseudorabies-free gilts by a combined vaccination program with modified live and inactivated vaccines

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Introduction: Pseudorabies (PR) is a common threat to pig farms in China nowadays. The key point for eradication of the disease on a farm with in-house gilt replacement is the production of PR-free pigs1. This study demonstrates an effective way to produce PR-free pigs on an in-house gilt replacement farm in China by using a combined vaccination program with a modified live vaccine (MLV) and an inactivated vaccine.

Materials and Methods: A commercial farm with 700 sows in Hunan province was selected for this study. Figure 1 represents the PRV gE status before the start of the study. Before the study, boars and sows were massively vaccinated, 4 times a year, with a live Bartha K-61 strain. Newborn piglets were intranasally sprayed with the vaccine on the first day of life, followed by an intramuscular vaccination with the same strain at 45 and 90 days of age respectively. Gilts were vaccinated intramuscularly twice before mating. During the study period, a combined vaccine program was implemented on the farm. Sows were vaccinated three times a year with AUSKIPRA® GN (a live Bartha K-61 strain) + A3 diluent with a booster injection with AUSKIPRA® BK (the same strain as AUSKIPRA® GN, but inactivated) 4 weeks before farrowing. Newborn piglets were intranasally sprayed with AUSKIPRA® GN + red diluent on the first day of life and the first and second intramuscular injection of AUSKIPRA® GN + A3 diluents were delayed until 70 and 100 days of age respectively. Boars were massively vaccinated 4 times a year with AUSKIPRA® GN + A3 diluent and gilts were vaccinated twice with AUSKIPRA® GN + A3 diluent before mating.

Results: Screening of the previous gE status shows that before the study, the gE antibody-positive rate in gilts was 100%; whilst in fattening pigs of 16, 20, 24 weeks of age it was 0, 17%, 67% respectively (Fig 1).

Figure 1. PRV gE status before the start of the study

Serological tests after the introduction of the new immunization program showed a decrease in the gE antibody-positive rate in gilts to 0%, and in fattening pigs of 16, 20 and 24 weeks of age, it decreased to 13%, 0%, 0% respectively (Fig 2).

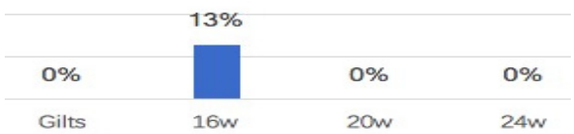

Figure 2. PRV gE status with the new immunization program.

Discussion: As shown in Figure 1, at the begin of the study, the gE-positive rate gradually increased during the fattening stage of pigs and until it was 100% in gilts, indicating that the immunization program could not offer sufficient protection for fattening pigs and gilts. With the new immunization program, combining MLV and inactivated vaccines, the fattening pigs remained free from PR in the later stage of the fattening period. Moreover, gilts also became gE negative, demonstrating that with the combined vaccination program of AUSKIPRA® GN and AUSKIPRA® BK, this farm could produce PR-free fatteners and gilts for in-house gilt replacement.

In conclusion, the results presented in this study show that the use of a combined vaccination program of AUSKIPRA® GN and AUSKIPRA® BK can produce negative gilts; a key element for the eradication of PR from a farm.

Reference:

[1] WU, Z.J.et al. Study on the immune effect of different vaccine immunization programs for ADV in pigs[J]. Swine production, 2016(01):105-107.

Trans-041

Rapid and specific detection of serotype O foot-and-mouth disease virus by reverse transcription loop mediated isothermal amplification

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease of domestic cloven-hoofed animals as well as many wild species. Rapid and accurate diagnosis of FMD virus (FMDV) is essential for the prompt control of FMD outbreaks. [1] An improved RT-LAMP assay using newly designed primers was developed for detection of serotype O FMDVs. In addition, for improving the sensitivity of the RT-LAMP, a swarm primers-applied RT-LAMP (sRT-LAMP) [2] was developed by applying a swarm priming strategy in this study.

Materials and Methods: Based on the conserved sequences of VP3 region of FMDVs, a set of six primers were manually designed for the LAMP assay aided by Primer Explorer V4 software (Fujitsu System Solutions, Ltd., Japan). In addition, two swarm primers (F1S and B1S) were designed to improve the sensitivity of the assay. The reaction conditions were optimized by performing test amplifications at temperatures ranging from 53°C to 68°C and reaction times of 20 to 50 min. The specificity and sensitivity of the assay were assessed. Clinical evaluation of the developed sRT-LAMP was carried out with different serotypes of FMDVs and compared with those of the reverse transcription-polymerase chain reaction (RT-PCR) and real time RT-PCR (qRT-PCR).

Results: The sRT-LAMP was completed in 40 min at 62°C. The assay specifically amplified VP3 gene of serotype O FMDV but not amplified other serotypes of FMDVs and other viruses. The limit of detection (LOD) of the assay was 10^2 $TCID_{50}$/mL, which is 100 times lower than that of the RT-LAMP assay without swarm primers. The new assay is 10 times more sensitive than RT-PCR and is comparable to the sensitivity of qRT-PCR (Fig. 1). Evaluation of the assay using different serotypes of FMDV strains showed 100% agreement with the RT-PCR results.

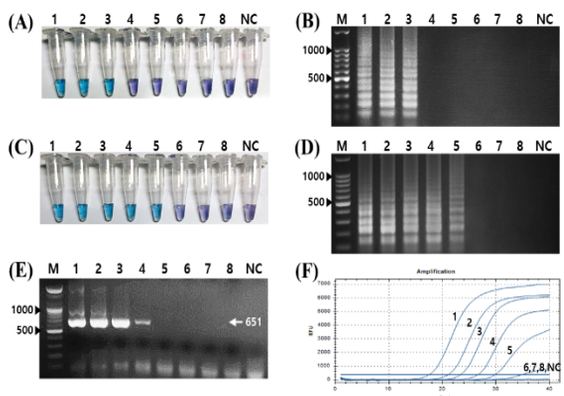

Fig. 1. Comparison of sensitivities of the RT-LAMP, sRT-LAMP, RT-PCR and qRT-PCR assays.

LOD of the RT-LAMP assay without swarm primers (A and B), sRT-LAMP assay with swarm primers (C and D), RT-PCR assay (E), and qRT-PCR assay (F) for the amplification of FMDV RNA. Tubes, lanes and lines 1-8, 10-fold serial dilutions (from 10^6 to 10^{-1} $TCID_{50}$/mL) of FMDV (O/Andong/KOR/2010); Lane M, 100 bp DNA marker; NC, negative control.

Conclusions: The sRT-LAMP assay with improved primers can rapidly and accurately diagnose serotype O FMDV circulating Pool 1 region including Korea, and it will be an alternative diagnostic tool for conventional RT-PCR and qRT-PCR.

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[1] OIE, 2016. Foot and Mouth Disease, OIE Terrestrial Mannual, pp. 1-31 (Chapter 2.1.8).

[2] Martineau. et al., 2017, Anal. Chem. 89, 625-632.

Trans-042

Rapid and visual detection of all serotypes of foot-and-mouth disease viruses by reverse transcription loop- mediated isothermal amplification

Da-Rae Lim[1], *Hye-Ryung Kim*[1], *Min-Ji Park*[1], *Ha-Gyeong Chae*[1], *Bok-Kyung Ku*[2], *Jin-Ju Nah*[2], *So-Yoon Ryoo*[2], *Sung-Hwan Wee*[2], *Sang-Geon Yeo*[1], *Choi-Kyu Park*[1*]

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious disease of domestic cloven-hoofed animals and can cause significant economic losses. Rapid and accurate diagnosis of FMDV is essential for the effective control of the FMD outbreaks. Molecular assays such as reverse transcription-PCR (RT-PCR) and real-time RT-PCR (qRT-PCR) are currently used for routine diagnosis of FMDV as OIE-recommended diagnostic assays due to their higher specificity and sensitivity and rapidity concurrently [1]. However, these PCR-based assays require sophisticated equipment and complicated procedures for the detection of amplified products. Since the development of loop-mediated isothermal amplification (LAMP) in 2000, the assay has been recognized as a valuable tool for the detection of various pathogens, with high sensitivity and specificity, rapidity, and simplicity [2]. In this study, we developed a sensitive and specific reverse transcription-loop mediated isothermal amplification (RT-LAMP) assay for rapid visual detection of foot-and-mouth disease virus (FMDV).

Materials and Methods: Conserved nucleotide sequences within the 3D region were identified by multiple alignment using the BioEdit Sequence Alignment Editor program. Based on the conserved sequences, a set of six primers, were manually designed for the LAMP assay aided by Primer Explorer V4 software (Fujitsu System Solutions, Ltd., Japan). The reaction conditions were optimized by performing test amplifications at temperatures ranging from 56°C to 66°C and reaction times of 20 to 60 min. Reactions were terminated by heating at 80°C for 5 min. In addition, specificity and sensitivity of the assay were assessed.

Results: The RT-LAMP was completed in 40 min at 62℃ and the results of the assay directly detected by naked eye without any detection process. The assay specifically amplified all 7 serotypes of FMDV RNAs but not amplified other viral and cellular nucleic acids. The sensitivity of the RT-LAMP was 102, 103 and 103 TCID50/mL for serotype O, A and Asia 1 FMDV, respectively, which was comparable to RT-PCR and relatively lower than that of qRT-PCR. Clinical evaluation of the RT-LAMP using different serotypes of Korean and foreign FMDV strains showed that the results of the RT-LAMP was a 100% (35/35) agreement with the results of the RT-PCR and qRT-PCR.

Table 1. Comparative diagnostic efficiency between RT-PCR, qRT-PCR and RT-LAMP for the detection of different serotypes of Korean and foreign FMDV strains

Serotype of FMDV	Results (No. of positive/tested)		
	RT-PCR	qRT-PCR	RT-LAMP
O	21/21	21/21	21/21
A	10/10	10/10	10/10
Asia 1	3/3	3/3	3/3
C	1/1	1/1	1/1
Total	35/35	35/35	35/35

Conclusions: The RT-LAMP assay is simple, rapid, highly specific, and highly sensitive, and the assay results can be observed directly by the naked eye without the need for any analysis equipment. Therefore, this assay will be a valuable tool for the rapid diagnosis of FMDV in clinical samples, even in under-equipped laboratories.

References:

[1] OIE, 2016. Foot and Mouth Disease, OIE Terrestrial Mannual, pp. 1-31 (Chapter2.1.8).
[2] Notomi et al., 2009, J. Infect. Chemother. 15, 62-69.

Trans-043

Reverse transcription loop-mediated isothermal amplification assay for specific detection of serotype A foot-and-mouth disease viruses circulating pool 1 region countries

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Introduction: Foot-and-mouth disease (FMD) outbreaks result in significant economic losses due to reduced animal productivity and drastic trade restrictions. Reverse transcription-polymerase chain reaction (RT-PCR) and real-time quantitative RT-PCR (qRT-PCR) have been an OIE-recommended diagnostic assay [1] for routine diagnosis of FMDV. However, these PCR-based assays are unsuitable for under-equipped laboratories in developing countries. In this study, to overcome these shortcomings, a simple, rapid, and cost-effective reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed and evaluated for the sensitive and specific detection of serotype A FMDV circulating in the pool 1 region countries.

Materials and Methods: Based on conserved VP1 sequences of the FMDVs, a set of six primers were manually designed for the RT-LAMP assay that can detect serotype A FMDVs circulating in pool 1-region countries during 2000-2017, aided by Primer Explorer V4 software (Fujitsu System Solutions Ltd., Japan). The reaction condition of RT-LAMP was optimized the specificity and sensitivity of the developed RT-LAMP was carried out with different serotypes of FMDVs and compared with those of the RT-PCR [2] and qRT-PCR [3]. Clinical evaluation of the assay was performed using Korean and foreign FMDV strains and the results of the developed RT-LAMP assay were compared with previously reported RT-PCR, qRT-PCR and RT-LAMP [4] assays as well.

Results: The amplification could be completed in 40 min at 62°C, and the results could be visually detected by the naked eye without any additional detection systems. The assay specifically amplified the VP1 gene of the Sea-97 genotype of serotype A FMDV, but it did not amplify other viral nucleic acids. The limit of detection of the assay was 10^2 $TCID_{50}$/mL, which is 10 times more sensitive than RT-PCR and is comparable to the sensitivity of qRT-PCR. Evaluation of the assay using different FMDV strain serotypes showed 100% agreement with the results of RT-PCR. The previously reported serotype A-specific RT-LAMP did not detect serotype A FMDV circulating Pool 1 region countries including Korea because the primers and template sequences have multiple mismatches.

Table 1. Comparative diagnostic results for the detection of different serotypes of FMDVs

Serotypes of FMDV	Results of different methods (No. of positive/tested)			
	qRT-PCR	RT-PCR	preRT-LAMP	RT-LAMP
O	22/22	0/22	0/22	0/22
A	8/8	8/8	0/8	8/8
Asia 1	3/3	0/3	0/3	0/3
C	1/1	0/1	0/1	0/1
SAT 1	1/1	0/1	0/1	0/1
SAT 2	1/1	0/1	0/1	0/1
SAT 3	1/1	0/1	0/1	0/1
NTS	0/10	0/10	0/10	0/10
Total	37/47	8/47	0/47	8/47

Conclusions: The newly developed RT-LAMP assay using tailored primers can rapidly and accurately diagnose serotype A FMDV circulating Pool 1 region including Korea. The results can be observed directly by the naked eye, and it will be an alternative diagnostic tool for conventional RT-PCR and qRT-PCR.

References:

[1] OIE, 2016. Foot and Mouth Disease, OIE Terrestrial Manual, pp. 1-31 (Chapter2.1.8)
[2] Le et al., 2011, J. Virol. Methods 175, 101-108
[3] Callahan et al., 2002, J. Am. Vet. Med. Assoc. 220, 1636-1642.
[4] Madhanmohan et al. 2013. J Virol. Methods, 187, 195-202.

Trans-044

RNA secondary structure variation of CRE of FMDV is related with host sensitivity of infection in Korea

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Introduction: Foot-and-mouth disease (FMD) is a highly contagious and fulminating infectious disease in mammals. RNA templated RNA synthesis is a central step in the lifecycle of FMD viruse (FMDV), which replicate and transcribe their genomes in the cytoplasm of the infected host cell. RNA replication is carried out on membranous structures by the viral RNA-dependent RNA polymerase, in conjunction with other viral and cellular proteins and cis-replicating RNA element (CRE). The replication initiated by a protein primer is the most unique one, in which the virus encodes a protein, viral protein genome-linked (VPg). The VPg uridylylation process in FMDV acts in a template-dependent manner by using a small stem loop structure of CRE as the natural template. The secondary structure of FMDV CRE might play a critical role in forming an effective RNA replication complex of FMDV amplification.

Materials and Methods

FMDV stock production: Viruses were amplified in the species they were isolated from bovine or porcine. One aliquot of each viral stock was subsequently thawed for titration in cells and to inoculate animals.

Analysis of sequence arrangement: We compared the full genome sequence of FMDV dependent on countries and years through NCBI database. The viral gene sequences and protein sequences were analyzed by using ClustalW Multiple alignment.

Modeling of RNA secondary structure: The RNA secondary structure was produced by Fold Web Server (http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Fold/Fold.html) with the lowest free energy calculation.

Results

Sample collection and sequence variation analysis of FMDV non-coding region: Serotype O strains were amplified in bovine and named O/GH/SKR/2010, O/AD/SKR/2010, O/ BE/SKR/2017, O/JE/SKR/2017. Serotype O strains were amplified in porcine and named O/GJ/SKR/2016, O/JC/SKR/2014, O/US/SKR/2014, O/GC/SKR/2016, O/AD/SKR/2010, O/YJ/SKR/2010. Serotype A strains were amplified in bovine and named A/YC/SKR/2017, A/PC/SKR/2010. Serotype A strain was amplified in porcine and named A/GP/SKR/2018. After obtaining the strains, we analyzed full sequencing of each strains and compared sequence variation.

Comparison of secondary structure of RNA of FMDV CRE: Since CRE region is essential for viral replication, we focused on the gene variation of the CRE region. We found the RNA nucleotide differences among the CRE region dependent on host species and years. We converted their RNA sequences into the secondary RNA structure following Fold Web Server program.

Host species difference of secondary structure of RNA of FMDV CRE: For efficient FMDV gene replication in the infected host cells, the RNA replication complex requires CRE non-coding regulatory RNA, FMDV 3B/3D proteins, and host cell-specific replication factors. The species-specific replication factors of cow or pig might interact with the RNA replication complex of FMDV.

Discussion: Given these observations, it appeared likely that FMDV have acquired a mechanism to efficiently replicate their own genomic RNA via an exquisite interplay of regulatory mechanisms involving virally encoded proteins, a number of host factors, and at least three structural RNA elements. VPg association with this complex is weak, suggesting that formation of a complex containing all necessary components of the reaction is rate-limiting for the reaction. The secondary structure of CRE RNA provides a docking site for FMDV viral 3B and 3D proteins.

References

[1] Yuan Gao et al., 2016. J. Virol. 13:107.
[2] Yuna Sun et al., 2014. Current Opinion in Virology 9:24-30

Trans-045

Separation of foot-and-mouth disease virus and non-structural protein using a hydrophobic interaction chromatography

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Introduction: The Korean foot-and-mouth disease (FMD) vaccines are under developing to replace the imported FMD vaccines. One of the important elements in FMD vaccine antigen production is the elimination of viral non-structural proteins (NSPs) because antibodies to NSPs can be used as indicators of distinguishing between vaccinated from naturally infected animals. However, FMD vaccines made by polyethylene glycol (PEG) precipitation method contain residual NSPs [1]. Therefore, in this study, hydrophobic interaction chromatography (HIC) based on the principle of hydrophobicity instead of charge was applied to separate FMD virus particles (146S) away from NSPs [2].

Materials and Methods: BHK-21 suspension cells (6×10^6 cells/ml) were inoculated with O/Jincheon/SKR /2014 virus at a multiplicity of infection (MOI) of 0.001 for 16 hours. FMDV was inactivated with 6mM of binary ethylenimine (BEI) at 26℃ for 28 hours. Chromatographic analysis was carried out using FPLC (AKTA™ Pure) with fraction collector F9-R (GE Healthcare). The FMD virus supernatant was loaded onto the HIC column with 50mM potassium phosphate buffer containing ammonium sulfate and then eluted with the buffer without ammonium sulfate. FMD virus particles were quantified by sucrose density gradient analysis and NSPs were confirmed by Western blot analysis.

Results: The chromatogram showed a graph obtained by injecting the supernatant into the column (Fig 1A). Analysis of flow-through and elution fraction showed different 146S antigen recovery and NSP removal according to ammonium sulfate concentration. The optimal concentration for the hydrophobic binding of NSPs to the column was investigated. Under the established condition, 146S antigen was washed out with a recovery rate of 86% (Fig 1B) and NSPs were noticeably separated from 146S antigen by binding to hydrophobic ligand in the flow-through fraction (Fig 1C). After purification, 146S antigen in the flow-through fractions of the HIC column was confirmed to be intact based on a spherical shape and diameter of 30nm by TEM (Fig. 1D).

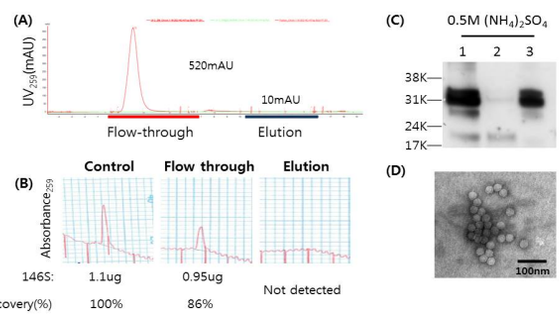

Figure. 1. Separation of 146S antigen and NSPs by HIC (A) Chromatogram showing purification process on the AKTATM purifier (B) Quantification of 146S antigen using UA-6 absorbance detectors (Teledyne lsco, USA) after sucrose density gradient centrifugation (C) Detection of NSP by Western blot analysis with anti-FMDV 3B antibodies. Lane1: FMDV crude, 2: Flow-through fraction, 3: Elution fraction (D) Observation of purified virus particles using transmission electron microscopy (TEM)

Conclusions: A chromatographic method separating 146S antigen and NSP was successfully established. This method is much more economical and time-saving than conventional PEG precipitation method. Therefore, it could be applied as domestic technology for FMD vaccine production using local FMDV strains.

Acknowledgement: This research was supported by the grant (B-1543386-2018-19-02) from the Animal and Plant Quarantine Agency, Republic of Korea.

References:

[1] Barteling SJ, 2002. Rev Sci Tech. 21. 577-588
[2] Hao Li, et al., 2015.Pro.Expr. puri. 113. 23-26

Trans-046

Single-dose E2 subunit vaccine "Tian Wen Jing"confers full protection against lethal challenge of classical swine fever virus

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Introduction:

Classical swine fever (CSF) still threats swine industry worldwide,and vaccination with modified live attenuated vaccine (MLV) is still the main strategy to control CSF in the endemic countries.In China, extensive vaccination with MLV C-strain has largely controlled the CSF epidemics. However, the efficacy of C-strain vaccine can be influenced by multiple factors including maternal antibody, cold chain storage and transportation, co-infection with immunosuppressive pathogens and so on, these may explain why sporadic outbreaks of CSF still occurred in the C-strain vaccinated swineherds in China. More important fact is that C-strain vaccination makes DIVA strategy impossible. In this case, marker vaccines companioned with discriminatory Erns antibody diagnostic ELISAs are necessary for CSF eradication. In 2018, the first E2 subunit vaccine (Tian Wen Jing, TWJ) was commercialized in China and developed by TECON, the efficacy of single dose of this vaccine was evaluated in the present study.

Materials and Methods:

About 200 pregnant sowsin the field were individually immunized with two doses ofTWJ-E2 and HCLV (C-strain) vaccineat 3 weeks interval in the middle stage of pregnancy.Two hundred piglets farrowed by E2-vaccinated sows were immunized with single dose of E2 subunit vaccine when the blocking rate of E2 antibody was <70%, and those from C-strain vaccinated sows (200) were immunized with two doses of C-strain vaccine at 4 and 8-week old respectively. Both E2 and C-strain vaccinated piglets were kept in the field until 24-week old, and sera were collected for detection of E2 antibody responsefrom 4 to 24 weeks at 1 to 4-week intervals. At the age of 24 weeks, 5 pigs from each vaccinated group were randomly selected and another 5 mock unvaccinated pigs from the C-strain vaccinated sows were used as the control, which were kept at the negative-pressure facilities affiliated to TECON.After observation for 3-5 days, all pigs were challenged with CSFV highly virulent Shimenstrain. After challenge, rectal temperature, clinical signs were daily recorded, and all euthanized, moribund or death pigs were subjected to necropsy for pathology. Whole blood samples were also collected for determining CSFV antigen with performed with quantitative real-time RT-PCR. The artificial infection test was reviewed and approved by the Experimental Animal Welfare Ethics Committee of the Tecon Biopharmaceutical Business Unit.

Results:

Before vaccination (4 weeks old), 77.8% piglets from C-strain vaccinated sows were positive for E2 antibody, which was significantly lower than those fromE2-vaccinated sows (100%), which was lasted to 8 weeks and decreased to 76.5% at 12 weeks old. After the firstvaccination with C-strain, the positive rate of piglets with E2 antibody decreased, butincreased after booster and reached the peakat the age of 10-12 weeks (94.4%), but decreased to 74.2% at 24 weeks.For the E2-vaccinated pigs the positive rate of E2 antibody reached 100% after single dose vaccination and decreased to 86.7% at 24 weeks. After challenge, all E2 and C-strain vaccinated pigs survived the lethal challenge, not showing fever, clinical signs, pathological alterations, and viremia, but the mock unvaccinated pigs displayed persistent fever, typical CSF clinical signs and pathological lesions, and long-lasting viremia.

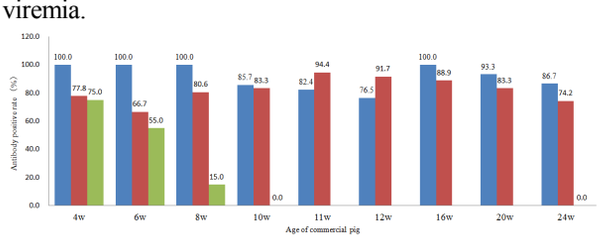

Fig. 1Dynamics of E2 antibody response after vaccination with TWJ-E2 and C-strainvaccine

Conclusions: Single-dose E2 subunit vaccine can provide effective protection against lethal challenge of CSFV, which is comparable to that elicited by two-dose of C-strain vaccine.

References:

[1] Blome S, et al., 2017, Viruses, 9(4): 1-25.

Trans-047

Strategies to prevent antigen loss during FMD vaccine production and storage

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Introduction: Foot-and-mouth disease virus (FMDV), an *Aphthovirus* within the family *Picornaviridae*, is a highly infectious, antigenically variable pathogen of cloven-hoofed animals [1]. Since 2000, there have been constant FMD outbreaks in South Korea. Especially, the outbreak during 2010-2011 was so catastrophic that Korean government implemented a vaccination policy and set out to research about development of domestic FMD vaccine. One of the candidate FMD virus strains for Korean domestic vaccine development is O/SKR/JC/2014, which was isolated from Jincheon county in South Korea 2014. Although it was able to produce fresh vaccine antigens with O/SKR/JC/2014 more than 2 ug/ml, it was difficult to preserve them for sufficient time due to its characteristic instability.

Materials and Methods:

Preparation of virus.

The FMDV O/SKR/JC/2014 (O Jincheon) strain was inoculated with 0.002 multiplicity of infection (MOI) in 6×10^6 cells/mL of BHK-21 suspension cells with ProVERO-1 media. During shaking incubation in the 5% CO_2 incubator at 37 ℃, viruses were harvested at 16 hours (h) post-infection (p.i.) and clarified by centrifugation at 4000 rpm for 20 min at 4 ℃ to remove cell debris.

Determination of optimal storage buffer and excipient

Purified FMDV O Jincheon antigens were dissolved in each buffer; Tris/NaCl (TN), Phosphate Buffered Saline (PBS), PBS/$MgCl_2$ ($MgCl_2$), PBS/EDTA (EDTA), Potassium Phosphate (PP) buffer, and buffer X. During storage at 4 or -70°C for long term, the amount of vaccine antigen was examined. Combinational effects of excipients such as sugar, protein, amino acid complex, and polyol were analyzed by heating and storage test.

Evaluation of viral stability

146S particle content of the samples were analyzed after concentration using PEG. Samples were loaded onto sucrose density gradient and then separated by ultracentrifugation. Based on the absorbance at 259 nm in the density gradient fractionation system, the amount of 146S vaccine antigen was estimated.

Results: When the vaccine antigen was stored either in 4℃ or -70℃, buffer X was the most stable preservation buffer regardless of the storage temperature. Addition of sucrose and amino acid complex into the buffer X potentiated stabilizing effect.

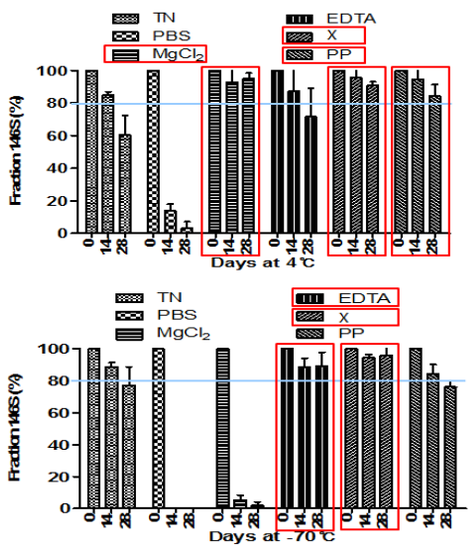

Figure 1. Stability of 146S antigen dependent on the storage buffer: (upper) 4℃, (lower) -70℃.

Conclusions: While the most widely used TN buffer was not suitable for O Jincheon strain, potassium-based buffer X and combinational use of excipients highly increased its stability during the production process and storage. These results can be usefully applied to other unstable FMD vaccine strains.

Acknowledgement: This research was supported by the grant (B-1543386-2018-19-01) from the Animal and Plant Quarantine Agency, Republic of Korea.

References:

[1] Scott KA et al., 2017. J. Virol. 91: e02312-16.

Trans-048

Surveillance of swine vesicular disease and vesicular stomatitis in the Republic of Korea during 2017-2018

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Introduction: Swine vesicular disease (SVD) is a contagious disease of swine caused by an Enterovirus of *Picornaviridae*. Vesicular stomatitis (VS) is an infectious vesicular disease of horses, cattle and pigs, induced by a Vesiculoviris of *Rhabdoviridae*. Both vesicular diseases are hard to be distinguished clinically from foot-and-mouth disease (FMD). Therefore, laboratory diagnosis is essential for the investigation and surveillance for both diseases. Recognition and declaration of free status from SVD and VS by the World Organization for Animal Health (OIE) is important to countries for access to lucrative foreign markets. Providing sufficient evidence that the country is free from these exotic diseases requires an effective level of surveillance to be performed. In this study, serosurveillance was conducted to demonstrate freedom from SVD and VS in the Republic of Korea.

Materials and Methods: Surveillance model was designed by considering the appropriate sampling strategy, characteristics of the disease, stratification, diagnostic method, calculation method and surveillance procedure. Sera for tests were collected by the Local Veterinary Services from 2017 to 2018. The samples were tested by enzyme-linked immunosorbent assay (ELISA) as a vscreening test and virus neutralization test as a confirmatory test. ID Screen Swine Vesicular Disease Competition ELISA kit (ID-Vet, Montpellier, France) in 2017 and PrioCHECK SVDV ELISA kit (Thermo, USA) in 2018 were used for detecting the antibody against SVD. Reagents of VSV ELISA from National Veterinary Services Laboratories (NVSL) were used for detecting antibody to VS-New Jersey (NJ) and VS-Indiana (IND) strains.

Results: Sera of 3,668 pigs from 723 farms were all negative for antibodies to SVD. 2,146 cattle sera from 497 farms and 2,651 cattle sera from 604 farms were negative for antibodies to VS-NJ and VS-IND, respectively. 1,936 pig samples from 484 farms were shown to be sero-negative for both strains.

Conclusions: These results could be the supporting data for that the Republic of Korea maintain the free status from SVD and VS during 2016-2018. These data will be accumulated to the national surveillance program for SVD and VS, and the program will be conducted continually.

Acknowledgement: This study was supported by a grant from Animal and Plant Quarantine Agency (APQA), Ministry of Agriculture, Food and Rural Development (MAFRA), Republic of Korea

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[1] OIE. Swine vesicular disease and Vesicular stomatitis. In Manual of Diagnostic & Vaccines for Terrestrial Animals, World Organization for Animal Health (OIE), Paris, France. 2019
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[4] Alvarado JF et al., 2002. J Vet Diagn Invest. 14: 240-247.

Trans-049

The duration of Classical swine fever virus Erns antibody in C strain vaccinated sows

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Introduction:

Classical swine fever (CSF) is one of the most important diseases in domestic pigs and wild boar and is caused by CSF virus (CSFV). An immune response against CSFV is induced against the envelope proteins E2 and Erns as well as against the non-structural protein NS3. Particularly, virus-neutralizing E2-specific antibodies play a key function in a protective immune response. TECON developed the first E2 subunit marker vaccine against classical swine fever in China in 2018. Thus Erns antibody ELISAs can be used to differentiate between CSFV-infected animals and animals vaccinated with an E2 subunit vaccine. Modified live virus (MLV) vaccines such as the CSFV C strain vaccine is widely used in China. However, the knowledge of the duration of C strain induced Erns antibody in sows is lacking and is a perquisite to interpret Erns antibody as CSFV infection in a farm during its transition period from C strain to E2 vaccine. Therefore, this study was conducted to fill up the above gap.

Materials and Methods:

A commercial sow farm free of CSFV was selected to replace the C strain vaccine with E2 vaccine since April 2017 to perform 3 vaccinations per year to observe the natural decline of C strain induced Erns antibody in sow. 10 sows per parity were sampled quarterly from 1st quarter in 2017 to 1st quarter in 2018 and detected the Erns antibody with the prototype pigtype Erns ELISA kit (Indical Bioscience, Germany)[1]. The Erns positive rate in each parity was averaged to exclude the effect of parity to be considered as the Erns positive rate for the sows.

Results:

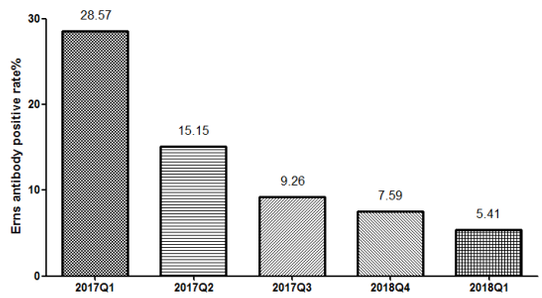

Fig. 1 Duration of CSFV Erns antibody in C Strain vaccinated sows

The Erns positive rate at 2017Q1 suggests that at least 50% sows vaccinated with C strain are Erns antibody negative. There is a continuous decline of Erns antibody in sows with the cease of C strain and it takes one year for Erns positive rate to drop from 28.57% to 5.41%.

Conclusions:

Our study shows the Erns positive status resulted from the C strain vaccination may last 1 year. The Erns antibody positive cannot be interpreted as a result of CSFV infection during this stage.

References:

[1] D. Meyer et al. The double-antigen ELISA concept for early detection of Erns-specific classical swine fever virus antibodies and application as an accompanying test for differentiation of infected from marker vaccinated animals. Transbound Emerg Dis 2017; 1-10

Trans-050

Type O foot-and-mouth disease vaccine, O JC-R, induce complete protection against SEA topotype viruses occurred in South Korea

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Introduction: There are seven serotypes of the foot-and-mouth disease virus (FMDV), of which the O serotype most frequently causes an outbreak of FMD globally [1]. In Korea, a SEA topotype FMDV outbreak lasted for five months-from December 2014 to April 2015 [2]-but no virus strains similar to this SEA topotype were observed for a while until the outbreak in 2016 from January to March [3]. Unlike other virus strains, we guess that this FMD would not be eradicated, but persisted for 3-5 months after its first case in 2014 and 2016.

Materials and Methods: The infectious cDNA plasmid, which was already secured by removing the $3B_1B_2$ site and manipulating the site into $3B_3B_3$, was used; in addition, an infectious clone where the 142 residue [C142T] in the 3C was manipulated, such that C was replaced by T was also used. The vaccine was prepared using the method used in Ko et al.'s (2019) study. To briefly explain the method, 15 μg (1 dose) of purified 146S antigen of O JC-R was mixed with ISA206VG (Seppic, Paris, France) in a ratio of 1:1 (volume [v]/v); then, 10% aluminum hydroxide gel (Rehyragel® HPA; General Chemical, NJ, USA) and Saponin 0.5 μg were added into that mixture to prepare the vaccine in the form of water-in-oil-in-water.

Results: Based on the results of the virus neutralization test and the challenge test, the vaccine strain developed in this study is believed to be able to provide protection against the SEA and ME-SA topotypes. Antibody titers against the Cathay topotype were somewhat lower, but 75% of the animals had a level of 1:45 VNT or higher, which is generally known as the protection antibody level. This suggests that sufficient protection would be provided in most cases, but a secondary immunization is required. This claim could be supported by the results of the challenge test after immunization, which showed that animals were protected at low antigen concentrations after the challenge of both the SEA and ME-SA topotype strains, as predicted in the challenge test results in mice.

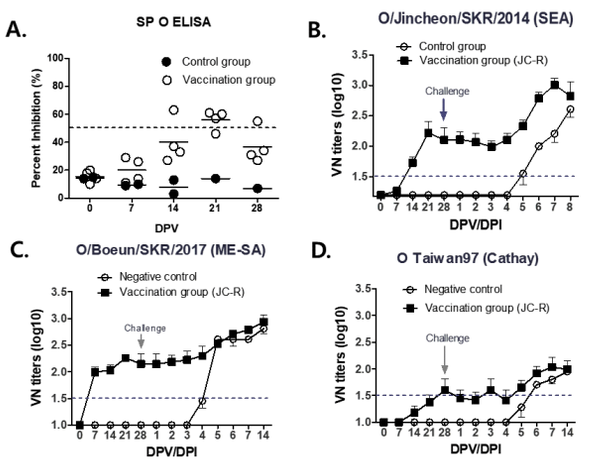

Fig. 1. Antibody titers in the pigs vaccinated with the experimental FMD vaccine, O JC-R.

Conclusions: It was confirmed that pigs immunized with the vaccine strain developed in this study were completely protected against the SEA virus, and sufficient neutralizing antibody titers that can protect against the ME-SA virus at the same level as the SEA virus circulating in Southeast Asia were detected.

Acknowledgement: We thank the staff of the Center for FMD Vaccine Research and Mr. Jung-Won Park for providing assistance with electron microscopy at the Animal and Plant Quarantine Agency.

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[1] Brito et al., 2017. Transboundary and emerging diseases 64, 316-332
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Trans-051

Unique genetic alterations observed in the 3′UTR region of classical swine fever virus LOM isolates detected from pigs with clinical symptoms in Jeju island

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Introduction: Classical swine fever virus (CSFV) is a disastrous viral disease characterized by high mortality and morbidity in pigs. For the effective control of CSFV, live attenuated vaccine strains, such as HCLV, C-strain, Thiverval strain, have been used worldwide for decades. Likewise, in Korea, national vaccination programs based on LOM strain, derived from a low virulent Miyagi strain isolated in Japan, has been implemented during the past 40 years. However, our group has shown that the LOM strain is not safe enough to be used for a vaccine strain in an immunologically naïve pig population [1]. Since an accidental introduction of LOM strain into Jeju island in 2014, the vaccine strain continues to spread across the western part of Jeju island with genetic mutations. The purpose of this study is to report and characterize unique genetic changes observed in 3′ -untranslated region (UTR)

Materials and Methods: Clinical samples such as aborted fetuses and tissue samples from several commercial pig farms suffered from LOM-associated abortions and wasting were submitted for laboratory analysis.
Those specimens underwent pretreatment procedure and RT-PCR analysis for CSFV infection. Complete genome sequence analysis was performed on positive samples as previously described [2].
Obtained sequences were aligned using BioEdit software and analyzed by comparison with a reference LOM sequence (GenBank accession no. EU789580). Subsequently, two strains with significant genetic changes in 3′ UTR region were subjected for secondary structure prediction to identify if there occurred structural changes. The prediction was performed using Mfold web server (http://unafold.rna.albany.edu/?q=mfold).

Results: Complete genome analysis revealed that two LOM variants (provisionally named strain 1 and 2) harbored altered 3′ UTR sequences; Two uridine deletions around adenine at nucleotide at 12134 (position based on reference LOM sequence) and poly uridine insertions staring at nucleotide 12225 with various levels were confirmed. An average of 20 and 3 uridine insertions were identified in strain 1 and 2, respectively. Through structure prediction analysis, it was identified that the structure of step loop (SL) 3 were changed and the distance between SL1 and SL2 become longer (Fig. 1).

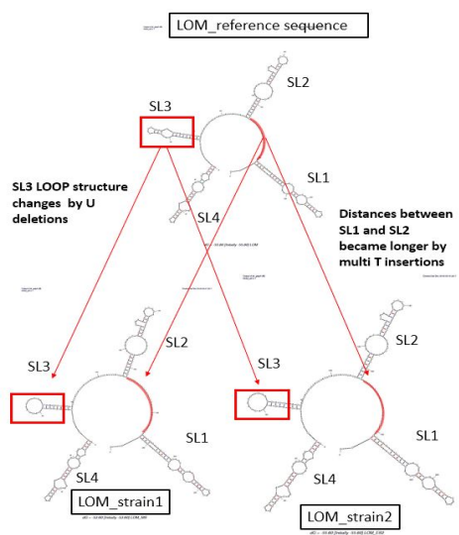

Fig. 1. Predicted secondary structure of 3′ UTR region of LOM variants. The structure is composed of 4 stem-loop (SL) structures. Reference strain, dG=-55.8; Strain1, dG=-53.6; Stain 2. dG=-55.6.

Acknowledgement: This work was supported by grant from the Department of Homeland Security Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) at Kansas State University, USA.

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[1] Je SH et al., 2018. Emerg. Infect. Dis., 24, 798-800.
[2] Yoo SJ et al., 2018. Transbound. Emerg. Dis. 65, 735-745

Trans-052

Using Sentinel® CSFV E2 Ab Rapid Test to Detect Classical Swine Fever E2 Antibody and Evaluate the vaccination efficacy

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Introduction: Classical Swine Fever (CSF) caused by CSF Virus (CSFV) infection is highly contagious and can rapidly spread with high morbidity and fatality in swine [1, 2]. In epidemic areas, attenuated virus vaccine or CSFV E2 subunit vaccine have been used to prevent the disease. The titer of neutralization antibody against CSFV E2 induced by the vaccine is related to the protection from CSFV. Therefore, measuring the serum neutralization antibody titer specific to CSFV is an important step in seeking to prevent CSF. We have developed a rapid chromatographic strip test (Sentinel® CSFV E2 Ab RT) which was designed to detect anti-CSFV E2 antibody in swine blood within 10 minutes. We use the CSFV E2 Ab RT to detect the anti-CSFV E2 antibody and determine the antibody positive rates of weaners from 6 to 18 weeks old. The results of our rapid test were compared with those of a competitive CSFV antibody ELISA.

Materials and Methods: A total of 988 time-course swine blood samples were harvested at 6, 7, 8, 9, 10, 14, 15, 16, 17, 18 weeks of age from the Best Genetics Group, China. Pigs were vaccinated with an attenuated virus vaccine (lapinized Chinese strain vaccine) at 10 weeks of age. For the CSFV E2 Ab RT, 50 μ L serum sample was added into a sample well of CSFV E2 Ab RT. Subsequently, three drops (~100 μ L) of running buffer were added to the sample application well. The results were interpreted at 10 minutes. Positive result showed two distinct lines (both Control Line and Test Line). The anti-CSFV E2 antibody titers also were determined by the IDEXX CSFV E2 ELISA Test. The blocking percentage (PI %) < 50% indicates negative result and PI ≧50% indicates positive result.

Results: For the 6, 7, 8, 9, 10 weeks old pigs, the results of CSFV E2 Ab RT show the positive rates were 38%, 34%, 14%, 4% and 3.1%, respectively. And, the results of CSFV E2 Ab ELISA show the positive rates were 43%, 50%, 17%, 4% and 1%, respectively. Both of methods indicated the anti-E2 antibody decreased to basal levels in 10 week old pigs, before vaccination. After vaccination in 10 week old pigs, the positive rates of anti-E2 antibody became 94% in 14 week old pigs and 99% in 17 week old pigs on CSFV E2 Ab RT. The positive rates were 83% and 98% in 14 and 17 week old pigs on CSFV E2 Ab ELISA. Both of results indicated the positive rates of anti-E2 antibody were significantly increased from 4 to 7 weeks after vaccination. For the 18 week old pigs, the positive rates of anti-E2 antibody was 89% in both CSFV E2 Ab RT and CSFV E2 Ab ELISA. Based on the virus neutralization test, the sensitivity and specificity of CSFV E2 Ab RT were 90.1% and 88.4%, respectively. The statistical correlation was 89.4% between CSFV E2 Ab RT and CSFV E2 Ab ELISA.

Table 1. The correlation between CSFV E2 Ab RT and commercial competitive CSFV E2 Ab ELISA

		CSFV E2 Ab ELISA		
		+	-	Total
CSFV E2 Ab RT	+	511	49	560
	-	56	372	428
	Total	567	421	988
Statistical correlation: 89.4%				

Conclusions: The Sentinel® CSFV E2 Ab RT exhibits great capability to detect anti-CSFV E2 antibody. The Sentinel® CSFV E2 Ab RT is an effective tool to evaluate the vaccination efficacy. The Sentinel® CSFV E2 Ab RT has high potential to be used to generate the vaccination programme.

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[2] Brown VR et al., 2018. Front Vet Sci. 5:11.

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ASIAN PIG VETERINARY SOCIETY CONGRESS 2019

Publication Date : August 23, 2019

Publication Information : Asian Pig Veterinary Society Congress 2019 (APVS 2019) ABSRACT

Organized by APVS 2019 Organizing Committee, Yangsu Mirae Foundation, and Korea Association of Swine Veterinarians
Managed by InnoN co,. LTD.