# The 7th Asian Pig Veterinary Society Congress

# October 25-27, 2015

# Scientific Posters

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# Porcine Respiratory Disease Complex

# PRDC

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# Monitoring PCV2 Maternally Derived Antibodies

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## Introduction

Data has been published before on suspected Maternally Derived Antibody (MDA) interference with PCV2 vaccination (1) and on variation in MDA titers (2,3). The lack of uniformity in MDA titers is reflected in the %CV (co-variation) and can be avoided by breeding stock vaccination, however this will increase MDA levels. Serological response after vaccination is more often seen in pigs with lower levels of MDA. A group of pigs with a better sero-conversion and a significant reduction of PCV2 viremia had a better economic performance (4). Monitoring MDA levels is important for vaccination timing and vaccine selection when sero-conversion is considered important

## Materials and methods

2 Groups of sows in 1 breeding herd were followed and serum samples were taken before farrowing in the sows and at Day 1 of age in piglets. Group 1: no PCV2 sow-vaccination. Group 2: sows were vaccinated against PCV2 according to leaflet instructions of the vaccine used. Samples were analyzed with the BioCheck PCV2 ELISA and results were presented in a quantitative manner.

## Result

Data obtained from the samples originating from Group 1 sows are presented in Figure 1 and 2. The large variation in titers in the breeding herd is reflected in the titers measured in the day old piglets. Data from Group 2 sows are shown in figure 3 and 4. The data presented in figure 3 and 4 are much more uniform. Note the %CV.

*Figure 1: Sows no PCV2 vaccination (%CV 26)*

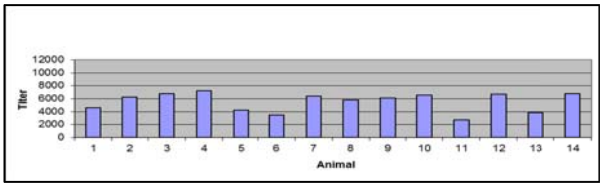



*Figure 2: Day old piglets from non-vaccinated sows*

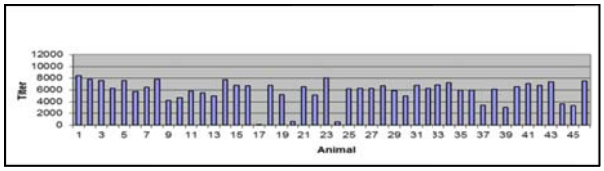



*Figure 3: Sows with PCV2 vaccination (%CV 9)*

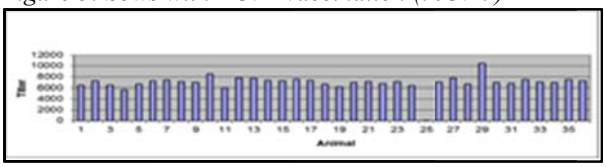
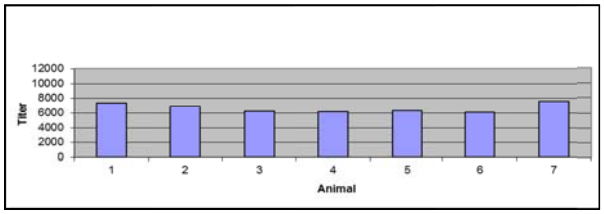



*Figure 4: Piglets born from PCV2 vaccinated sows*




## Discussion

Uniformity is very important in pork production. Uniformity is reflected in low %CV. The %CV in the sows improved after PCV2 vaccination. As a result also the %CV in the piglets improved from 33 to 21% but their uniformity also depends on factors like the colostrum intake management system. With sow vaccination more uniform batches of pigs were produced with higher MDA levels. When sero–conversion is important the timing of vaccination needs to be considered and/or the choice of the vaccine used in the piglets. Monitoring the PCV2 intervention strategy is important. Sero–conversion and viremia can be monitored using BioChek PCV2 ELISA and BioChek PCV2 qPCR (5).

## Reference

[1] Palzer; IPVS 2010
[2] Sibila; ESPHM 2015
[3] Martelli; ESPHM 2014
[4] Atlagich; ESPHM 2014
[5] Esch, van; APVS 2015

# Innovative polymeric adjuvant for PCV2 vaccination

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## Introduction

Porcine circovirus associated diseases (PCVADs) are economically important diseases of domestic pigs caused by porcine circovirus type 2 (PCV2). PCV2 vaccination is usually performed with adjuvanted inactivated formulations and is necessary to control PCVADs and subclinical PCV2 related body weight losses in pig farming. An important issue with PCV2 vaccine formulation is that PCV2 antigenic media often have properties which destabilize vaccine formulations.
We have developed a new robust polyacrylic polymer based liquid adjuvant formulation which is ready to disperse. Here we show that Montanide™ Gel 02 (Gel 02) allows the formulation of stable, safe and efficient PCV2 vaccines.

## Materials and Methods

First, new resisting adjuvant Montanide™ GEL 02 (polymer) was compared to reference adjuvants for galenic properties. To assess the stabilizing properties of the adjuvant, PCV2 vaccines were formulated with all adjuvants and stored at 4°C (normal conditions) or 20°C (destabilizing conditions) for up to 1 year. Stability of formulations was assessed after storage by visual observation and default intensity quotation. Efficacy and safety profile of Montanide™ Gel 02 was then assessed in swine. 3 weeks old pigs were vaccinated at D0 with a commercial PCV2 vaccine or a PCV2 vaccine based on Gel 02 adjuvant. Safety was assessed by temperature and behavioral measurement during the trial and macroscopic and histological examination of the injection site after slaughter at 18 weeks post injection. In a second swine trial, 3 weeks old pigs were vaccinated at D0 with a commercial PCV2 vaccine or a recombinant PCV2 vaccine based on Gel 02. All pigs were seronegative against circovirus at the beginning of the trial. Vaccination efficacy was assessed by specific antibody titration and body weight gain during the trial until slaughter of the animals at day 120 after vaccination.

## Results

Vaccines based on Gel 02 were perfectly stable over time, even in destabilizing conditions in which vaccines based on reference adjuvants showed stability defects. In swine trials, resisting adjuvant showed an acceptable safety profile, similar to commercial formulations. Specific antibody titers showed that the animals had been as expected in contact with circovirus during the trial, and had therefore been submitted to a natural challenge. Vaccine based on Gel 02 induced a similar protection (measured as the average body weight gain during the trial) compared to a commercial PCV2 vaccine.

## Discussion and Conclusions

These results show that the polymeric adjuvant Montanide™ Gel 02 can allow the development of safe and efficient PCV2 vaccines with an improved stability.

# A case report of PRRSV control effort at 2 farms in Japan

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**Introduction**

Porcine reproductive and respiratory syndrome (PRRS) causes respiratory symptoms as well as reproductive problems such as premature piglets and stillborn. Also asymptomatic PRRS causes economic loss (1).

Modified live vaccine for PRRS (Ingelvac PRRS MLV, Boehringer Ingelheim Vetmedica Japan Co.,Ltd.) is commercially available to control PRRS in Japan. The aim of this study is to identify conditions to elicit optimal efficacy of the modified live vaccine for PRRS (PRRS MLV) by comparing different outcomes of same PRRS control program implemented at 2 farms (Farm A and B).

**Materials and methods**

Farm A consists of 2 sites with 2,000 sows. Pigs are weaned at 24 days of age and moved to fattening site at 110 days of age. Farm B is 1,800 sow farm with same pig flow as farm A. Both farms are located in the same pig dense area of Kyusyu, Japan. PRRS was identified as the primary cause of the problem based on history, clinical signs and laboratory results. Blood samples from pigs at 30, 60, 90 days of age and older were confirmed positive by PCR for PRRS virus (PRRSV), mortality rate increased 2 to 3 weeks after weaning at both Farm A and B when the situation of the 2 farms were investigated for the first time.

Breeding herd and all pigs at weaning age and over were mass vaccinated with Ingelvac PRRS MLV twice with one month interval under veterinary supervision ( Oct. and Nov. 2014). Breeding herd is mass vaccinated quarterly and pigs are vaccinated at weaning since the initial mass vaccination.

**Result**

Figure 1 shows mortality rate of weaned pigs from Oct.2014 to Apr.2015. It decreased from 13.6% to 10.8% and from 13.9% to 7.7% at Farm A and B respectively.

6 month after implementation of the vaccination program, umbilical blood samples were collected from each parity sow group and pooled by group of 2 to 3 samples and tested by PCR for PRRSV to investigate vertical transmission status. As a result, all samples of all parity at Farm A were negative and samples of parity 2 and 4 at Farm B showed positive (Table 1).

***Figure 1 Trends in mortality of weaned pigs***

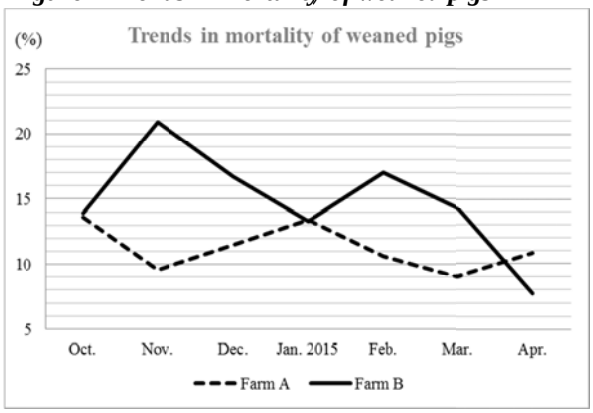


***Table 1 Status of vertical transmission in farrowing house***

| PRRS-PCR | Farm A | Farm B |
|---|---|---|
| | Umbilical blood | Umbilical blood |
| Parity 1 sow group | ( - ) | ( - ) |
| Parity 2 sow group | ( - ) | ( + ) |
| Parity 3 sow group | ( - ) | ( - ) |
| Parity 4 sow group | ( - ) | ( + ) |
| Parity 5 sow group | ( - ) | ( - ) |

**Discussion**

Farm A showed mortality decrease after PRRS MLV vaccination (Figure 1). Horizontal transmission was observed at 20 days of age. Tightening biosecurity and revising farrowing system are necessary to further improve mortality at farrowing house.

Farm B also showed mortality decrease but it's taken longer to stabilize the farm situation compared to Farm A. One possibility of the deference between the 2 farms is the vertical transmission status.

Improving pig flow, tightening biosecurity and also immunological stabilization of breeding herd by eliminating wild type PRRSV are important to maximize the efficacy of PRRS control program utilizing PRRS MLV. Optimal conditions to control PRRS in pig dense area will be further investigated.

**Reference**

[1] Rossow K.D. (1998) Porcine reproductive and respiratory syndrome. *Vet Pathol* **35:** 1–20

# Antibiotic susceptiblity of major pathogens bacterial pathogens in Korea

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## Introduction

For the control of pig bacterial diseases, choice of proper antibiotic is important. The choice of antibiotic was usually done based on practitioner's experience, magazine, and marketing leaflet of manufacturers.
We analyzed the antibiotic sensitivity data in 2013 and in 2014 done and suggest which antibiotics could be selected for controlling each bacterial pathogen.

## Materials and Methods

We've sent sick pigs to government veterinary laboratory (QIA, Quarantine and Inspection Agency). QIA has sent diagnose report including antibiotic sensitivity test.
We classified the diagnose reports into specific pathogen and scored the sensitivity of each antibiotics to this specific pathogen.

## Results

Results are as below.

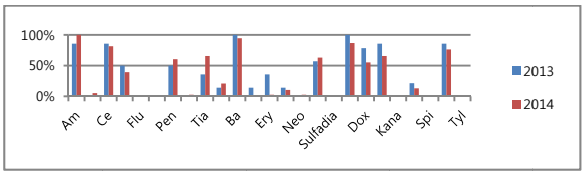


Fig1. The sensitivity result of *Streptococcus spp.* (2013=25 results, 2014=19 results)

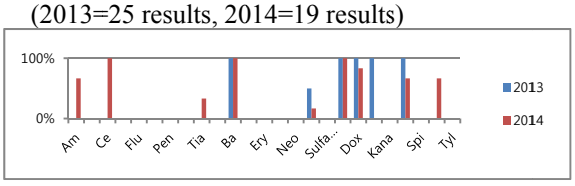


Fig2. The sensitivity result of *Staphylococcus hycus* (2013=1 results, 2014=3 results)

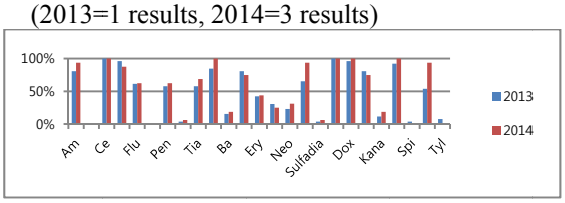


Fig3. The sensitivity result of *Pastuerella multocida* (2013=13 results, 2014=8 results)

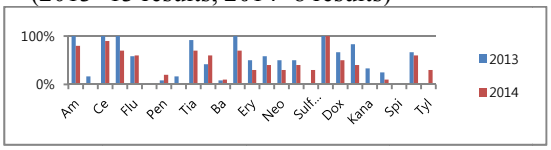


Fig4. The sensitivity result of *Actinobacillus pleuropneumoniae* (2013=6 results, 2014=5 results)

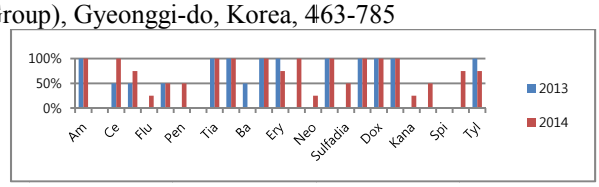


Fig5. The sensitivity result of *Hemophilus parasuis* (2013=1results, 2014=2results)

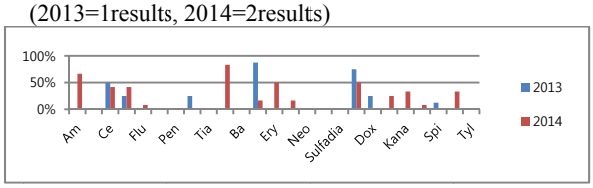


Fig6. The sensitivity result of *Salmonella spp* (2013=4results, 2014=6results)

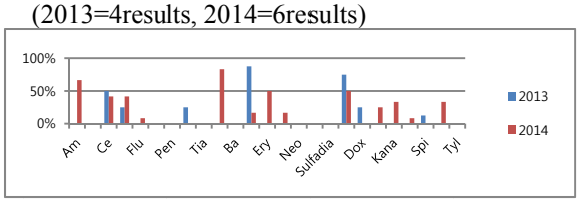


Fig7. The sensitivity result of *pathogenic E.coli* (2013=9results, 2014=17results)

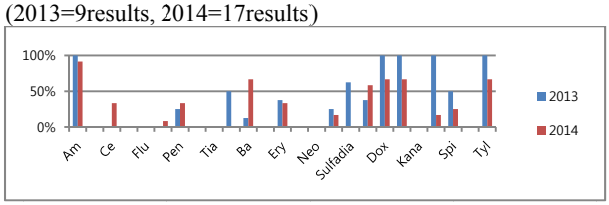


Fig8. The sensitivity result of *Clostridium spp* (2013=4results, 2014=6results)

## Discussion

The result of antibiotic sensitivity test can be a guide to choose the proper antibiotic for control bacterial disease. It is helpful to reduce the cost of treatment and the cost of disease. We analyzed the data of antibiotic sensitivity test and summarized as the table1 as below.

Table1. The summarized results for choosing antibiotics.

| Bacterial pathogen | 1st choice | 2nd choice | 3rd choice |
|---|---|---|---|
| **Streptococci** | **Amoxicillin** | Ceftiofur | Sulfadiazine |
| **Staphylococci** | **Ceftiofur** | Doxicycline | Oxytetracycline Sulfadiazine |
| **P. multocida** | **Ceftiofur** | Doxicycline | Oxytetracycline |
| **APP** | **Ceftiofur** | Amoxicillin | Enrofloxacin |
| **H. parasuis** | **Amoxicillin, Ampicillin, Ceftiofur, Doxicycline Oxytetracycline, Tiamulin** | | |
| **Salmonella** | **Ampicillin** | Ceftiofur | Amoxicillin |
| **E.coli** | **Colistin** | Ceftiofur | Gentamycin |
| **Clostridium** | **Amoxicillin** | Doxicycline, Florophenicol, Tylosin | |

## Reference

1. YC Moon, et al, Control strategies of bacterial pathogens in Danji. APVS2013 Vietnam.

# The prevalence of *Chlamydia* spp. in Polish pigs with and without clinical symptoms

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**Introduction**

Four *Chlamydia* species have been described in pigs: *C. abortus*, *C. psittaci*, *C. pecorum* and *C. suis*. The most common in pigs is *C. suis* and very often occurring in mixed infection with *C. abortus* and *C. pecorum*. Chlamydia infection in pigs are related to reproductive disorders e.g. conjunctivitis, eteritiitis, pneumonia. Prevalence *Chlamydia* spp. have been noted in pig farms in different countries. The aim of the studies was evaluation occurrence of Chlamydia in pig in Poland with and without clinical diseases.

**Materials and methods**

The samples of rectal or fecal swabs originated from pig herds with clinical symptoms mainly pneumonia (20 herds) and asymptomatic (20 herds). The herds were selected based on previous serological studies and only from seropositive herds samples were taken. All samples were immersed in chlamydia transport medium. DNA extraction and purification on rectal or fecal swabs was performed by using commercial tests. The samples of DNA were subjected to *Chlamydiaceae* specific real-time PCR targeting *omp*A gen[1] and next specific real-time PCR for individual species[2]. Samples with a Ct-value above 35 were judged as positives. As the positive control genomic DNA of reference strain of *C. suis* S45 (ATCC-VR-1474) were used. To detect false negative PCR results due to inhibiting agents, an internal amplification control was used. The results between two compared groups of herds were analyzed by chi-squared test.

**Result**

The 5/20 (25%) and 2/20 (10%) herds with and without clinical symptoms, respectively were positive for *Chlamydiaceae*. Thirty samples from pigs with clinical signs giving a strong positive reaction ($C_t$<29) and eleven samples had Ct >32 for *C. suis*. While only eight positives samples were from herds without clinical disorders and giving Ct >34. The most of *Chlamydiaceae* positive samples were confirmed as *C. suis* but in one herd besides *C. suis* the presence of *C. pecorum* was confirmed. The mixed chlamydia infection was detected in pigs without clinical signs. The highest value of Ct were noted for samples from pigs with clinical symptoms. The significant differences between compared groups of swine were noted when statistical analysis was performed.

**Discussion**

*C. suis* predominantly occurs in pigs. The presence of Chlamydia infection in pigs is not always connected with clinical disorders. The correlation was noted between samples with a high value of Ct and the presence of clinical signs. The case of mixed chlamydia infection could be related with close contact this herd with cattle on the farm. Some authors speculated that development of Chlamydia infection and clinical lesions may depend on different factors such: route of infection, age or immunological status of the host.

**Reference**

[1] Ehricht R.et al. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mole Cell Probe 20, 2006, 60-63.

[2] Pantchev A., String R. et al. Detection of all *Chlamydophila* and *Chlamydia* spp. of veterinary interest using species – specific real-time PCR assay. Comp Immunol Microbiol Infect Dis. 33, 2010, 473-484.

# Chlamydia infection in pigs with pneumonia and arthritis

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## Introduction

Massive outbreaks of chlamydiosis associated with bronchopneumonia or abortion in pigs were reported in Eastern European countries between 1960 and 1970. In the 1990s and 2000s chlamydia were isolated from pigs in different countries both from animals with clinical signs and without clinical disorders. *Chlamydiaceae* still are considered as non-important pathogens of pigs because the data about prevalence are underestimated. Moreover, *Chlamydia* spp. are often found in association with other pathogens. The aim of the studies was detection of *Chlamydia* spp. in pigs with clinical disorders and evaluation relation between Chlamydia infection and prevalence of pneumonia and artritis in pigs.

## Materials and methods

The samples were taken from 60 herds of pigs in Poland. The herds were selected based on positives serological results. Totally, from each herds 30 samples were tested and the rectal and nasal swabs were taken. The DNA was extracted by using DNA mini kit (Qiagen), according to the manufacturer's protocol. A 1 µl of extracted DNA was subjected to *Chlamydiaceae* specific real-time PCR (targeting the *omp*A gen). Samples with a Ct-value below 35 cycles were classified as positives. All positive samples were retested according the methodology described by Ehricht et al.[2] and real-time PCR assays (target *omp* A) for *C. pecorum*, *C. felis*, *C. caviae*, *C. abortus* and 23S rRNA for*C. suis* were performed.

## Result

The real-time PCR confirmed presence of *Chlamydiacae* in seven seropositive herds with pneumonia and in 4 seropositve herds with artritis. In the *Chlamydiacae* posive herds with artritis only *C. suis* was detected. While in herds with pneumonia mainly *C. suis* was presented but in two herds the coinfection with *C. abortus* was noted. Ct values in real-time PCR-positive swabs varied from 24.6 to 31.5 and 29.35 to 34.88 in herd with pneumonia and artritis, respectively. The statistical analysis showed that *Chlamydia* spp. was significantly related to pneumonia when individual results were compared at the herd level. While the presence of *Chlamydia* spp. in nasal or rectal swabs was not significantly correlated with artritis. Moreover, much higher Ct values for Chlamydia positive samples was observed in pigs with pneumonia. Most of qPCR positives results were noted in pigs with pneumonia when tested material were nasal swabs, while in swine with artritis much often positives results were for rectal swabs.

## Discussion

The pig is considered as the natural reservoir for *C. susi*. Nevertheless the other Chlamydia species can be presented in swine. There are preliminary studies and the hypothesis about significant significance of *C. susi* in pigs with pneumonia needs confirmation in further studies that will included more pig herds and additional assays detecting other pathogens.

## Reference

[1] Ehricht R.et al. Optimized DNA microarray assay allows detection and genotyping of single PCR-amplifiable target copies. Mole Cell Probe 20, 2006, 60-63.

[2] Pantchev A. et. al. Detection of all *Chlamydophila* and Chlamydia spp. of veterinary interest using species – specific real-time PCR assay. Comp Immunol Microbiol Infect Dis. 33, 2010, 473-484.

# PCV2 virus Monitoring; The Value of Diagnostics

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## Introduction

Differences of opinion have always been present whenever it comes to interpretations of PCV2 virus infections. Right from the start of detecting PCV2 virus in diseased pigs scientists have debated the importance of this finding. PCV2 virus could be and can still be found in sick and in apparently healthy pigs. In this abstract an overview of the related literature is presented and the results of a new qPCR for detecting PCV2 virus.

## Materials and methods

Literature was screened for PCV2 field studies using a positive control group. Studies with different strategies to control PCV2 virus were selected. These strategies could be the same vaccine at different time points (1), the same vaccine at the same time point but in piglets with different levels of Maternally Derived Antibodies (MDA) (2) or different vaccines at the same time point (3). The PCV2 qPCR results in these studies provided information on good and better protection against PCV2 related economic damage. Because of the importance of accurately detecting the PCV2 viral load special attention was given during the development of the BioChek PCV2 qPCR test kit to the sensitivity and specificity of the kit. Part of this process involved analyzing spiked samples and samples with a known history. The results of these assays with the BioCheck PCV2 qPCR are presented.

## Result

When the same vaccine was used in commercial pigs at different time points (1), a difference in serological response was noted and a difference in PCV2 qPCR results. The 2 test groups performed better than the controls and the group with the lowest qPCR had a significant higher ADWG.

In another study and testing the same vaccine in piglets of the same age but with different levels of MDA showed lower qPCR values for the group vaccinated in the presence of lower MDA titers and a better ADWG (18 g higher and a $p>0.05$). Testing different vaccines showed a difference between 2 vaccines for PCV2 viral load and serological response, resulting in better FCR (3).

*Figure 1: BioCheck PCV2 qPCR example of Cq values of different PCV2 virus positive samples .*

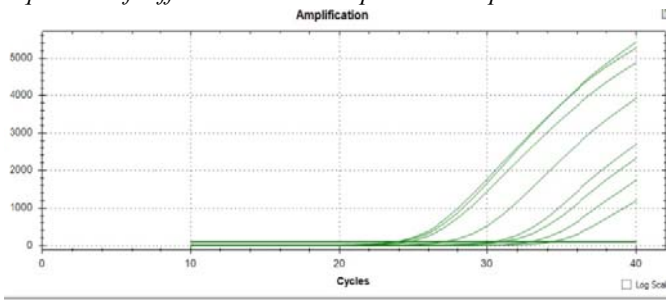
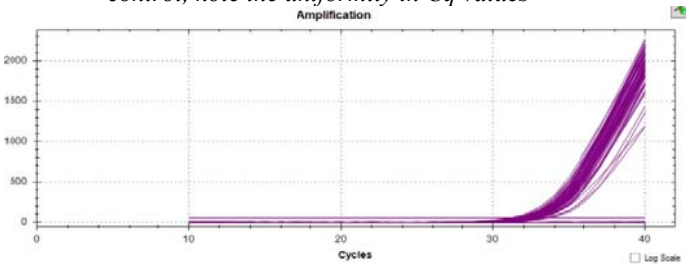



*Figure 2: BioChek PCV2 qPCR internal control, note the uniformity in Cq values*




## Discussion

PCV2 virus infection is controlled by vaccination at a cost. Return on investment is related to the level of PCV2 control. The return is higher when PCV2 virus is controlled better resulting in a lower PCV2 viral load. PCV2 viremia can be monitored using BioChek PCV2 qPCR. When PCV2 viremia occurs in vaccinated pigs, it is advisable to reconsider the vaccination scheme used. Monitoring for PCV2 virus is a clear example of the value of diagnostics.

## Reference

[1] Palzer IPVS 2010
[2] Sibila, ESPHM 2015
[3] Atlagich (ESPHM 2014

# Loop-mediated isothermal amplification (LAMP) assay for the rapid detection of Porcine circovirus 2

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## Introduction

Porcine circo virus 2 (PCV2) is the most common disease in commercial pork production worldwide. Laboratory diagnosis of PCV2 is carried out on tissues of infected animals using histopathology associated with the detection of PCV2 DNA by in situ hybridization (ISH) or viral antigens by immunohistochemistry (IHC) or indirect immunofluorescence (IIF). However, most of these methods require expensive and specialized instruments and reagents. On the other hand Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method in which reagents reacts under isothermal conditions with high specificity, efficiency and rapidity. Therefore, the main objective of this study was to develop a LAMP for rapid detection of PCV2. In addition, One of the most attractive features of this LAMP assay is that the results can be observed and determined by hydroxynaphthol blue (HNB) dye-mediated visualization using the naked eye and without opening the tubes after amplification.

## Materials and methods

Primer sets that could detect the PCV2 were designed. Nucleotide sequence data for PCV2 strains from the Genbank were aligned by using Clone Manager 6 to identify regions that equal between the genotype. Target ORF1~ORF2 gene specific primers were designed using a Primer Explorer V4 program. Six primers including outer primers (F3/B3), inner primers (FIP/BIP), and loop primers (LF/LB) for targeting ORF1~ORF2 genes. LAMP reaction mixture containing 1ul Bst DNA polymerase (8 U/ul, New England Biolabs, Ipswitch, MA, USA), 5ul template, 2.5ul dNTPs (10 mM), 8ul Betaine (250 mM), 1ul MgSO4 (150 mM), 1ul HNB (3mM, Lemongreen, Shanghai, China) and 1ul of each primer (F3 and B3: 5 pmol/ul; BIP and FIP:40 pmol/ul; LF and LB: 20 pmol/ul). To optimize of reaction condition for LAMP detection, the reactions were carried out at 61, 62, 63, 64, 65℃ for 60 min and performed the LAMP for different reaction times. The sensitivity of the LAMP assay was determined and compared with UNG-based direct polymerase chain reaction (udPCR) and real-time PCR using the same template at identical concentrations.

## Result

By the PCV2 LAMP developed in this study, PCV2 was visually detected in 40 min reaction time at 63℃ reaction temperature. The sensitivity of the PCV2 LAMP was higher than previous reported PCR assay and same as the real-time PCR assay.

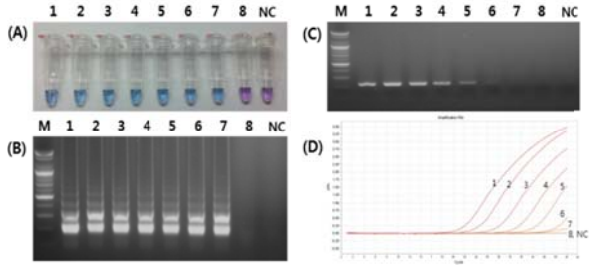


Fig. 1. Detection limit of loop-mediated isothermal amplification (A and B), polymerase chain reaction (PCR) (C) and real time PCR (D) for amplification of the PCV2 DNA. Lane M; 100 bp DNA marker, Lane 1-8; 10-fold serial dilution of PCV2 DNA.

## Discussion.

In this study, we developed a visual and rapid detection method for PCV2 using the optimized LAMP technique. Compared to conventional PCR analysis, the LAMP method has advantages such as time-saving, low cost and ease of operation. The LAMP extends previous methods for PCV2 detection and provides an alternative approach for detection of PCV2.The method is simple and obviates the need for expensive equipment such as real-time PCR instruments. So it is useful for clinical diagnosis in developing countries and clinically laboratories..

## Reference

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[2] EG Kim et al. 2009. Korean J Vet Serv. 32:299-306

# Reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of swine influenza viruses

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## Introduction

Swine influenza virus (SIV) is caused by influenza A viruses. To control the influenza A infection, early detection with a rapid, cost-effective and efficient assay is need, particularly in developing countries. Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method in which reagents reacts under isothermal conditions with high specificity, efficiency and rapidity. So, in this study we developed a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) based system for rapid and specific detection of swine influenza virus (SIV). In addition, one of the most attractive features of this RT-LAMP assay is that the results can be observed and determined by hydroxynaphthol blue (HNB) dye mediated visualization using the naked eye and without opening the tubes after amplification.

## Materials and methods

Primer sets that could detect the SIV were designed. Nucleotide sequence data for SIV strains from the Influenza Sequence Database were aligned by using Clone Manager 6 to identify regions that equal between the genotype. Target M gene specific primers were designed using a Primer Explorer V4 program. Six primers including outer primers (F3/B3), inner primers (FIP/BIP), and loop primers (LF/LB) for targeting M genes. RT-LAMP reaction mixture containing 1ul Bst DNA polymerase (8 U/ul, New England Biolabs, USA), 5ul template, reverse-transcriptase (10 U/ul, Invitrogen, CA), 2.5ul dNTPs (10 mM), 8ul Betaine (250 mM), 1ul MgSO4 (150 mM), 1ul HNB (3mM, Lemongreen, China) and 1ul of each primer (F3 and B3: 5 pmol/ul; BIP and FIP: 40 pmol/ul; LF and LB: 20 pmol/ul). To optimize of reaction condition for RT-LAMP detection, the reactions were carried out at 45, 50, 55, 58, 60, 62 and 65℃ for 50 min and performed the LAMP for different reaction times (20, 30, 40 and 50 min). The sensitivity of the RT-LAMP assay was determined and compared with RT-PCR and real-time PCR using the same template at identical concentrations.

## Result

The RT-LAMP for SIV developed in this study was confirmed that can visually detect all subtype of the SIVs in 40 min reaction time at 58℃ reaction temperature. The sensitivity of the SIV RT- LAMP was higher than conventional RT-PCR and same as real-time PCR previously repored.

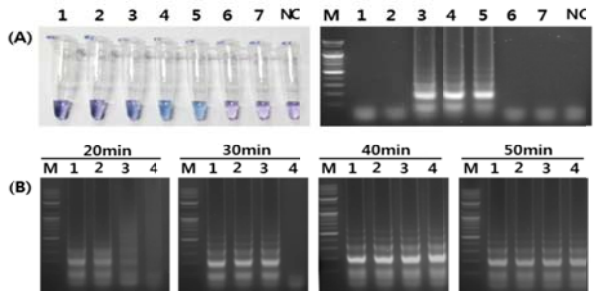


Fig. 1. Optimization of RT-LAMP condition for amplification of swine influenza virus with different reaction temperature (A) and reaction time (B). (A) Lane M; 100 bp DNA marker. Lane 1-7; 45, 50 55, 58, 60, 62 and 65 ℃ respectively. (B) Lane M; 100 bp DNA marker, Lanes 1 to 4, serially diluted SIV by 24, 20, 2-4 and 2-8 HA unit/25μL, respectively.

## Discussion.

The RT-LAMP for SIV developed in this study was shown to be highly specific and sensitive. The RT-LAMP can be completed within 40 min and is faster than the conventional RT-PCR and real-time RT-PCR approaches. The assay is highly sensitive and specific and can detect low copy number of viruses. And the method is simple and obviates the need for expensive equipment such as real-time PCR instruments. So it is useful for clinical diagnosis in developing countries and clinical laboratories.

## Reference

[1] Shin YK et al. 2011. J Vet Med Sci. 73:55-63.
[2] Kim HR et al. 2013. Virology J. 10:85
[3] Ma XJ et al. 2010. J Virol Methods. 167:214-217

# Efficacy of APM777 vaccine in pigs challenged with *Actinobacillus pleuropneumoniae* serotype 2

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## Introduction

Respiratory syndrome in swine, porcine respiratory disease complex (PRDC) has been described as an important cause of decreased productivity in swine.

*Actinobacillus pleuropneumoniae* (App) and *Mycoplasma hyopneumoniae* (Mhp) are the most important pathogens associated with PRDC. The objective of this study was to evaluate the efficacy and safety of APM777 vaccine, assess its ability to induce specific antibodies, and determine its ability to reduce App pneumonia.

## Materials and methods

### Safety and efficacy of APM777 vaccine in mice

The vaccine contains 7 components in alum adjuvant, inactivated bacterin of Mhp, three inactivated App cells (serovars 1, 2 and 5), three toxoids (recombinant ApxI, ApxII and ApxIII). The tests for Safety and efficacy were carried out according to the procedure described in the Japanese Standard of Veterinary biological Products. For safety test, each of 10 mice was injected intraperitoneally (i.p.) with 0.5 ml of the vaccine. Health status and body weight were monitored and recorded just before and 7 days after the injection. For App efficacy test, mice were immunized i.p. with 0.5 ml of the vaccine preparation diluted 1:20 with PBS. Two weeks after the immunization, the immunized and control mice (10 each) were challenged with App serovars 1, 2 and 5. Mouse mortality was monitored daily for the following 7 days. For Mhp efficacy test, each of 10 mice were immunized i.p. with 1 ml of the vaccine. Blood samples were collected at week 4 after the immunization for the determination of anti-Mhp antibodies by ELISA.

### Safety and efficacy of APM777 vaccine in pigs

**Trial. 1.** SPF pigs (five per group) were vaccinated intramuscularly with 2 mL of APM 777 vaccine twice at 4-week intervals. The vaccinated pigs were daily observed for adverse reactions following injection of the vaccine. Eight weeks after the second immunization, the vaccinated and control pigs were challenged with about 10^3 cfu/head of App 2 via intratracheal route.

**Trial. 2.** Pigs were vaccinated at 3- or 5-week intervals. Two weeks after the second injection, the vaccinated and control pigs were challenged as mentioned above.

In both trials, after the challenge, clinical signs and death were monitored and recorded. Dead pigs were autopsied on the day of death, and pigs that survived were euthanized and autopsied 1 week after challenge.

## Result and Discussion

### Safety and efficacy testing in mice

None of the mice treated with APM 777 showed any abnormal clinical signs. The average body weight returned to the starting level on day 4. After challenge with App serovars 1, 2 and 5; 9 to 10 (90 to 100%) of the control mice died within 4 days. In contrast, 9 to 10 (90 to 100%) of the immunized mice survived until day 7, when the experiment was terminated. The anti-Mhp antibody titers in sera of 90 % of the immunized mice were 2,560 or more, whereas those in sera of the control mice were 20 or less.

### Safety and efficacy testing in pigs

Abnormal clinical signs or local reactions were not observed in all pigs following the vaccination. After challenge with App serovar 2; 4 of 5 control pigs died, while 4 of 5 vaccinated pigs survived. All the animals developed pulmonary lesions, pigs immunized with APM 777 vaccine had significantly lower lesion scores of App pneumonia than the control pigs (5.8 vs 11.2). Antibody titers to the App serovars 1, 2 and 5 as well as ApxI, ApxIII and Mhp antigens in vaccinated group were higher than those of control group at 8 weeks after the second immunization.

### Interval between 1st and 2nd vaccination in pig

After vaccination, no pigs got fever. Four of the five controls died, while all vaccinated pigs in 3 and 5 weeks intervals survived. All the animals developed pulmonary lesions, with a mean lesion score of 6.0, 3.8 and 9.0 in the 3, 5 weeks and control groups, respectively. The antibody response of 3 and 5 interval vaccinated group at 2 weeks after the second injection was higher than control group.

# Efficacy of a novel COMBO vaccine against porcine reproductive and respiratory syndrome (PRRS) virus and porcine circovius type 2 (PCV2) virus in field trials

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) results in high economic losses in animal husbandry every year. PCV2 can cause productive failure including abortion, stillbirth and weak born. In fields, majority of PCV2 infected pig are asymptomatic but the information regarding host immune status is limited. Some embodiments of the present study relate to PRRS/PCV2 COMBO vaccines, which are composed of four separate PRRS subunit antigens (PRRSFREE®, Taiwan) with modified PCV2 capsid antigen. This combination vaccine induced remarkable immune response, both the CMI response and the humoral immune response. Clinical course and cell-mediated responses were monitored for 84 days post-vaccination (PV). IFN-gamma release assay was used for evaluation of cell-mediated immunity. Our results show that level of IFN-gamma in the vaccinated group was significantly higher than in the non-vaccinated group. The tests show that the average daily weight gain of vaccinated group was significantly increased when paralleled with non-vaccinated control group. The survival rate of vaccinated group was significantly increased when compared with non-vaccinated group. The aim of this trial study is to evaluate the novel COMBO vaccine to consider the influence of PRRSV and PCV2 control on swine productivity and industry.

## Materials and methods

Samplecollection

A total of 200 three-week-old pigs were allocated to 1 of 2 groups (vaccine and placebo groups).

Isolation of PBMC and IFN-gamma release assay

PBMC were isolated from the swine heparinized blood by centrifugation using Histopaque 1077 (Sigma, St. Louis, Mo.), suspended to $5\times10^5$ cell/ml with RPMI complete medium (RPMI 1640 containing 10% FBS) and seeded to 96-well flat-bottom plates at 100 µl per well. Each cells sample was plated in triplicate. The culture was stimulated with PRRSFREE antigens. After incubation for 72 h at 37°C with 5% CO2, the supernatant cytokine levels from PBMC cultures were measured using commercially available ELISA kits for swine IFN-γ (Invitrogen, Carlsbad, CA)

Determination of Immunoreactivity of piglets' sera by PRRS/PCV2 antigen ELISA

ELISA was conducted according to the procedure. Half µg of PRRSFREE vaccine antigens in 50 µl PBS (pH 7.4) was coated onto each well of an ELISA plate. After blocking and washes, 100 µl of diluted piglets' sera were added and incubated at room temperature for 1 h. Following washes and incubation of 100 µl of peroxidase-conjugated goat anti-swine IgG (1:3000 dilutions).

Statistical analysis

Differences in average daily weight gain, survival rate and IFN-gamma levels were analyzed using analysis of variance (ANOVA).

## Discussion

The CMI response, as determined by the IFN-□ concentration, in vaccinated groups was higher than that in the PBS group (control), indicating that the CMI response was induced upon vaccination. From the results, it is clear that both approaches are effective in inducing immuno responses, including CMI response and humoral response. Between these two approaches, the PRRS/PCV2 combo vaccine composed of 2 antigens shows better efficacy in three out of four immune responses examined. This study demonstrates that PRRS/PCV2 combo vaccine composed of PRRS chimeric fusion antigen and PCV2 ORF2 antigen is a better choice than that composed of 5 antigens. Nevertheless, both approaches are useful for inducing immune responses inananimal.

## Reference

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[2] Lyoo YS, Kleiboeker SB, Jang KY, Shin NK, Kang JM, Kim CH, Lee SJ, Sur JH. A simple and rapid chromatographic strip test for detection of antibody to porcine reproductive and respiratory syndrome virus. J Vet Diagn Invest. 2005;17(5):469-

# Comparison of two different porcine circovirus type 2 vaccines in a commercial farm in Korea

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## Introduction

It is generally accepted in the swine industry that Porcine Circovirus Associated Diseases (PCVAD) can be very well controlled by the use of commercial porcine circovirus type 2 (PCV2) vaccines. However some producers think that the price of PCV2 vaccines is high. So they see that changing from one PCV2 vaccine to another one can help to reduce costs. The present observation describes what happened after the change of PCV2 vaccines in a commercial herd in Korea.

## Materials and methods

The field observation was carried out in a 2-site production system with 1,000 sow. In this system every two weeks a batch of about 800 to 1000 feeder pigs is moved from the farrow-to-feeder to a finishing site. Before the study was carried out Ingelvac CircoFLEX® (Boehringer Ingelheim Vetmedica Korea ltd.) was used at the age of 3 weeks to control PCV2. The mortality rate during finishing used to be below 5 %. For the study 4 consecutive batches were vaccinated with an alternative vaccine used according to label and also at 3 weeks of age. These 4 batches were compared for mortality with 4 batches of pigs (2 previous and 2 consecutive batches to the 4 trial batches) that were vaccinated with Ingelvac CircoFLEX.

## Result

Pigs that were vaccinated with the alternative vaccine showed coughing, diarrhea and wasting. A pig showing clinical signs of wasting was sent to the QIA (Korean Quarantine and Inspection Agency) for laboratory investigation. The diagnostic results are as below (Table1). PCV2 was found at all kinds of tissues of sick animal and PRRS virus was also found at lung, tonsil, lymph node and brain. And Pasteurella multocida found at lung and Salmonella typhimurium was found at large intestine and feces. The mortality rate was increased from 4% to 17.6% in the pigs that were vaccinated with the alternative vaccine (Fig. 2). In the two consecutive batches that were again vaccinated with Ingelvac CircoFLEX® the mortality came back to the original level.

**Table.1** *The summary of PCR results for virus*

| | PCV | SIV | CSF | PRRS | PR | PPv | EMC | JE | TGE |
|---|---|---|---|---|---|---|---|---|---|
| ***Lung*** | + | - | - | + | - | - | - | - | - |
| ***Tonsil*** | + | - | - | + | - | - | - | - | - |
| ***Lymph node*** | + | - | - | + | - | - | - | - | - |
| ***Heart*** | + | - | - | - | - | - | - | - | - |
| ***Kidney*** | + | - | - | - | - | - | - | - | - |
| ***Liver*** | + | - | - | - | - | - | - | - | - |
| ***Spleen*** | + | - | - | - | - | - | - | - | - |
| ***Brain*** | + | - | - | + | - | - | - | - | - |
| ***Intestine*** | + | - | - | - | - | - | - | - | - |

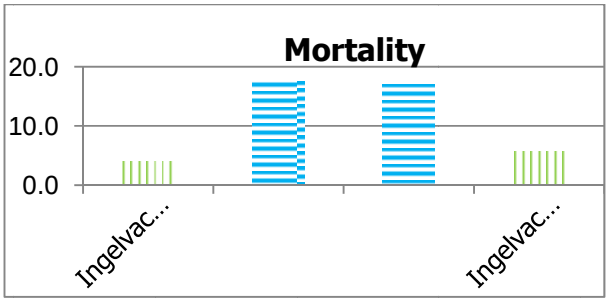


**Figure. 2** *The mortality rate before and after changing PCV2 vaccine at fattener period*

## Discussion

In the present case the mortality increased by 11 % when pigs were vaccinated with an alternative PCV2 vaccine. The diagnostic investigation indicates that the main cause of high mortality was PCV2. Based on the difference in vaccine price the farmer could save one US dollar per dose when changing from Ingelvac CircoFLEX® to an alternative vaccine. However this higher cost in vaccine price resulted in a return of investment of 1:5.5 when taking into account the differences in mortality. This case illustrates that risk of changing vaccination protocol even if there are no clinical signs present.

# Improvement of mortality rate after changing to Ingelvac CircoFLEX®

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## Introduction

Porcine circovirus type 2 (PCV2) is regarded as causal agent in the development of a number of disease syndromes which have been called Porcine Circovirus Diseases (PCVD). In 1998, PCV2 was isolated for the first time in Korea, and the first PCV2 vaccine was introduced to the Korean market in 2008. Ninety seven percent of Korean swine farms vaccinated against PCV2 in 2013 (1). Fifteen different commercial PCV2 vaccines are available in Korea. Some of them are manufactured and marketed by global pharmaceutical companies others are produced by local Korean companies. The advantages of PCV2 vaccination are already well known in Korea, However, it is not well established whether and what kind of differences there are between the different PCV2 vaccines. The purpose of this study was to evaluate whether there are differences in vaccine efficacy and in performance parameters when pigs are vaccinated with different PCV2 vaccines.

## Materials and methods

This field observation was conducted in a farrow to finish farm with about 350 sows in Jeon-La province, Korea. After the farm changed from Ingelvac CircoFLEX® to a local PCV2 vaccine because of vaccine price, the farmer observed an increase in wean to finish mortality. To be able to measure the effect of the different vaccines on pig performance the following study was conducted. 4 batches of 675 pigs in total were vaccinated with a local PCV2 vaccinate 1 and 3 weeks of age (group A). The following batches (675 pigs in total) were vaccinated at 3 weeks of age with Ingelvac CircoFLEX® (group B). All pigs were weaned at 28 day of age and transferred to the nursery unit and at around 10 weeks of age they were transferred to the finishing unit. For both treatment groups the number of dead pigs during the nursery and finishing period was recorded. BECAL (Boehringer Ingelheim Economic CALculator) (2) was used for an economic evaluation of the two vaccination protocol.

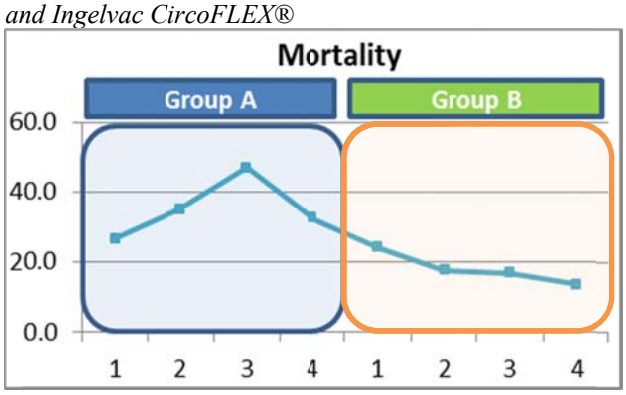
## Result

The mortality rate for treatment group A was 35.1% compared to 18.2% in treatment group B. In this case the ROI (Return on investment) of the additional cost of Ingelvac CircoFLEX® compared with local PCV2 vaccine was 1:25 based on a carcass price 4,000 KRW/kg, feed price 500 KRW/kg, and a difference in vaccine price of 1,050KRW per pig.

**Table. 1** *Number of dead pigs per production phase and wean-to-finish mortality in group A & B*

| | | local PCV2 vaccine (group 'A') | Ingelvac CircoFLEX® (group 'B') |
|---|---|---|---|
| Number of pigs | | 675 | 658 |
| Number of dead pigs | nursery | 116 | 60 |
| | grower-finish | 121 | 60 |
| Mortality (%) | | 35.10% | 18.20% |

**Figure. 1** *Improvement of mortality for 8 consecutive batches between local PCV2 vaccine and Ingelvac CircoFLEX®*




## Discussion

The results of this study are in line with others that demonstrate that PCV2 vaccines can have an explicit effect on mortality. In conclusion this trial highlights that the decision on which vaccine to use for PCV2 protection should not be based on vaccine price but primarily on vaccine efficacy and ROI.

## Reference

[1] Park et al. (2014) Swine disease report 2013, Korean Pork Producer Association
[2] M. Adam et al. (2013) Proc 5th ESPHM symposium, Edinburgh, United Kingdom, P124

# Efficacy of Ingelvac CircoFLEX® comparing to Korean local PCV 2 vaccine

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## Introduction

Porcine circovirus (PCV2) has been associated with various disease syndromes in pigs, primarily post-weaning multisystemic wasting syndrome (PMWS). In Korea, PCV2 was isolated for the first time in 1998, and the first PCV2 vaccine was introduced in 2008. Ninety seven percent of Korean swine farms vaccinated against PCV2 in 2013 [1]. Fifteen different commercial PCV2 vaccines are available in Korea. The advantages of PCV2 vaccination are already well known in Korea, especially the effect of vaccination on mortality and the improvement in production performance. However, differences between PCV2 vaccines are not well described in regards to their effect on mortality, production performance and economic benefit. Moreover there is the general assumption in Korea that 2-dose vaccine protocols are more efficacious than 1-dose protocol. The objective of this study was to compare one local with a global PCV2 vaccine in terms of mortality as well as the number of vaccinations.

## Materials and methods

The field observation was conducted on a 2site production system with 2,500 sows. Pigs were weaned at 28 days of age, and transferred to the nursery house. Around 70 days of age, pigs were transferred to the grower/finisher house. In this trial, we used Ingelvac CircoFLEX® as a global PCV2 vaccine and one of the local PCV2 vaccines. Per batch of pigs one treatment group was included: The first batch (group A) was vaccinated with one dose of 1 ml of Ingelvac CircoFLEX® (1,313 pigs) at 21 days of age. The second batch (group B) was vaccinated with one dose of a local PCV2 vaccine (1,177 pigs) at 21 days of age. The third batch (group C, 1,306 pigs)) was treated like group A with one dose of Ingelvac CircoFLEX® at 21 days of age. The fourth batch (group D, 1,294 pigs) was vaccinated with the same vaccine like group B but as a 2-dose at 21 and 35 days of age. Differences in mortality between groups were evaluated using BECAL (Boehringer Ingelheim Economic CALculator) [2].

## Result

By comparing Ingelvac CircoFLEX® and local PCV2 vaccine, we detected a difference in mortality. In 'A' group, mortality is 3.65%. In 'B' group, mortality is 10.45%. In 'C' group, mortality is 3.90%. And in 'D' group, mortality is 6.49% (Table.1).

Other indicators of production, such as average daily weight gain (ADWG), feed conversion rate (FCR) and average market days were also different in favor of Ingelvac CircoFLEX (Table.2). The result between group 'A' and 'b' of returns on investment(ROI) using BECAL is 16.6:1 in case of carcass price 4,000 KRW/kg, feed price 500 KRW/kg and difference of vaccine price per pig 1,200KRW.

***Table.1*** Number of dead pigs per production phase and wean-to-finish mortality in each group

| | | Group A | Group B | Group C | Group D |
|---|---|---|---|---|---|
| Number of pigs | | 1,313 | 1,177 | 1,306 | 1,294 |
| Number of dead pigs | Nursery | 17 | 40 | 15 | 43 |
| | Grower-Finisher | 31 | 83 | 36 | 41 |
| Mortality(%) | | 3.65 | 10.45 | 3.90 | 6.49 |

***Table.2*** The variations of FCR, ADWG and average market days in each group

| | Group A | Group B | Group C | Group D |
|---|---|---|---|---|
| FCR | 2.82 | 2.87 | 2.81 | 2.91 |
| ADG(g) | 797 | 775 | 812 | 714 |
| Average market days | 187 | 192.7 | 182 | 197.4 |

## Discussion

As demonstrated before, PCV2 vaccines can have a significant effect on mortality in Korean pig production. This comparative trial shows that the effect of PCV2 vaccines on performance parameters can vary between commercial products. This is critical for decision making on PCV2 vaccines. Not the price of vaccines should make the decision on product choice but the efficacy of the different vaccines.

## Reference

[1] Park et al. (2014) Swine disease report 2013, Korean Pork Producer Association
[2] M. Adam et al. (2013) Proc 5th ESPHM symposium, Edinburgh, United Kingdom, P124

# Comparative field efficacy study of Ingelvac Circoflex® and Fostera PCV in a Korean farm

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## Introduction

Porcine circovirus type 2 is a well-known virus that causes PCVAD. But after the market entry of PCV2 vaccine, PCVAD symptoms disappeared in many farms. So PCV2 vaccines have been used as the most efficient tool to control PCVAD. Different PCV2 vaccines have been developed and are now marketed in Korea by global or local pharmaceutical companies. It has been shown that these vaccines differ in terms of efficacy and safety. The purpose of this study was to compare two commercial PCV2 vaccines in terms of efficacy

## Materials and Methods

The field observation was conducted in a farrow to finish farm with 150 sows. 272 pigs of 4 consecutive production batches of pigs were vaccinated with Ingelvac CircoFLEX® at 21 days of age (group A). 281 pigs of the following four batches were vaccinated with Fostera PCV at the same age.

The next four weekly batches of pigs were again vaccinated with Ingelvac CircoFLEX® (280 pigs) at 3 weeks of age. All pigs were kept under similar conditions in the same facility. Pigs of treatment group A and C were vaccinated with Ingelvac MycoFLEX® which was used combined with Ingelvac CircoFLEX at 21 days of age. In treatment group B Ingelvac MycoFLEX® was given at 14 days of age.

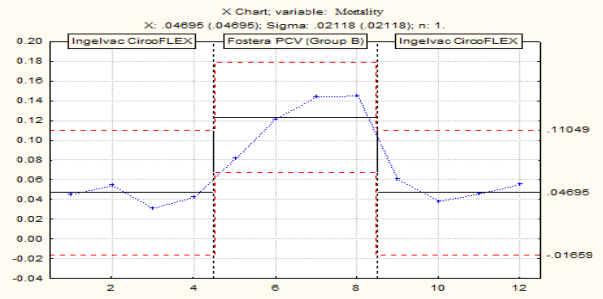
## Results

The levels of mortality per treatment group and per batch are given in table 1 and figure 1.

***Table 1.*** *Number of pigs and dead pigs per group and post weaning mortality in A, B and C group*

| | Ingelvac CircoFLEX® (group 'A') | Fostera PCV (group'B') | Ingelvac CircoFLEX® (group 'C') |
|---|---|---|---|
| Number of pigs | 272 | 280 | 281 |
| Number of dead pigs | 12 | 35 | 14 |
| Mortality | 4.41% | 12.50% | 4.98% |

***Fig 1.*** *Post-weaning mortality comparison between Ingelvac CircoFLEX® and Fostera PCV in 12 consecutive batches using control SPC chart*



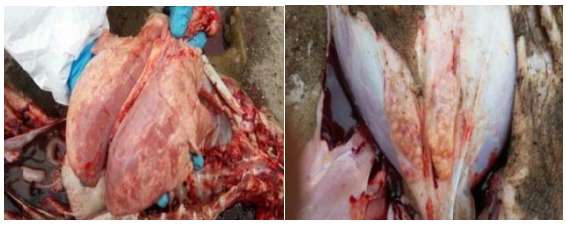



**Figure. 1** *Enlarged and swollen lung and lymph node in group B*

In treatment group B, some pigs showed coughing and wasting and a couple of those that perished were subjected to necropsy where enlarged and non-collapsed lungs and enlarged lymph nodes were observed. Of these pigs lung samples were tested positive by PCR for PCV2.

## Discussion and Conclusions

As shown before in Korea (1), In this case Ingelvac CircoFLEX® vaccinated pigs showed higher and more consistent performance than pigs vaccinated with Fostera PCV. Clinical signs, necropsy findings and lab testing indicate that clinical PCVAD was the main reason for higher mortality in group B compared to group A and C. After group B, Ingelvac CircoFLEX® was applied again next four batches to increase the correlation between mortality and vaccine efficacy. The results show that the choice of PCV2 vaccine is critical when it comes to PCV2 control.

## Reference

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# In Vitro Study of Organic Releasing Chlorine Disinfectant (Virusnip®) Contact Time for Porcine Circovirus Type 2

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## Introduction

Porcine Circovirus type 2 (PCV2) is considered as one of the most important viral pathogens in swine industry worldwide, causing significant economic losses.[1] It plays an important role in several clinical symptoms that are summarized as PCV-associated disease (PCVAD) including post-weaning multisystemic wasting syndrome (PMWS), porcine dermatitis and nephropathy syndrome (PDNS), porcine respiratory disease complex (PRDC), enteric disease and reproductive failure.[1,2] There are many measures to control PCV2 infection. Biosecurity, hygiene and husbandry practices by applying disinfectant was proved to reduce viral load and minimize re-infection as the previous study.[3] This study aims to identify the contact time of Virusnip® for PCV2.

## Materials and methods

*Disinfectant:* Virusnip® (Lot.: 009237)

*Virus and cell:* PCV2 (stock virus (11NP96)) from Veterinary Diagnostic Laboratory Center, Chulalongkorn University was used at $10^{4.55}$ $TCID_{50}$/ml.

*Virucidal activity*: Virusnip™ was prepared in 4 concentrations in distil water including 1:100, 1:200, 1:400 and 1:1000 and mixed with the virus stock. The mixture was incubated at room temperature for 3 and 10 minutes, filtrated through Sephadex 20™ column. The filtrated solutions were inoculated into PK15 cell line. Report of viral growth in each dilution had been evaluated (Modified EN 14675 method).

## Result

The virus titer of all treatments and control are shown in Table 1.

**Table.1 Viral titration after contacting with agents in each concentration (Conc.) and contact time (CT)**

| Virusnip Conc. | Virus titer (log 10) at CT 3 min. | Virus titer (log 10) at CT 10 min. |
|---|---|---|
| 1:100 | 0 | 0 |
| 1:200 | 0 | 0 |
| 1:400 | 0 | 0 |
| 1:1000 | 0 | 0 |
| Control | 4.15 | 4.15 |

## Discussion

The results demonstrate that the disinfectant (Virusnip®) was capable to kill PCV2 at every contact time. The lowest concentration and shortest contact time is at 1:1000 for 3 minutes. This can be applied for usage under field conditions as an effective measure to control PCV2 infection by reducing viral environmental load and limiting the transmission rate within the herd.

## Reference

1. Segales J. et al., 2013, Vet. Micobiol. 165 (2013) 13-20.
2. Opriessing T. et al., 2012, Vet. Pathol. 50 (1) 23-38.
3. Poonsuk K. et al., 2013, APVS 2013 Proceedings. PO44.

# The effects of *Actinobacillus pleuropneumoniae* and Porcine circovirus type 2 combined vaccine (APM-X®) in pigs

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## Introduction

*Actinobacillus pleuropneumoniae* (APP) is one of the most important pathogens that causes a highly contagious respiratory disease in pigs of all ages. In Korea, the isolation rate of APP serotype 1 (APP 1) has significantly increased during the past few years, however vaccines for APP 1 are rarely available in the domestic market. Therefore, there is a growing need to develop a vaccine that can protect against various strains (serotype 1, 2, and 5) of APP.

Porcine circovirus type 2 (PCV2), which is the main causative agent of porcine circovirus associated disease (PCVAD) such as post-weaning multi-systemic wasting syndrome (PMWS) and porcine respiratory disease complex (PRDC), is also a pathogen that largely and negatively impacts the the Korean swine industry. Thus, vaccination against PCV2 is largely conducted in the country. However, the current problem is that in many farms, there is a tendency for the virus to reemerge at the finishing stage even after vaccination.

In this study, we performed an experiment to see whether APM-X® is effective or not for preventing various strains of APP and PCV2 infection simultaneously.

## Materials and methods

**Groups and vaccinations:** Two hundred sixty two pigs at 5 weeks of age were divided into test group (n=135) and control group (n=127). They were mixed and raised together with their ears tagged. The test group was vaccinated twice at 5 and 7 weeks of age.

**Serology:** In order to confirm the seroconversion and the Ab duration, 20 blood samples were collected from each group at 5, 7, 10, 15, and 20 weeks of age, and then ELISA Ab titer (S/P ratio) against PCV2 and APP 1, 2, and 5 were measured. The content of PCV2 viremia was determined by real time PCR.

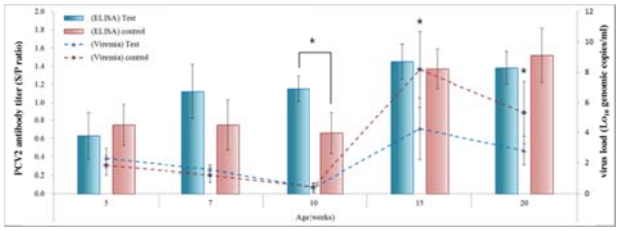
**Productivity evaluation:** The mortality rate, average body weight (ABW, kg), and average daily gain (ADG, kg) of each group were identified until marketed.

## Result

**Serology:** The Ab titer of PCV2 and APP in the test group significantly increased at 10 weeks of age compared to those of the control group (Figure 1 and 2). The content of PCV2 viremia in the test group was significantly less ($p$<0.05) than the control group at 15 and 20 weeks of age (Figure 1.).

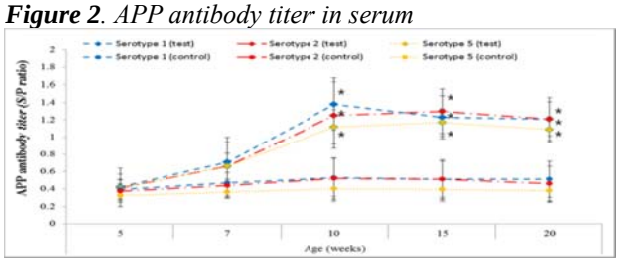
**Productivity evaluation:** In the test group, the mortality rate significantly decreased while ABW and ADG were significantly higher than those of the control group.

***Figure 1***. *PCV2 antibody titers and viremia in serum*




* Significantly different compared with control group ($p$<0.05)

***Figure 2***. *APP antibody titer in serum*




* Significantly different compared with control group ($p$<0.05)

***Table 1***. *Mortality rate and growth performance*

| Group | Mortality rate | ABW (kg)[b] | ADG (kg)[b] |
|---|---|---|---|
| Test | 5/135 (3.7%)[a] | 110.4±4.56 [a] | 0.60±0.02 [a] |
| Control | 12/127 (9.4) | 109.1±5.06 | 0.57±0.03 |

[a]Significantly different compared with control group ($p$<0.05).
[b]Mean±SD

## Discussion

In this study, after vaccinating APM-X®, the Ab against PCV2 and three APP serotypes lasted until >20 weeks of age, and the PCV2 viral load was significantly reduced during the late finisher period. As a result, the mortality rate and average daily gain in pigs remarkably improved with APM-X® vaccination.

## Reference

[1] P. Carasova. et al., The levels of PCV2 specific antibodies and viremia in pigs., Research in Veterinary Science 83 (2007) 274–278.

# Efficacy of a porcine circovirus type 2(PCV2) chimeric vaccine inoculated to 10-week-age piglets from sows using PCV2 vaccine

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## Introduction

Porcine circovirus type 2 (PCV2) is a causative agent for porcine circovirus-associated disease (PCVAD). In Japan, 4 inactivated vaccines for piglets or sows have been commercially distributed and contributed to the PCVAD control. It has been reported that maternally-derived antibody (MDA) of PCV2 should interfere with the humoral immune response of the vaccination for suckling pigs [1]. The objective of this study is to confirm the efficacy of PCV2 vaccine administered at 10 weeks of age, for the purpose of avoiding MDA interference, in a farm adopting PCV2 vaccine for sows.

## Materials and methods

The study was conducted in a commercial farm of 700 sows with PCV2 infection, located in the southern region of Japan between August 2014 and February 2015. Pregnant sows were injected with Circovac® (Merial) 5 weeks before farrowing. Healthy pigs of 250 born in August 2014 were randomly allocated into 2 groups, vaccine (130 heads) and control (120 heads) groups. In the vaccine group, Fostera®PCV (Zoetis), an inactivated PCV2 chimeric vaccine, was inoculated at 10 weeks of age. In the control group, meanwhile, any PCV2 vaccines were not administered to piglets.

The average daily gain (ADG) during the fattening period was calculated by measuring the live body weights (LBW) at the time of moving to the fattening barn and shipping. PCV2 antibody titers and the genomic copies in sera were inspected at 60, 100 and 150 days of age using ELISA and real-time PCR methods, respectively.

## Results

While ADG in the fattening period of the vaccine group was 76g higher than that of the control group (Table.1), there was no significant difference between the 2 groups.

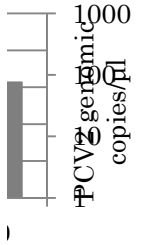
PCV2 genes were only detected at 150 days of age in the vaccine group, whereas in the control group, observed at both 100 and 150 days of age. Furthermore the amount of PCV2 genomic copies in the vaccine group at 150 days of age was clearly lower than that in the control group.

PCV2 antibody titer responses did not have any significant difference between the 2 groups, but the titer of the vaccine group at 100 days of age tends to be higher compared to the control group (Figure.1)

*Table.1* LBW and ADG

| Group | Moving to fattening burn (LBW) | Shiping (LBW) | ADG |
|---|---|---|---|
| Vaccine | 39.4kg | 126.6kg | 992g |
| Control | 39.6kg | 116.8kg | 916g |
| Differences | -0.2kg | +9.8kg | +76g |

*Figure.1*




## Discussion

This present study showed that the vaccination of Fostera®PCV to young pigs at 10 weeks of age in the commercial farm with PCV2 infection was efficacious for improving the immune response to PCV2 vaccination as well as reducing the number of the genomic copies, even if sows of those piglets were administered with PCV2 vaccine before farrowing. As a result, it seemed that the productivity in the fattening period was improved.

## Reference

[1] Yeonsu Oh, Vet Microbiol, 2014, 172;371-380

# Comparison of growth performance between different commercial PCV2 and *Mycoplasma hyopneumoniae* vaccines in Japan

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## Introduction

Porcine Circovirus 2 (PCV2) and *Mycoplasma hyopneumoniae* (M. hyo) are important pathogens which cause respiratory problems and economic losses in pig production (1). In this study, pigs vaccinated with different commercial PCV2 and M. hyo vaccines were compared in a side-by-side trial in field.

## Materials and methods

The study was performed in a farrow-to-finish, 1,000 sow farm in Kanto area in Japan. 312 piglets in one batch were included. On D0, 1-7 dayold piglets per litter were equally divided into group A (n=150) and B (n=162). Animals were individually identified by ear tags. Group A was vaccinated with 2 ml of mixture of PCV2 and M. hyo vaccines in one injection at D21. Group B received 2 ml of M. hyo vaccine at D0 and 2 ml of PCV2 vaccine at D21. The two treatment groups were kept commingled until market. Individual body weight (BW) was measured at D0, 21, 68 and 147. At the same points, blood samples were collected from 7 pigs in each group randomly selected for monitoring of PCV2, M. hyo and PRRSV. Lung lesion (LL) score related to M. hyo infection was checked at slaughter in 55 and 50 heads in group A and B, respectively. Scoring was based on Japanese guideline for evaluation of inactivated M. hyo vaccine (Score 0 to 4). All data was statistically analyzed using Minitab® 17.1.0.

## Result

PCV2 and PRRSV infection was detected between D68-147 by PCR and ELISA. M. hyo seroconversion was mostly detected at D147 in the study herd. Those infections seemed to be subclinical. No significant differences was detected in lab test results between groups.

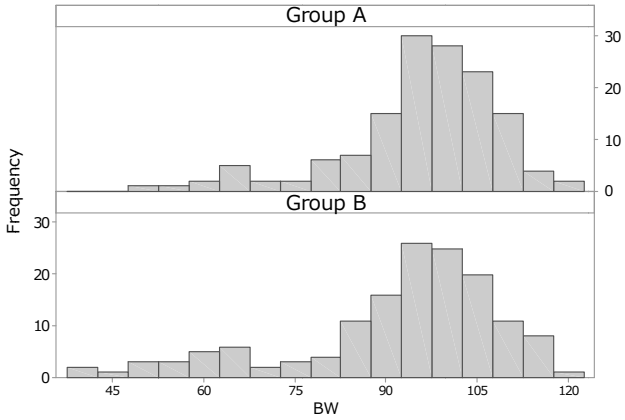
Group A showed a significant higher BW at D147 than group B and ADG from D0 to 147 (Table 1). Moreover, a significant difference in BW variability was detected at D147 with a higher variability in group B (Fig. 1,$p<0.01$, Levene's test). For mortality and LL scores no significant difference between the two treatment groups was detected.

***Table.1 Performance results in study groups***

| | group A | group B | A-B |
|---|---|---|---|
| # of dead pigs | 5 | 7 | - |
| D0 BW (kg) | $1.9^{a}$ (±0.5) | $1.9^{a}$ (±0.5) | 0.0 |
| D21 BW (kg) | $6.9^{a}$ (±1.6) | $6.7^{a}$ (±1.8) | 0.2 |
| D68 BW (kg) | $31.8^{a}$ (±5.1) | $30.8^{a}$ (±5.6) | 0.9 |
| D147 BW(kg) | $95.5^{a}$ (±13.0) | $91.9^{b}$ (±17.2) | 3.6 |
| D0-147 ADG (g/d) | $642.1^{a}$ (±87.8) | $618.0^{b}$ (±116.0) | 24.1 |
| ave. LL score | 0.8 | 1.0 | - |

a, b; $p<0.05$, Welch *t*-test

***Figure 1 Live weight distribution at D147***




## Discussion

Significant better performance was shown in Group A which received one-shot of mixed vaccine for PCV2 and M. hyo In group A, the number of lightweight pigs at D147 was lower than in group B. In conclusion the decision on which products are used for PCV2 and Mhyo control is critical to achieve optimal performance.

## Reference

[1] Choi YK *et al*., Can Vet J 2003;44:735-737.

# Importance of pre-weaning management: heavier pigs around weaning provide better growth in finisher

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## Introduction

Body weight (BW) around weaning has been reported to be important in pig growth performance in the grower/ finisher phase (1). In this study, association of pig growth performances between pre-weaning and finisher stages and factors influencing pre-weaning pig performance were investigated.

## Materials and methods

This research was done from Aug 2014 to Jan 2015 in a farrow-to-finish, 1,000 sow farm in Kanto area in Japan. In this farm, subclinical infections of Porcine Circovirus 2 (PCV2), *Mycoplasma hyopneumoniae* (M. hyo) and porcine reproductive and respiratory syndrome virus (PRRSv) are present in finisher.

At D0 of the study 312 suckling piglets from 1 farrowing batch were individually ear tagged at the age of 1-7 (about 4) days. Litters were equally divided into 2 groups and were vaccinated with commercial M. hyo and PCV2 vaccines. To evaluate the impact of early vaccination, one group (early vax group) received 2 ml of a commercial one-shot M. hyo vaccine at D0 at the age of 1-7 (about 4) days. Therefore 2 ml of a commercial PCV2 vaccine was administered around weaning (D21, 21-28 (about 25) days of age). The other group (non-early vax group) received 2 ml of another commercial M. hyo vaccine mixed with PCV2 vaccine at D21 (about 25 days of age). Pigs were individually weighed at day 0, 21, 68 and 147 of the study.

Statistical analyses were done using Minitab® 17.1.0.

## Result

Positive correlation between average daily gain (ADG) from D0 to 21 and BW at D147 was observed (Fig. 1). Briefly, better ADG during suckling period leads to better performance in finisher.

Only in case of males, ADG from D0 to 21 was significantly lower in early vax group than non-early vax group (Table 1). Significant difference was not observed in post-weaning ADG between groups. However, early vax group tended to be lighter and the variation of individual BW data of early vax group at D147 was significantly larger than another group ($p<0.05$, Levene's test).

***Figure 1 Relativeness between ADG of all study pigs from D0 to 21 and BW at D147***

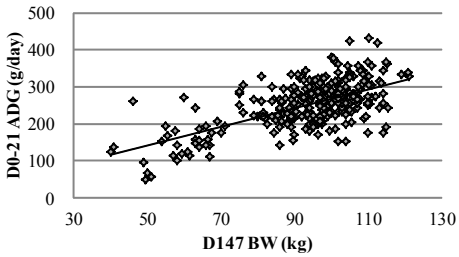


***Table.1 ADG (g/day) of male pigs in groups of early vax and non-early vax during suckling period***

| | Early vax + castration | Non-early vax (only castration) | Δ | p-value |
|---|---|---|---|---|
| D0-21 | 240.0±72.5 | 261.5±63.9 | 21.5 | **0.048** |
| D21-68 | 511.0±100.0 | 526.0±89.9 | 15.0 | 0.325 |
| D68-147 | 775.0±184.0 | 813.0±137.0 | 38.0 | 0.143 |

(Welch's *t*-test)

## Discussion

It was shown that there was correlation between pig performances during suckling period and post-weaning growth. There are various factors which influence pre-weaning ADG and this study showed early M. hyo vaccination is one of the important factors as previous report (2). In this herd, castration was done within a few days of the early vaccination and this combination of stressors might induce worse performance of male piglets. Some of the impacts seemed to continue in finisher.

The conclusion is that a good condition around weaning is followed by better performance in finisher. It is important to consider whole stages of pig lifetime as well as the specific stage if a problem occurred there.

## Reference

[1] Tokach M.D. *et al*., KANSAS STATE UNIVERSITY SWINE DAY 1992:19-21.

[2] Naito M. *et al*., Proceedings of Allan D. Leman conf. 2012: 221.

# The initiative of PRRS area regional control/elimination in Japan (P-JET: PRRS-Japan Elimination Team)

Satoshi Otake[1, 15], Sanae Aizawa[14], Sachiko Arai[2], Tomohiro Furuichi[10], Makoto Furukawa[7], Yuiko Hayakawa[9], Keiko Ikeda[12], Sayoko Ishizeki[3], Hiroshi Iseki[4], Fumiko Koike[5], Yoshihiro Mizukami[8], Mali Miyashita[11], Shingo Nakatake[13] , Makiko Notsute[6], Yugo Watanabe[3], Rika Kano[11], Toshihisa Shibuya[11], Hiromichi Ishikawa[3] & Hiroshi Tsunemitsu[4]

*[1]Swine Extension & Consulting, Inc. [2]Azabu University, [3]Summit Veterinary Services, [4]National Institute of Animal Health Japan, [5]SMC, [6]Notsute Swine Clinic, [7]Toyoura Veterinary Clinic, [8]Akabane Animal Clinic, [9]IDEAS Swine Clinic, [10]Nippon Formula Feed Manufacturing Co., Ltd., [11]Boehringer Ingelheim Vetmedica Japan, [12]Merial Japan Limited., [13]Miyazaki Prefectural Economic Federation of Agricultural Co-operative, Section of Swine, [14]IDEXX Laboratories, K.K., [15]Swine Disease Eradication Center, University of Minnesota*

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## Background

- PRRS is one of the most economically significant diseases in the Japanese swine industry. The annual economical loss due to PRRS in Japan was reported as \28 billion ($373 million)[1]
- To initiate PRRS area regional control/elimination in Japan, P-JET (PRRS-Japan Elimination Team) has been founded since July 2011.

## Objectives

- To organize a working group that consists of swine practitioners, researchers, and industrial partners who focus on PRRS area regional control/elimination in Japan.
- To establish and provide a network, technical know-how, and educational support for pig producers and veterinarians who are active in their PRRS area regional control/elimination projects in Japan.
- To create and publish a hands-on manual of PRRS control/elimination, which will be tailored to the some of the specifics of the Japanese pig industry. The manual will be available for pig producers and veterinarians in Japan.

## Demographics *(update by Jun 2015)*

- 10 regions in 8 prefectures (see Figure 1)
- Approximately 300 sites, 50,000 sows
- Type of production system: Farrow-to-grow (20%), Farrow-to-finish (80%)

## Main Strategies

- Routine P-JET member meeting (periodically)
- Workshop for each project region and case
- Presentation at industrial and academic seminars
- Publication for industrial and academic journals
- Producing and providing technical/educational materials for pig producers and veterinarians


**Figure 1.** Optional map of regions *(updated by Jun 2015)*

## Achievements

- This is the first initiative of PRRS area regional control/elimination in the history of the Japanese swine industry.
- To date (Jun 2015), a total of 24 P-JET member meetings and 4 P-JET workshops have been completed.
- To date (Jun 2015), a number of seminars and publications have been made.
- P-JET herd classification has been established and is being widely used among pig producers and veterinarians in Japan.
- P-JET biosecurity educational brochure has been established and is being widely used among pig producers and veterinarians in Japan.
- P-JET biosecurity risk assessment tool has been established and its usage is already started.

## Next Steps

- To hold workshops that are more technical-oriented, adapted specifically for each project region or case.
- To establish P-JET management recommendation manual.
- To establish P-JET sampling/testing manual.
- To support to complete a mapping in all the project regions.

## References

1. Yamane, et al. (2009) The proceedings of APVS, 70.

# Lung lesion survey using Ceva Lung Program in the Philippines and Vietnam compared to other countries

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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. Moreover, there is a clear relation between lung lesions present at slaughterhouse and economic impact of respiratory disease [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 Animal Health recently developed a tablet-based software tool allowing for rapid scoring of batches of lungs, without the use of paper with automated processing and storage of data. This application is a part of Ceva Lung program (CLP).

## Materials and Methods

In between February 2014 and June 2015, a total of 508 batches of lungs were scored using the CLP app..

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 scoring, to describe EP-associated secondary pleurisy (score 0-1).
- Scarring, describing prevalence of fissures in the cranial lobe associated with older EP-like lesions (score 0-1).
- Dorsocaudal pleurisy score, to describe Actinobacillus pleuropneumoniae (APP)-like lesions (scale 0-4).
- Actinobacillus pleuropneumoniae Index (APPI), using prevalence and grade of dorsocaudal pleurisy.

In total, 40399 lungs were scored originating from the Philippines (15 batches), Vietnam (10 batches) and outside Asia (ROW, 483 batches in 10 countries). Results outside Asia were pooled, and compared with values obtained for the Philippines and Vietnam.

## Results

*Table 1. EP-like lesions*

| | The Philippines | Vietnam | ROW |
|---|---|---|---|
| % affected lungs ($Q_1$-$Q_2$-$Q_3$) | 72%-75%-90% | 60%-83%-92% | 31%-52%-69% |
| % lung affected ($Q_1$-$Q_2$-$Q_3$) | 1%-15%-27% | 5.8%-9.2%-11.2% | 1.5%-3.4%-6.5% |
| Cranial pleurisy score ($Q_1$-$Q_2$-$Q_3$) | 0.24-0.40-0.53 | 0.15-0.18-0.22 | 0.03-0.1-0.21 |
| Scar score ($Q_1$-$Q_2$-$Q_3$) | 0.00-0.06-0.09 | 0.01-0.14-0.31 | 0.02-0.13-0.29 |

*Table 2. APP-like lesions*

| | The Philippines | Vietnam | ROW |
|---|---|---|---|
| % affected lungs ($Q_1$-$Q_2$-$Q_3$) | 4%-15%-33% | 1%-7%-23% | 3%-13%-35% |
| APPI index ($Q_1$-$Q_2$- $Q_3$) | 0.12-0.50-1.05 | 0.03-0.21-0.64 | 0.08-0.33-0.97 |

*$Q_1$=1st quartile, $Q_2$=median, $Q_3$= 3rd quartile*

## Discussion

Both EP-like and APP-like lesions have a high prevalence in pig lungs investigated at the slaughterhouse. Interestingly the incidence of A.p. –like lesions in Vietnam is lower than in other countries, while that of M. hyo-like lesions in higher both in Philippines and Vietnam than in ROW. This finding together with variation between farms suggests improvement of lung health is feasible.

### References

[1] Hill MA, Scheidt AB, Teclaw RF, Clark LK, Knox KE and Jordan M. Relationship between the indicators of performance and the weight of pneumonic lesions from pigs at slaughter. Res Vet Sci. 1994; 56: 240-4.
[2] Krejci R, Bijasa R and Lopez A. Lung lesion survey in the Philippines. Proceedings 6th Asian Pig Veterinary Society Congress, 2013, OR6.

# Duration of immunity of Hyogen® in fattening pigs against Mycoplasma hyopneumoniae

Miklós Tenk, Tamás Szalai, Marianna Ivók, Éric Brunier, Zoltán Nagy, Zoltán Rozsnyai, Zoltán Pénzes

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## Introduction

Mycoplasma hyopneumoniae (M.hyo) is a major infectious agent in swine, it is of worldwide relevance, and responsible for persistent dry cough, retarded growth rate and reduced performance.
The objective of the study was to test the efficacy and duration of immunity of Hyogen® vaccine by M.hyo challenge at six months after vaccination.

## Materials and methods

Forty (40) 3-week old pigs, seronegative to M.hyo were randomly divided into two groups of twenty animals. In the first group (Group 1), pigs were vaccinated at 3 weeks of age with Hyogen® (2 ml I.M.), while in the second group (Group 2) the pigs were injected with PBS as a placebo (2 ml I.M.). Both the vaccinated and the placebo-injected control groups were challenged at 181 days (~6 months) after vaccination with $2x10^8$ CCU/animal dose of M.hyo strain L1. The challenge was repeated on the following day. After a 28 day observation period the animals were euthanized, and the following parameters were collected: mortality and clinical signs, lung lesions (scored as described in the European Pharmacopoeia: 2448), humoral immune response (Oxoid ELISA kit) and cellular immune response (CMI, Porcine IFNγ ELISpot kit). Histology (haematoxiline-eosin stain) and M.hyo-specific real time PCR were used to confirm the presence of M.hyo in the lung samples.

## Results

Clinical signs and mortality. In general, in both groups the clinical signs observed during the post challenge period were very rare and light, therefore these data were not statistically analysed. In the placebo-injected control group (Group 2) one animal died at 25 days post-challenge. The pathological examination confirmed M.hyo as the cause of the death.

Lung lesion scores

Lung lesion were evaluated and scored according the European Pharmacopoeia at 28 days post M.hyo challenge (Table 1). The challenge was valid, as high mean weighted lung scores (40.1) were obtained in the placebo-injected control group (Group 2). The mean weighted lung lesion scores of the vaccinated group (Group 1) were significantly lower (2.8) (Wilcoxon ranksum test, p=0.0001). Histology and M.hyo-specific real time PCR were used to confirm that the lesions observed were due to M.hyo.

*Table 1. Lung lesions scores at 28 days post M.hyo challenge.*

| | n | Mean | Median | SD | Min | Max |
|---|---|---|---|---|---|---|
| **Group 1 Vaccinated** | 20 | 2.8 | 0.0 | 6.1 | 0 | 20 |
| **Group 2 Control** | 20 | 40.1 | 29.0 | 42.3 | 0 | 161 |

Serology. All study animals were seronegative at the time of vaccination. At 23 days post vaccination 80% of the vaccinated pigs (Group 1) became seropositive to M.hyo, and 70% of them remained seropositive till 181 days (~6 months) after vaccination. The placebo-injected control animals (Group 2) remained seronegative during the whole study.

CMI. The number of M.hyo-specific porcine IFNγ secreting cells was measured at 76 days post vaccination. The vaccinated group (Group 1) exhibited significantly higher number of M.hyo-specific IFNγ secreting cells (8.3 cells per $10^6$ PBMC) compared to the control Group 2 (0.5 cells per $10^6$ PBMC; p=0.0057).

## Conclusions

Three-week-old pigs were vaccinated with Hyogen®, and the pigs were challenged with M.hyo at six months after vaccination. At the end of the fattening period Hyogen® provided excellent protection, as the lung lesions scores of the vaccinated group were significantly lower compared to the placebo-injected control group. In addition, in the vaccinated group high level of seropositivity to M.hyo, and significantly increased number of M.hyo-specific porcine IFNγ secreting cells were observed, indicating good vaccine take and strong cell mediated immunity.

# Evaluating the in-use stability of Coglapix® by testing its efficacy against serotype 9 Actinobacillus pleuropneumoniae

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## Introduction

Porcine pleuropneumonia caused by *Actinobacillus pleuropneumoniae* (A.p.) is a highly contagious respiratory disease, characterized by rapid onset, short course, high morbidity and mortality. The disease occurs worldwide with varying incidence and severity. Controlling the disease is difficult, but vaccination can provide efficient protection by decreasing the prevalence and extension of pneumonia and pleuritis, and reduce consequent weight loss of pigs.

Coglapix® vaccine contains inactivated serotype 1 and 2 A.p. strains and their RTX toxins (Apx I, Apx II, and Apx III) in order to provide protection against a broad range of A.p. serotypes.

In order to avoid excessive vaccine wastage we investigated the efficacy of Coglapix using a vaccine vial having been partly used and then the rest of the vaccine kept refrigerated (+2-8°C) for a week ("open-vial Coglapix").

## Materials and methods

Six weeks old pigs were vaccinated either with unopened Coglapix (Coglapix T0) or "open-vial Coglapix" (Coglapix T7) in a prime-boost regime, with three weeks interval. Six weeks after prime vaccination (D42) both the vaccinated and the control pigs were challenged with an A.p. serotype 9 strain in an aerosol chamber by applying approximately $10^8$ CCU/pig of the bacterium suspension. Clinical signs were monitored daily throughout the 7 days post-challenge (pch) observation period. Humoral immune-responses to Coglapix® was measured by ELISA (APX II: in-house method of Ceva-Phylaxia, Budapest; ApxIV: Idexx CHEKIT APP-ApxIV kit). All animals were euthanized and subjected to post mortem examination on D7 pch (D49). Vaccine efficacy was calculated [1] based on post-mortem lesions in the lungs (lung lesion score, LLS [2]) and on the pleura. Serological results, body weight gain, and LLS data were analysed by ANOVA. Differences were considered significant at $p<0.05$.

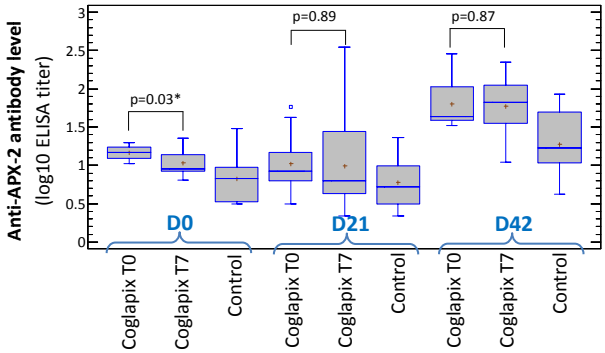
## Results

There was no significant difference between the "normal regime", using new bottle of vaccine for each vaccination and the "open-vial Coglapix" vaccination regime, concerning (i) humoral immune-response to RTX toxins and (ii) the calculated vaccine efficacy against challenge with A.p. 9.

***Table.1*** *Calculated vaccine efficacy values.*

| group | calculated vaccine efficacy (%) |
|---|---|
| Coglapix normal regime | 70.3 |
| Coglapix 7 days open | 70.0 |

*Figure 1. Anti-Apx2 antibody responses after Coglapix vaccinations.*




*NB.: Results of one-way ANOVA are shown above the boxes (p<0.05 denotes statistically significant difference).*

## Discussion

In conclusion, the results obtained confirmed the in-use stability of Coglapix at least for a week if the partially used vaccine kept refrigerated at +2-8°C.

## References

[1] Jones, GF. et al.: J. Swine Health Prod. 2005; 13(1):19-27.
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# Field comparison of two commercial vaccines for controlling mutant porcine circovirus type 2 viremia

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## Introduction

Recently a new strain of porcine circovirus type 2 has been identified in the US based on genetic sequencing.[2] This virus has a similar sequence pattern to a virus previously identified in China and is often referred to as the "Chinese mutant" or mutant PCV2 (mPCV2). Some concern has been expressed regarding the ability of current US commercial vaccines to protect against this new strain. As part of a producer initiated PCV2 vaccine evaluation, we were provided the opportunity to monitor the PCV2 viremia and antibody status of pigs undergoing field exposure to mPCV2.

## Materials and Methods

The pigs originated from a herd free of PRRSv and *Mycoplasma hyopneumoniae* (Mhp) and were part of a larger field evaluation comparing the performance between two commercial PCV2 vaccines: Fostera™ PCV (FOST) (Zoetis, Florham, NJ) and Circumvent® PCV (CVENT) (Merck Animal Health, Summit, NJ). This study used a "barn level" design. The pigs that were monitored for PCV2 viremia by PCR and PCV2 antibody by 4-dilution IFA were housed in two adjacent finisher barns on the same site. The FOST pigs were vaccinated once at weaning (3 weeks of age). For CVENT vaccination, the producer elected to administer the two vaccinations at processing (3 days of age) and at weaning. The pigs were tagged after arrival to separate nursery rooms and the same pigs were sampled at 4, 11, 16 and 19 weeks of age. Forty pigs from the source sow herd were sampled at 10 days of age. All laboratory testing was performed by routine methods at the ISU-VDL. Samples for PCR were tested in pools of 5. To confirm the presence of mPCV2 in each barn, oral fluids from 5 pens and blood from 10 non-tagged, light-weight pigs were collected at 19 weeks of age. Several positive samples were sequenced and all sequences indicated mPCV2. Based on serotesting at 19 weeks of age, the pigs remained free of PRRSv and Mhp. Statistical analysis was performed by ANOVA and a P value <0.05 was considered significant.

## Results

The 10-day-old pigs from the source herd were not viremic and had a geomean IFA titer of 190.3 (Data not shown). The table below presents the PCR and IFA results from the tested serum. IFA titers were significantly higher in the CVENT pigs compared to the FOST pigs at 4, 11 and 16 weeks of age. At 19 weeks of age, the titers of the FOST pigs were significantly greater than the CVENT pigs.

The average cycle times (CTs) for the positive pools from the FOST pigs at 16 and 19 weeks of age were 27.90 and 23.84, respectively. For the light-weight pigs, the two FOST pools had CTs of 25.00 and 22.10. The one CVENT positive pool CT was 26.4. For the oral fluids, the average CTs for the FOST and CVENT barns were 23.54 and 33.53, respectively.

| Age (wks) | PCR – Pools Pos./Tested | | Geomean IFA Titers | |
|---|---|---|---|---|
| | CVENT | FOST | CVENT | FOST |
| 4 | 0/5 | 0/5 | 498.7[a] | 131.8[b] |
| 11 | 0/5 | 0/5 | 560.8[a] | 86.9[b] |
| 16 | 0/4 | 5/5 | 266.6[a] | 139.3[b] |
| 19* | 0/5 | 5/5 | 216.3[b] | 844.3[a] |
| 19** | 1/2 | 2/2 | 139.3[b] | 735.2[a] |

[a,b] If different within a row, $P < 0.05$.
* Tagged pigs. ** Light weight pigs.

## Conclusions and Discussion

The data presented clearly illustrates the ability of CVENT to protect against mPCV2 viremia and brings into question the ability of FOST to provide a similar level of protection. In addition, the IFA titers indicate a greater level of protection based on the declining titers of CVENT pigs and rising titers of FOST pigs at 19 weeks of age. This finding has been reported in a previous field study that compared non-viremic, CVENT vaccinated pigs (declining titers) to viremic, non-vaccinated controls (rising titers).[1] The oral fluid results indicate a lower level (approximately 1,000 times less) of virus shedding in the CVENT barn. This suggests that CVENT vaccinated pigs shed less virus than FOST vaccinated pigs. Accordingly, the benefit of vaccination may extend beyond controlling infection and disease in the pig to controlling the level of environmental contamination. The level of PCV2 in the environment may impact the onset of infection, disease severity and the performance of vaccines over time.

## References

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# Field comparison of PCV2 vaccines: A retrospective production data analysis

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## Introduction

In this case study, a retrospective production record data analysis is presented that found significant differences in PCV2 vaccine performance and estimates economic impacts of these differences. The operation purchases weaned pigs from two separate flows. One flow consists of terminal line animals where all progeny are sold for slaughter. The other flow consists of maternal line animals where barrows and some gilts are sold for slaughter while most gilts are sold as replacements. Both flows are considered to be of high health status and are managed the same. Originally, a one dose PCV2 vaccine, CircoFLEX® (CFLEX) (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was used in both flows. The maternal flow was switched to Circumvent® PCV (CVENT) (Merck Animal Health, Summit, NJ) in response to concerns about viremia in replacement gilts. The producer judged that the maternal flow was outperforming the terminal flow and decided to switch the terminal flow to Fostera™ PCV (FOST) (Zoetis, Florham, NJ). The performance of the terminal flow still lagged behind the maternal flow so the producer requested assistance with analyzing the operation's production records along with an economic assessment.

## Materials and Methods

Production data in this operation was collected by site as each group was closed-out and the site was emptied. Only finisher data was evaluated; evaluation of nursery performance revealed no differences between the two flows. For statistical comparison, data was organized into two time periods. In Time Period 1, CFLEX was compared to CVENT. In Time Period 2, FOST was compared to CVENT. Overall average values for average daily gain (ADG), feed conversion ratio (FCR), mortality rate and cull rate were weighted by the number of pigs placed in each group. The genetic supplier indicated that ADG and FCR would be impacted such that the maternal line would have lower ADG (0.064 lb/ day) and increased FCR (0.100 less efficient). Accordingly, the data was analyzed with (genetic line adjusted-ADJ) or without (actual-ACT) group adjustments. The data was analyzed on a group basis by ANOVA or an individual pig basis by Chi square. A swine enterprise budgeting model was used to determine the economic differences between vaccines within each time period. The outcome is reported as difference in profit per pig.

## Results

For both periods, the mortality and cull rates were significantly ($P<0.05$) lower in CVENT vaccinated groups compared to CFLEX or FOST (Table 1). For ADG, no differences were found using actual or adjusted values. For FCR, no differences were found between vaccines within time period using actual values. However, FCR was significantly better in CVENT groups using adjusted values.

For individual pig based analysis, for CFLEX and FOST pigs, the odds of dying were 1.87 and 1.98 times greater compared to CVENT pigs and the odds of being culled were 1.90 and 1.76 times greater, respectively.

Economic analysis revealed that the increase in profit/pig by CVENT compared to CFLEX ranged from \$0.99 to \$6.27 and compared to FOST from \$0.70 to \$6.16, depending on the parameter values used in the model. The largest differences were calculated using ADG and FCR adjusted values.

**Table 1**. Summary of group close-out performance.

| Parameter | Results | | | |
|---|---|---|---|---|
| Flow | TERMINAL | | MATERNAL | |
| Period | 1 | 2 | 1 | 2 |
| Vaccine | CFLEX | FOST | CVENT | |
| Groups | 29 | 30 | 25 | 20 |
| No. pigs | 56,830 | 60,847 | 66,611 | 54,202 |
| % Died | 2.67% | 3.01% | 1.45% | 1.55% |
| % Culls | 1.61% | 1.31% | 0.87% | 0.76% |
| ADG-ACT | 1.91 | 1.97 | 1.89 | 1.91 |
| ADG-ADJ | | | 1.95 | 1.97 |
| FCR-ACT | 2.74 | 2.63 | 2.74 | 2.63 |
| FCR-ADJ | | | 2.64 | 2.53 |

## Conclusions and Discussion

This situation provided a unique opportunity to compare PCV2 vaccine performance under field conditions. The operation switched the terminal flow to Circumvent PCV and close-out data from several initial groups reveals reduced mortality and cull rates similar to the CVENT vaccinated maternal flow.

# Apparent absence of PCV2 exposure in a Circumvent® PCV M vaccination timing field study

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**Introduction**

Timing of vaccination in a herd depends on several factors including onset of disease, labor availability, combining with other routine procedures and maternally-derived antibody (MDA) levels. The data reported here was part of a larger study that evaluated the impact of timing of Circumvent® PCV M vaccination on growth performance. Here we present the serological data that was generated from the study.

**Materials and Methods**

The study was conducted at the JBS United Burton-Russell Research Farm. Pigs from 4 consecutive weaning groups (every 4 weeks) were allocated to treatment group at 1 week of age (WOA). Three treatment groups, as directed by the producer and herd veterinarian, were evaluated: 1) A - vaccination at processing (1 WOA) and weaning (3 WOA); 2) B - vaccination at processing and at 6 WOA; and 3) C - vaccination at weaning and 6 WOA (per label directions). Each treatment group contained approximately 475 pigs. Five pigs/weaning group/ treatment (n = 20) were randomly selected for repeated blood sampling. Also, one pen in each of the first two weaning groups was left unvaccinated and 10 pigs from these pens were bled at each time point. All samples were tested for PCV2 by PCR. A subset of samples was randomly selected for determining PCV2 serum antibody levels by the Four-Dilution (160, 320, 640 and 1280) IFA and to *Mycoplasma hyopneumoniae* (Mhp) by ELISA (IDEXX, Westbrook, ME). All testing was performed by Iowa State University Veterinary Diagnostic Laboratory.

**Results**

All PCV2 PCR assays were negative including samples collected from non-vaccinated control pigs (Table 1). Control pigs remained PCV2 seronegative throughout the study with the exception that two pigs had titers of 160 and 320 17 WOA. In vaccinated groups, the maximum IFA titer at 6 WOA for group A-1/3 and 9 WOA for groups B-1/6 and C-3/6. Titers declined thereafter and were low 24 WOA. All groups had a moderate level of Mhp MDA at 1 WOA (Table 2). The MDA had the greatest impact on the 3 week post-vaccination titers of group A-1/3. By 17 WOA, control pigs started to seroconvert and antibody levels in vaccinated pigs increased with exposure to Mhp.

**Table 1**: **PCV2 IFA Geomean Reciprocal Titers**

| Age (wks) | Para- meter | Treatment Group | | | |
|---|---|---|---|---|---|
| | | CONT | A-1/3 | B-1/6 | C-3/6 |
| 1 | Pos. | ND | 0/10 | 0/10 | 0/10 |
| | Geom. | ND | <160 | <160 | <160 |
| 3 | Pos. | 0/9 | 0/10 | 0/10 | 0/10 |
| | Geom. | <160 | <160 | <160 | <160 |
| 6 | Pos. | 0/9 | 10/10 | 7/10 | 8/10 |
| | Geom. | <160 | 735.2 | 278.6 | 320.0 |
| 9 | Pos. | 0/9 | 10/10 | 10/10 | 10/10 |
| | Geom. | <160 | 970.1 | 1114.3 | 1280.0 |
| 17 | Pos. | 2/9 | 4/10 | 8/10 | 10/10 |
| | Geom. | 100.8 | 121.3 | 183.8 | 367.6 |
| 24 | Pos. | 0/9 | 2/10 | 5/10 | 7/10 |
| | Geom. | <160 | 98.5 | 130.0 | 171.5 |

**Table 2: Mhp ELISA S/P Ratios**

| Age (wks) | Para-meter | Treatment Group | | | |
|---|---|---|---|---|---|
| | | CONT | A-1/3 | B-1/6 | C-3/6 |
| 1 | Pos. | ND | 7/10 | 6/10 | 7/10 |
| | S/P | ND | 0.538 | 0.658 | 0.653 |
| 3 | Pos. | 0/9 | 1/10 | 3/10 | 3/10 |
| | S/P | 0.090 | 0.201 | 0.321 | 0.291 |
| 6 | Pos. | 0/9 | 6/10 | 0/10 | 0/10 |
| | S/P | 0.010 | 0.534 | 0.092 | 0.084 |
| 9 | Pos. | 0/9 | 5/10 | 10/10 | 6/10 |
| | S/P | 0.000 | 0.461 | 1.061 | 0.653 |
| 17 | Pos. | 6/9 | 10/10 | 10/10 | 10/10 |
| | S/P | 0.759 | 1.144 | 1.908 | 1.147 |
| 24 | Pos. | 7/9 | 10/10 | 10/10 | 10/10 |
| | S/P | 1.102 | 1.543 | 2.056 | 1.470 |

**Conclusions and Discussion**

Based on negative PCV2 PCR results and failure of control pigs to seroconvert, pigs in this study were not exposed to PCV2. This finding has implications for conducting field studies that rely on natural exposure; some method is needed to confirm exposure. In this study, non-vaccinated control pigs served that purpose. Oral fluid samples may

also provide evidence of exposure. Mhp MDA appeared to decrease but not eliminate the antibody response to vaccination depending on the age of the pig at both the first and second vaccinations.

# Utilization of laboratory testing for monitoring Porcine Circovirus type 2 vaccination programs

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## Introduction

Vaccination for porcine circovirus type 2 along with M*ycoplasma hyopneumoniae* (Mhp) is performed in nearly all swine operations. Vaccination compliance along with logistic considerations related to other procedures, pig health at the time of vaccination and the potential for vaccine interference by maternally-derived antibody (MDA) need to be considered when developing and implementing vaccination programs. Serological monitoring is commonly done to assess potential MDA interference, to evaluate post-vaccination immune responses as an indicator for vaccination compliance and to determine the onset of infection.[1] The objective of the study reported here was to evaluate different serological assays for Circumvent® PCV M compliance monitoring.

## Materials and Methods

Pigs were vaccinated with Circumvent® PCV M at processing (3-4 days of age) and weaning (3 weeks of age) as directed by the herd veterinarian. Immediately after weaning, selected pigs were ear tagged and blood sampled at the nursery site. The initial plan was to bleed the pigs again at 6 weeks of age (WOA). Due to poor antibody responses at 6 WOA, additional samples were collected at 10, 15 and 21 WOA. A second group (B) was started 8 weeks after the first group (A) using the same altered protocol. All laboratory testing was performed at Iowa State University Veterinary Diagnostic Laboratory. PCV2 serum antibodies were measured by 4-Dilution PCV2 IFA, Ingezim Circovirus IgG (INGEL) (Ingenasa, Madrid, Spain) and PCV2 ELISA (PCVEL). Mhp serum antibodies were measured by ELISA (MHEL) (IDEXX, Westbrook, ME). Titer values are reported as group geometric mean. For the IFA test, a titer $\geq$640 was considered positive. S/P ratios and PCR cycle times (CT) are reported as group means. Selected time points were tested for PCV2 by PCR.

## Results

Overall, post-vaccination responses at 6 and 10 WOA were lower than expected (Table 1). MDA interference may have been responsible for lower Mhp titers but were not a consideration for low PCV2 IFA titers. The INGEL and PCVEL were not done at 3 and 6 WOA due to misplacement of the samples. Subsequent testing indicated exposure to PCV2 and Mhp by all assays. PCV2 PCR and IFA indicated onset of PCV2 infection between 15 and 21 WOA. PCV2 PCRs were negative up through 20 WOA (Table 2). The post-vaccination antibody response is consistent with proper vaccination. PCV2 MDAs were low and Mhp MDAs were lower than observed with Group A.

**Table 1: Group A Results**

| Age (wks) | No. positive/No. tested | | | | |
|---|---|---|---|---|---|
| | IFA | INGEL | PCVEL | MHEL | PCR |
| 3 | 8/20 | ND | ND | 17/20 | ND |
| 6 | 4/20 | ND | ND | 6/20 | ND |
| 10 | 4/19 | 15/19 | 17/19 | 3/19 | ND |
| 15 | 2/19 | 8/19 | 8/19 | 0/19 | 0/19 |
| 21 | 17/19 | 19/19 | 19/19 | 13/19 | 16/19 |
| | Titers | | S/P Ratios | | CT |
| 3 | 134.5 | ND | ND | 0.985 | ND |
| 6 | 109.3 | ND | ND | 0.462 | ND |
| 10 | 119.5 | 333.2 | 0.662 | 0.242 | ND |
| 15 | 92.6 | 122.5 | 0.416 | 0.100 | >37 |
| 21 | 856.9 | 1097.6 | 1.073 | 0.738 | 30.1 |

**Table 2: Group B Results**

| Age (wks) | No. positive/No. tested | | | | |
|---|---|---|---|---|---|
| | IFA | INGEL | PCVEL | MHEL | PCR |
| 3 | 0/25 | 25/25 | 25/25 | 7/25 | 0/25 |
| 6 | 22/23 | 23/23 | 23/23 | 23/23 | 0/23 |
| 10 | 19/24 | 24/24 | 24/24 | 23/24 | 0/24 |
| 16 | 17/22 | ND | 9/22 | 22/22 | 0/22 |
| 20 | 0/22 | ND | 13/22 | 24/24 | 0/24 |
| | Titers | | S/P Ratios | | CT |
| 3 | 80 | 653 | 0.748 | 0.303 | >37 |
| 6 | 1037 | 8299 | 0.987 | 1.737 | >37 |
| 10 | 640 | 1427 | 0.816 | 1.692 | >37 |
| 16 | 273 | ND | .299 | 1.542 | >37 |
| 20 | 128 | ND | .413 | 1.300 | >37 |

## Conclusions and Discussion

It appears that Group A was not properly vaccinated as all assays showed poor responses to PCV2 and Mhp. Group B appears to be properly vaccinated with nearly all pigs positive in all tests With regard to assays, IFA, INGEL and MHEL appear to be more discriminating than the PCVEL based on the titers or S/P ratios at 3 vs. 6 WOA in Group B.

## References

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# Outbreak and fade out of a genotype 2 Porcine Reproductive and Respiratory Syndrome virus in three German SPF-herds: Role of vaccination and herd closure

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## Introduction

PRRS-negative farms can be created by depopulation and repopulation with negative piglets and gilts from PRRS-free farms. Following strict hygienic procedures, these farms can remain PRRS-free in non-pig-dense areas like the eastern part of Germany. In the summer of 2011, three large German multiplying pig farms became infected with a US-PRRS virus (Type 2), possibly via infected sperm. All three farms had production problems like weak- and little piglets at farrowing with high mortality of pre- and post-weaning piglets. A Type 2 PRRSv was found via PCR in weak born piglets and had a sequence homology of 95-96% with VR2332. The farms decided to implement a control/eradication strategy in line with Vogelmayer and van Groenland. Porcilis® PRRS was the vaccine of choice based on evidence of cross-protection of EU-MLV vaccines against Type 2 PRRS and reduced transmission of Porcilis®PRRS compared to the US MLV vaccine (Ingelvac PRRS-MLV). To reduce the risk of transmission between pigs by needle injection, a needle-less vaccination system was used (IDAL®, intra dermal applicator of liquids, MSD Animal Health).

## Materials and Methods

*Herd*

Farm A (Thuringia) is a multiplying farm with 1200 sows, no nursery and SPF-rearing gilts arrive at 150 days. Farm B (Brandenburg) is a multiplying farm with 2000 sows, with a nursery on the same site and own rearing gilts until the moment of the PRRSv infection. Farm C (Saxony-Anhalt) is a multiplying farm with 5000 sows, with a nursery on the same site and SPF-rearing gilts arrive at different ages every 3-4 months.

*Farm management and vaccination protocol:*

On farm A and B, the strategy was divided in three stages: (1) 2 x a herd vaccination (4 wk interval) of sows with Porcilis PRRS (IDAL) and structural removal of all piglets older than 3 wks (inclusive non pregnant rearing gilts). (2) Stop import new gilts for at least 120 days followed by (3), input of PRRS free gilts which are sentinels for PRRS monitoring. Farm C did not opt to close the herd. All sows and rearing gilts were vaccinated 2x with an interval of 4 wks. Rearing gilts were vaccinated again after 4 wks (3th vaccination) and sows were vaccinated in 2nd wk after farrowing and 9th wk of gestation. Since March 2013 sows are vaccinated once per cycle, in 2nd wk after farrowing: rearing gilts are still vaccinated 3x after arrival.

*Monitoring*

Every 12 wks, 20 weak born piglets and their mothers were tested by PRRSv PCR. Incoming gilts in farm A and B were tested serologically (PRRS Elisa Idexx) 4-8 wks after arrival with an interval of also 12 wks.

## Results

| Farm | First PRRS diagnosis | pigs PRRSv (-) | sentinel gilts remain (-) | PRRSv present after control./eradication |
|---|---|---|---|---|
| A | Aug. 2011 | Dec. 2011 | Jan. 2012 | No |
| B | Sept. 2011 | Jan. 2012 | Jan. 2012 | No |
| C | Sept. 2011 | Jan. 2012 | Not Done | No |

## Discussion and Conclusions

In 2 farms, Porcilis®PRRS herd vaccination combined with herd closure was successful. In farm C, without herd closure, herd vaccination followed by continuous vaccination of sows and rearing gilts was successful in reducing genotype 2 PRRSv. In farm A and B, infection faded out together with absence of susceptible pigs, resulting in production of PRRSv (-) pigs.

In our study, herd closure ensured that during 120 days virus-carriers cannot infect susceptible pigs and might get free from the carrier status. Whether eradication is achieved is not sure because not all sows and piglets were tested by PCR. Eradication is achieved if all incoming SPF gilts remain serologically negative after removal of all sows that were present at the time of infection. In this study, further spread of Type 2 PRRSv infection was prevented by combining vaccination with removal of susceptible pigs. Therefore we conclude that EU-MLV vaccine likely contributed to cross-protection against Type 2 PRRS strains.

# Genetic evolution of PRRSV in pigs vaccinated with modified live prrs vaccineS of type I in comparison to type II in Thailand

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## Introduction

Two distinct genotypes of PRRSV, Type I (European) and Type II (North American), have been recognized and the co-existence of both genotypes has been increasingly evident in several countries, including Thailand, Korea and China[1-3]. In Thailand, where modified live vaccines (MLV) of both genotypes are commercially available, questions have been raised as to which should be used for control of PRRSV and how PRRSV evolution would occur. The objectives of the study were to investigate the genetic evolution of the ORF5 gene following MLV vaccination in previously infected pigs.

## Materials and Methods

Two hundred (200), 20 kg PRRSV-positive pigs were separated into three groups: Controls (n=30), Type I, and Type II MLV-vaccinated groups (each n=85). Each group was housed in a separate pen in the same building with a stocking density of 1.5 $m^2$/pig. Pigs in vaccinated groups were vaccinated intramuscularly at 10 days after arrival with either MLV of Type I or II genotype in accordance with manufacturer's instructions. Three pigs from each group were randomly selected, identified and blood sampled on 0, 7, 14, 21, 28 and 77 days post- vaccination (DPV). Sera were separated and assayed for the presence of virus by PCR. ORF5 genes were sequenced using a previously described method[3].

## Results

The phylogenetic analysis demonstrated that both types of PRRSV were isolated from the study. All Type I and II isolates were further divided into 2 and 4 clusters, respectively. Type I MLV belongs to the cluster 1 of Type I isolates along with other Type I Thai isolates. The cluster 2 of Type II PRRSV was highly pathogenic (HP) PRRSV. Type II MLV belongs to the cluster 3 of Type II isolates and the cluster 4 isolates were reported to be the progeny virus of Type II MLV[4].

Prior to and following MLV vaccination, pigs in all 3 groups were co-infected with both Type I and II PRRSV and pigs in all 3 groups were infected with HP-PRRSV.

The results of ORF5 genes demonstrated that Type I and II PRRSV were consistently detected in all 3 groups following vaccination, regardless of MLV type. Type I isolates in Type I MLV vaccinated group were not genetically identical to Type I MLV, unlike in Type II MLV vaccinated group, in which isolates identical to MLV type were isolated from pigs 7 days DPV (Table 1).

**Table 1** The cluster of PRRS genotype from 3 pigs each per DPV sampled

| Group | Type | D0 | D7 | D14 | D21 | D28 | D77 |
|---|---|---|---|---|---|---|---|
| Type I vaccine | 1 | C1 | C1 | C1 | C1 | C1 | |
| | 2 | C4 | C4 | C2, C4 | C2, C4 | C4 | |
| Type II vaccine | 1 | C1 | C1 | C1 | C1 | | C1 |
| | 2 | C2, C4 | C2, C3, C4 | C2 | | C2, C4 | C2, C4 |
| Control | 1 | | C1 | | C1 | C1 | |
| | 2 | C4 | C4, C4 | C2, C4 | C4 | C2, C4 | C2 |

## Conclusions and Discussion

Study results suggested that vaccination with Type I MLV did not influence PRRSV diversity in the herd once the endemic virus belonged to the similar cluster as MLV. This is unlike Type II MLV, which influences PRRSV strain development in the herd as previously reported[4].

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# Comparison over a 12 month period of Type I and Type II PRRS vaccine in commercial farm infected with Type II PRRSv virus

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## Introduction

Porcine Reproductive and Respiratory Syndrome is an economically important disease for the swine industry (1). To control PRRS in Thailand, it is important to coincide virus strain, vaccine strain and farm management (1). We have found 6 main clusters of PRRS genetic diversification in Thailand (2 for Type I and 4 for Type II) (2). Both Type I and II PRRS virus were used as vaccine in Thailand. The aim of this study was to compare pig performance in a farm which used Type I and Type II PRRS vaccine strain in the same farm but in different unit.

## Materials and Methods

This study was done in a 2,400 sow Farrow to Nursery farm, which was mainly infected with Type II PRRS virus strain. This farm organized their operation in 3 similar units. The laborers were rotated between units during the years. Between the end of 2012 and beginning of 2013, the farm experienced a minor PRRS outbreak. In Feb 2013, a new PRRS vaccination protocol was implemented. In Unit 1, all sows and Piglets were vaccinated with Type I PRRS vaccine and Units 2, 3 were vaccinated with Type II PRRS vaccine. The Performances of Sow herd and Nurseries were monitored from Jan -Dec of 2013.

## Results

Table 1. Performance in Sow Units

| | U1 | U2 | U3 |
|---|---|---|---|
| Sow no. | 2 091 | 2 217 | 1 966 |
| Weaned pigs | 10.0±2 | 10.0±2.1 | 9.3±1.1 |
| Weaning weight | 6.5±0.3* | 6.6±0.3 | 6.7±0.27 |
| % loss | 9.5 | 12.3 | 12.7 |

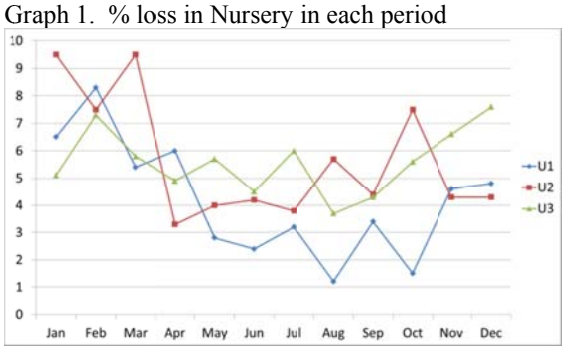
**Weaning weight in Kg*

During the 12 month observation period, no serious PRRS outbreak was reported even though a farm in the area experienced an outbreak in Q3. The performance of Sow and Nursery units are summarized in Tables 1 and 2. Trend of % loss in nursery decreased after implementing new vaccination protocol (Graph1), with exception of U3 where some seasonal increase occurred.

Table 2. Performance in Nursery Unit

| | U1 | U2 | U3 |
|---|---|---|---|
| Piglet no. | 17 926 | 18 310 | 16 591 |
| ADG | 394±28 | 381±30 | 382±17 |
| FCR | 1.37±0.1 | 1.42±0.11 | 1.44±0.05 |
| FCG | 28.22±2.4 | 30.66±3.4 | 30.08±1.6 |
| % loss | 4.36 | 5.63 | 5.59 |

Graph 1. % loss in Nursery in each period




## Conclusions and Discussion

Both Type of vaccine strains improved farm performance when combined with biosecurity measures, proper vaccine and pig flow management. The data supports that PRRSv type of vaccine isolate was not a good predictor of efficacy, i.e. a Type I PRRS vaccine can also be efficacious in farms infected with type II PRRS virus.

## References

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# Piglet strategic vaccination: a tool to control PRRSv infection in the nursery

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## Introduction

PRRS MLV vaccines have been demonstrated to reduce viremia and viral shedding, being therefore considered an effective tool to control viral dissemination in a farm or facility (1,2). The objective of this trial was to evaluate Temporary Strategic Piglet Vaccination as an effective tool to reduce PRRS viral transmission in the nursery.

## Materials and Methods

The trial was done in a closed herd of 350 sows located in Province of Barcelona (Spain). The farm was PRRS positive but classified as stable. Gilts were vaccinated and revaccinated before entering the farm, and the sows were herd vaccinated every 4 months (Porcilis® PRRS). Piglets were vaccinated against *M. hyopneumoniae* at 15 days of age, and against PCV2 just before weaning at 3 weeks of age.

In March 2013, some problems were detected, such as anorexia, retarded growth, respiratory disease, Glässer's Disease, etc. Nursery mortality increased significantly, reaching values of 7-10%. Serology and PCR of 6-9 week old animals confirmed involvement of PRRSv. In sows, no clinical signs were detected, but stability was confirmed through negative PCR of 3 wk old piglet blood samples. A Salmonella outbreak in late nursery complicated the situation, and PRRS control measures were not initiated until end of May, when it was decided to vaccinate 14 day old piglets with Porcilis® PRRS, intradermally with IDAL device, over a 12 wk period.

After vaccination, strategy success was determined by measuring absence of PRRSv in nursery. Mortality data of pre- and post-vaccination batches were also compared (ANOVA & Tukey Test).

## Results

The samples from the first non-vaccinated piglets after a vaccination period of 12 weeks confirmed that PRRS virus was not circulating in the nursery. Non-vaccinated piglets of 5, 7 and 9 weeks of age were bled, and serology (Idexx) and PCR were negative. Mortality rate was reduced by 70% comparing the results of the non-vaccinated batches to the PRRS vaccinated batches (from 6,7% to 1,9%, p<0,01) (Table 1). Variance analysis also showed that mortality variability was less in vaccinated animals than non- vaccinated (Graph 1)

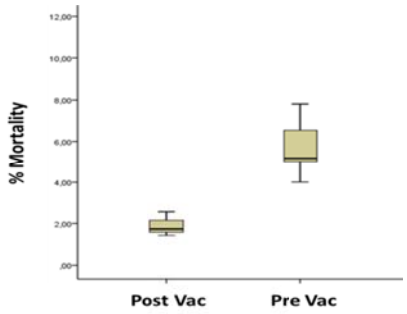
**Table 1.** Nursery Mortality pre- and post- Porcilis® PRRS vaccination

| | Pre-Vaccine | During Vaccine | Post-Vaccine |
|---|---|---|---|
| **Mortality (%)** | 5,7[a] | 6,7[a] | 1,9[b] |
| **PRRS PCR (5, 7 and 9weeks)** | Positive | | Negative |

[a, b] values with different superscript in the same row are significantly different (p< 0,01)

In addition, a clear reduction in respiratory disease and medication costs was observed, as well as an improved growth rate.

**Graph 1.** Variability on Mortality rate




Considering reduced mortality only, the investment of strategic piglet vaccination was returned after the first 12 wks of improved mortality results.

## Conclusions and Discussion

Strategic piglet vaccination is an effective tool to control viral transmission in the nursery, being therefore a valid alternative to a sanitary depopulation of the facilities. Besides the epidemiological effect, the improved productive parameters ensure the profitability of the vaccination.

## References

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# Comparative study between ID and IM vaccination and the course of seroconversion in PRRSV-negative gilts following vaccination with Porcilis®

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## Introduction

*Porcine reproductive and respiratory syndrome virus* (PRRSV) is still a major issue in the German swine industry. The virus leads to severe reproductive disorder in sows and also, mainly by a strong immunosuppressive effect, to massive respiratory infections within the porcine respiratory disease complex (PRDC). Economic losses can reach up to € 125.00 per sow per year[1]. The most effective way to control PRRSV is vaccination of sows and/or piglets with a modified live vaccine (MLV). The vaccine can be delivered either intramuscular (im) or, for one vaccine, intradermally (id) with a special air pressure injector (IDAL)[2]. The aim of the present study was to compare humoral immune response following vaccination via id or im route under field conditions.

## Materials and Methods

A PRRSV positive farm, housing 650 productive sows (Hypor Genetic), introduced new gilts serological negative for PRRSV and *Actinobacillus pleuropneumoniae* (APP) on a regular basis. In quarantine, gilts were vaccinated with: PRRSV upon arrival (Porcilis® PRRS, Intervet/MSD), one week later Influenza A (Respiporc Flu3®, IDT-Biologika), earliest from the 180th day of life Parvoruvac® (Merial). These vaccinations were boostered subsequently 3 weeks after first vaccination.

For the study, blood samples were taken from 20, individually tagged, clinically healthy gilts upon entering quarantine. Two groups of 10 animals each were formed randomly. All gilts were vaccinated with a genotype I MLV (Porcilis® PRRS); 10 im, 10 id with the IDAL – applicator. Three weeks later, prior to the second PRRS vaccination, blood samples were taken again. Pre-vaccination samples were analyzed in a commercial Laboratory for: APP (ApXII in-house ELISA), Influenza A (HAH), PRRSV Antibodies (Ab.) (IDEXX® PRRS X3 ELISA) and PRRSV genome (LDL Virotype® PRRS). The three weeks post-vaccination samples were just analyzed for PRRSV specific antibodies, using the aforementioned test.

## Results

On arrival, all gilts were negative in all performed tests, including the PRRSV Antibodies ELISA (Tab. 1&2). Three weeks post-vaccination, samples were consistently positive for PRRSV specific antibodies, regardless of vaccination route (Tab. 1 & 2).

*Tab. 1: PRRSV Ab before (b.v.) and post (p.v.) im vaccination*

| Gilt Nr. | 24474 | 24475 | 24472 | 24473 | 24474 | 24448 | 24452 | 24450 | 24454 | 24449 |
|---|---|---|---|---|---|---|---|---|---|---|
| Titre b.v. | 0,018 | 0,006 | 0,036 | 0,025 | 0,011 | 0,000 | 0,015 | 0,000 | 0,010 | 0,015 |
| Titre p.v. | 1,093 | 0,806 | 1,312 | 1,490 | 1,349 | 1,831 | 0,670 | 1,253 | 0,560 | 1,221 |

*Tab. 2: PRRSV Ab before (b.v.) and post (p.v.) id vaccination*

| *Gilt Nr.* | *24464* | *24468* | *24469* | *24467* | *24466* | *24461* | *24458* | *24463* | *24457* | *24436* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Titre b.v.* | *0,016* | *0,011* | *0,013* | *0,004* | *0,004* | *0,004* | *0,122* | *0,017* | *0,010* | *0,015* |
| *Titre p.v.* | *0,851* | *0,516* | *2,006* | *1,206* | *2,077* | *1,618* | *2,142* | *1,612* | *1,907* | *1,444* |

## Conclusions and Discussion

Our results demonstrate that vaccination with a PRRS genotype I MLV (Porcilis® PRRS) induces a humoral immune response in PRRS naïve pigs. There was no difference in antibody response between im and id vaccinated animals. These results and confirm previous experimental results on occurrence of PRRSV specific antibodies after vaccination of PRRSV-naïve animals via id vaccination with IDAL injector in the field. [2,3]

## References

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**Effect of PRRSv infection on PCV2 vaccination efficacy and measures to control the negative impact**

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**Introduction**

Common questions in modern pig production are how PRRSv recirculation impacts PCV2 vaccination, what the impact is on productivity parameters or what actions can be taken to prevent these situations. The objective of this study is to describe the interaction between PRRS and PCV2 and to demonstrate that PRRS control is key in PCV2 vaccination efficacy

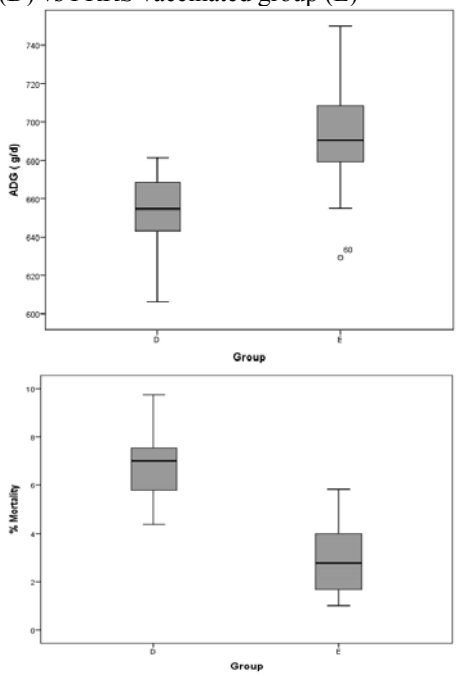
**Materials and Methods**

The study was conducted in a 3000 sow farm (site 1+2), PRRS positive but stable. In 2014, production rates of the fattening units began to deteriorate and clinical signs compatible with PCV2 infection were observed. Piglets were vaccinated against PCV2 in the nurseries, but it was detected that PRRSv was recirculating in nurseries, mainly at 8 weeks of age (serology and PCR positive results). In order to control PRRSv recirculation, it was decided to implement a strategic and temporal PRRS vaccination in 14 day old piglets (Porcilis®PRRS via intradermal). A total of 22 097 piglets were vaccinated over a 12 week period. Production data of the fattening units such as mortality, Average Daily Gain (ADG) and medication costs were recorded, and the results of the vaccinated batches, Group E (from 21/7/14 to 30/9/14) were compared with previous non-vaccinated animals Group D (from 23/4/14 to 20/7/14). Control of the PRRSv infection in nurseries was evaluated by serology. The Linear Method (GLM: program SPSS 15.0) was used for the statistical analysis

**Results**

Samples obtained from the first non-vaccinated piglets after 12 weeks of vaccination confirmed that PRRSv no longer circulated in the nursery. Non-vaccinated 3, 6 and 9 week old piglets were bled for serology and PCR and were confirmed negative. With respect to production data, the results of the non-vaccinated batches were not as good presumably because of the reduced PCV2 vaccine efficacy linked to a PRRS infection. Production parameters were significantly improved during and after PRRS vaccination, with similar results as prior to PRRSv infection. ADG of the fatteners improved from 652,2g/day in the non-vaccinated batches to 690,5g/d in the vaccinated animals ($p<0,001$). Medication costs were not significantly different (2,31vs 2, 42€ /pig, $p=0,709$). Mortality was significantly improved from 6,8% in the non-vaccinated animals to 2,9% in the PRRS vaccinated animals ($p<0,001$).

Graph 1 and 2: ADG and mortality in control group (D) vs PRRS vaccinated group (E)




**Conclusions and Discussion**

PRRSv recirculation in the nursery may affect the efficacy of PCV2 vaccination. Strategic temporary intradermal PRRS vaccination of 14 day old piglets with Porcilis® PRRS over a 12 week period helped to control PRRS infection in nurseries and resulted in a clear improvement in production parameters during the fattening period.

# Field evaluation of a separate and combined vaccination against Porcine Reproductive and Respiratory Syndrome and *Haemophilus parasuis* in nursery pigs and serologic profiling of the vaccinated groups using an oligopeptide permease A enzyme-linked immunosorbent assay

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## Introduction

The aim of the study was to compare a combined vaccination with a separate vaccination against Porcine Reproductive and Respiratory Syndrome (PRRS) and Haemophilus (H.) parasuis.

## Materials and Methods

The study was conducted in a 1200 head nursery farm. A total of 360 piglets at an age of 26 days were randomly allocated into three groups: Group A - H. parasuis (Porcilis® Glässer) and PRRS (Porcilis® PRRS) separately, Group B - combined Porcilis® Glässer and Porcilis® PRRS, Group C - unvaccinated control. Compatibility of both vaccination schemes was evaluated by body temperature and palpation score of the injection site 0, 4, 24 and 72 hours after vaccination. During the nursery and the fattening period, average daily weight gain (ADWG) and number of runts and losses was evaluated. Additionally blood samples were taken every 2 weeks during the nursery period to perform an oligopeptide permease A enzyme-linked immunosorbent assay (oppA-ELISA) and a PCR for PRRS virus. ADWG and body temperature were analyzed with t-test and palpation score with exact Mann-Whitney U test. Number of runts and losses as well as number of positive animals in the ELISA was compared between groups using 2x2 tables and chi-square-test. In general, a level of significance of 5 % with a correction for multiple comparisons (Bonferroni-Holm adjustment) was applied. Group A was compared to control group C to evaluate vaccination effect, then separate vaccination (group A) was compared with combined vaccination (group B).

## Results

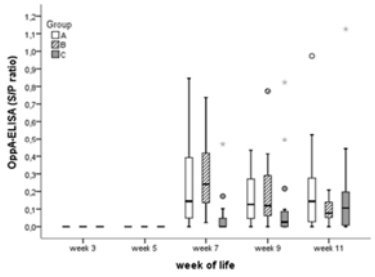
Body temperature did not differ significantly between group A and group C, while piglets vaccinated with the combined vaccination (group B) had a significantly higher body temperature 4 and 72h than group A (not shown). 4 and 24 h after vaccination the palpation score was significantly higher in group A compared to controls, but not between groups A and B. No significant differences were measured in ADWG during the nursery period, but ADWG was significantly higher during fattening in vaccinated groups. Number of losses during the nursery period was significantly higher in controls (Tab.1). Although number of positive animals in the oppA-ELISA did not differ between groups, vaccinated pigs seemed to respond earlier (Fig. 1). OppA-ELISA positive pigs in the vaccinated groups did not develop Glässer´s disease and remained in the study until slaughter.

**Table 1**: ADWG (±SD), number (and %) of losses and runts during nursery (N) and fattening (F).

| Group | ADWG | | Losses | | Runts | |
|---|---|---|---|---|---|---|
| | N | F | N | F | N | F |
| A | 396g (±75) | 808g[a](±117) | 0 (0%) [a] | 2 (1.7%) | 4 (3.3%) | 2 (1.7%) |
| B | 388g (±77) | 796g (±112) | 3 (2.5%) | 4 (3.4%) | 3 (2.6%) | 4 (3.5%) |
| C | 390g (±82) | 765g[b](±123) | 6 (5%)[b] | 6 (5.3%) | 11 (9.6%) | 11 (10.2%) |

[a,b] Values with different superscript within a column were significantly different ($p < 0.05$).

**Figure 1:** S7P-ratio of the OppA ELISA (HPS OppA) for all three groups




## Conclusions and Discussion

Both, separate and combined vaccination against PRRSV and *H. parasuis* positively impacted mortality and ADWG. Thus, a reduction of economic losses after vaccination in farms with clinical Glässer´s and PRRSV respiratory disease can be expected. Combined vaccination has no negative influence on vaccine efficacy but has a minor disadvantage on post-vaccination rectal temperature. In this study, vaccination with Porcilis®Glässer might have influenced the results of the OppA ELISA.

# A Clinical Case Study of Porcine Respiratory Disease Complex Involving PRRSv, Mycoplasma and Ascarids

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## Iroduction:

Porcine respiratory disease complex (PRDC) during fattening is found in all major production areas and has serious economic consequences. Pathogens, including PRRSv and *Mycoplasma hyopneumoniae*, are involved. *Ascaris suum* is also a pulmonary pathogen, both through larval migrations and immunomodulatory ability. This case from a 200 sow, farrow-to-finish farm in Brittany is an example of PRDC complexity in pig farming and the difficulties of accurate diagnosist. Well documented, it shows the significant impact of PRDC on the economy of affected farms.

## Materials and Methods:

The sow herd was PRRSv positive and does "mass" vaccination every 16 wks, using a live attenuated vaccine. Sows are dewormed once a year, with ivermectin in gestation feed or injection (lactating). Piglets are vaccinated against *M.hyo* once and dewormed with 15 ppm flubendazole in nursery feed for 2 wks after weaning. At slaughter, repeated lung lesion scorings (LLS) showed very good respiratory status. Parasitic hepatic lesions were never reported. Technical and economic data (TED) were good. During the winter of 2012–2013, a chronic cough began at 4 mo of age in all production batches, appetite became irregular and pigs required individual antibiotic injections. Last finishers left beyond 200 days and carcass weight decreased. Pulmonary scores at the slaughterhouse also deteriorated, moderately at first, then very clearly after the summer of 2013. The TED deteriorated and health expenditures increased (Table 1):

Table 1: change in TED in 2012, 2013 and early 2014

| | Feed Conv. Index 8–115 kg | Age at 115 kg | Nursery to finish amount of feed | Mortality and condemnation percentage | Health expenditure per sow per year |
|---|---|---|---|---|---|
| 2012 | 2.44 | 184 days | 272 kg | 5.6 % | € 106 |
| 2013 | 2.49 | 186 days | 277 kg | 6.7 % | € 149 |
| 1st half of 2014 | 2.42 | 180 days | 266 kg | 5.4 % | € 133 |

In Feb 2013, fatteners were PRRSv positive at 130 days (7/8) and PRRS vaccine was implemented in piglets Mar 2013. The situation continued to deteriorate during the summer in PRRS vaccinated batches at weaning, as evidenced by the very poor LLS from the inspection performed on Oct 22, 2013. A serological profile from Sept 6 revealed a *M.hyo* seroconversion at 132 days (6+/6). The decision was taken on Sept 20 to euthanize a 125-day-old pig with starting dyspnea. The autopsy (Labocéa22, Ploufragan, France) result included extensive pneumonia (LLS = 18/28) and, a "BALT" (bronchus-associated lymphoid tissue) hyperplasia with positive *M.hyo* IFA, and interstitial eosinophilic infiltration in lung and associated lymph nodes. PRRS PCR was positive and ORF5 88% homologous with the live vaccine strain. Mycoplasma and PRRSv appeared to have escaped vaccine protection. An ELISA test for *A. suum* (Serasca™) was done on 10 pigs at end of fattening with a mean titer of 0.924, highly suggestive of a high-intensity ascaris infection during fattening. The diagnosis of co-infection with *M.hyo*, PRRS and *A. suum* was done.

In late October, the deworming protocol was completely redesigned. Gilts were dewormed a few days before quarantine release. Sows are treated over 2 days, 5 days before farrowing. Piglet treatment is distributed in drinking water, 17 days after weaning. Finishing pigs are dewormed every 4–6 weeks to control fattening pen infestation. Fenbendazole is preferred for its larvicidal properties on migrating larvae of *A. suum*. At the same time, a two dose mycoplasma vaccination was implemented for piglets.

Early 2014, symptoms and TED significantly improved. Piglets dewormed after weaning did no cough or had reduced appetite while fattening. Medical antibiotic interventions stopped. Age when last finishers left for slaughterhouse returned to normal. Carcasses were heavier and rankings improved, especially from early April. A LLS performed on September 19, 2014 confirmed the control of the pulmonary disease.

## Conclusions and Discussions:

This clinical case illustrates the association between *M.hyo*, PRRSv and *A. suum*, which caused a serious respiratory complex during fattening. The technical and economic data for this field case quantify the economic impact of PRDC in this farm to a loss of €222/sow/ year; i.e., for a 200 sow farm, an annual loss of more than €44,000. The new health investment protocol is + €46/sow/year (PRRS vaccination of piglets + modified deworming protocol) and helped improve the initial technical-economic performances. The return on investment is at least 4.8 (€4.8 of economic gain per euro invested). The complex diagnosis demonstrates benefits of necropsy, histology and Ascaris serology test.

# Evaluation of welfare aspects in suckling piglets after intradermal vaccine application with the IDAL injector

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## Introduction

The purpose of this study was the scientific evaluation of welfare aspects of intradermal (id) vaccination for suckling piglets under field conditions. Local reactions within three days after vaccination, behavioural responses and performance data of the id vaccinated piglets were compared to piglets immunized by intramuscular (im) injection.

## Materials and Methods

The study was carried out on a commercial German pig farm. At 7 days of age, 672 suckling piglets in three batches were vaccinated with Porcilis® M Hyo ID ONCE; 338 with the IDAL injector, and 334 with conventional needle injection-systems. The following three days, injection site was scored for swelling from 0 to 5 (none, <pea, pea, bean, hazelnut, dove egg). Piglets were weighed individually one day before vaccination and eight days later. Video-recordings were performed for ten days,
starting two days before vaccination, in order to assess piglets' resting and activity behaviour. Per batch, two id vaccinated litters and two control litters were observed. Scan Sampling in chromatic intervals of five minutes between 6-10 a.m., 1-5 p.m. and 7-9 p.m. was carried out. For statistical analyses, IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA) was used.

*Figure 1*

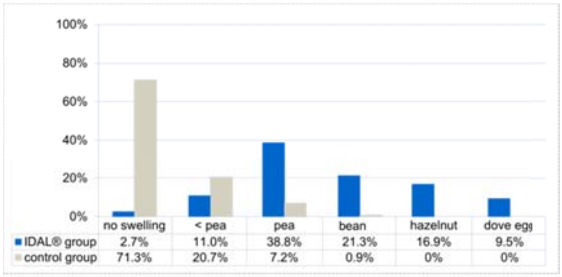



**Fig. 1: 1st day after vaccination: swelling of the injection site**

## Results

Vaccination with IDAL took 11 seconds, on average, whereas for conventional vaccination 17 seconds were needed. Daily weight gain was not significantly different between im piglets (258g/d) and id piglets (247g/d). Injection site swelling on the first day after vaccination was more apparent in id piglets, indicating a good immune response (Fig. 1), but abated within one week (Fig. 2). Piglets vaccinated with IDAL were more active and suckled more than piglets vaccinated with needle-systems (Fig 3).

*Figure 2*

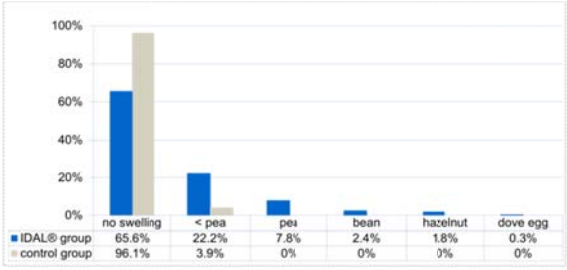
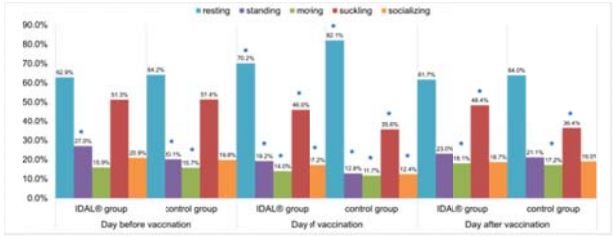



**Fig. 2: 7th day after vaccination: swelling of the injection site**

*Figure 3. Activity of id piglets (IDAL Group) and im piglets (control group)*




*** indicates a significant difference between IDAL group and control group ($p < 0.05$).**

## Conclusions and Discussion

The IDAL applicator is easy to handle and enables fast vaccination. In id vaccinated piglets, more soft tissue reactions were found, showing a desired local immune reaction. Vaccination method did not influence daily weight gain. Piglets vaccinated intradermally with IDAL showed less lying and more suckling behaviour after vaccination than intramusculary vaccinated piglets.

# Practical experience after using IDAL as an alternative to traditional intramuscular vaccination in pigs

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## Introduction

IDAL (IntraDermal Application of Liquids) is a device for applying vaccines intradermally (ID).
The ID route is preferred for several reasons: The maternal derived antibodies are at low concentrations in the skin, the IgA production is very well stimulated by the ID route, a low amount of liquid can be used, there is no pain at injection and the non-invasive injection will reduce significantly the risk of transferring pathogens. IDAL is expected to reduce labor time when vaccinating piglets, as one can inject along the back instead of in the neck by i.m. route.
The device was marketed in DK early 2013 and information of practical handling was missing. This report concludes an investigation about the practical use of IDAL on farms.

## Materials and Methods

IDAL was introduced in 33 farms and used to vaccinate with either Porcilis M. Hyo ID ONCE or Porcilis PRRS.
Vaccinations were mainly given to suckling piglets from 14 days of age and piglets at or after weaning. In all cases, an MSD AH field advisor was demonstrating the injection technique, cleaning procedure and other functionalities.
Farms were revisited 6 months after start of trial and a questionnaire was made focusing on advantage and disadvantage of the device compared to the i.m. injection of vaccines. The questionnaire did only focus on the practicalities of use and perception and satisfaction of working with the device.
Further a process was started with the Danish SPF-company in order to assess the biosecurity issues in relation to sending IDAL for service at Henke Sass Wolf (HSW) in Germany, as this might pose a risk of cross contamination from other devices in case mixing up devices would happen at the factory during service. Two Quality Auditors from Danish SPF-company visited HSW and all procedures from receiving to returning IDAL from one farm to the same farm were described and audited.

## Results

Thirty three (33) herds in total were included in the study. Thirty (30) pigs were vaccinated against *Mycoplasma hyopneumoniae* and 7 against *PRRS*. At the end of the test period, 21 farms continued using IDAL. All expressed a desire that the IDAL was lighter, although only 1 farm stopped use for this reason. All were satisfied with IDAL compared to i.m. vaccination. Time reduction of vaccination procedure with IDAL is limited in suckling piglets, but is up to 50%, including preparation and cleaning of the IDAL, in weaners.
During the test period 12 of the farms decided to stop using IDAL (see table below).

| Reason for termination | Number |
|---|---|
| Farm eradicated/no longer needs | 4 |
| Price of IDAL too high | 1 |
| Never started the vaccination | 1 |
| IDAL too heavy | 1 |
| Preferred earlier vaccination | 1 |
| No time saved by simultaneous intramuscular vaccination against other diseases | 4 |

Following the HSW QA visit by SPF auditors, a certificate of approval was issued and it was agreed to review the procedure during a yearly QA visit.

## Conclusions and Discussion

IDAL was well received as an alternative to i.m. vaccination in most farms. In suckling piglets, the time spent on vaccination was not reduced, but vaccination of weaners went faster and almost reduced the time in half. Only one farm stopped IDAL use due to technical issues. The remaining farms returned to i.m vaccination for reasons not directly related to the IDAL technical performance. The current biosecurity procedures at HSW were acceptable to fulfill Danish SPF-demands. In summary, vaccination with IDAL is a fast and safe alternative to i.m.-vaccination.

## References

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5. HSW manufactures and services the IDAL http://www.henkesasswolf.de/

# Serological response of APP unsuspicious gilts to vaccination with Porcilis® App

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## Introduction

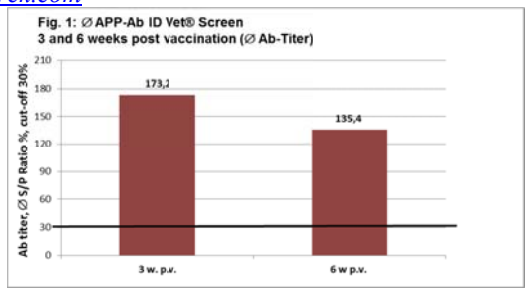
*Actinobacillus pleuropneumoniae* (APP) is one of the most important swine respiratory pathogens. In recent years, an increase of clinically relevant APP-infections is observed. Most successful diagnostic methods are testing by ELISA, PCR or culture. Serology is widely used in the field. Control of APP is often done by vaccination. The following study was conducted to evaluate the seroconversion with different ELISA test in pigs, vaccinated twice with Porcilis® APP.

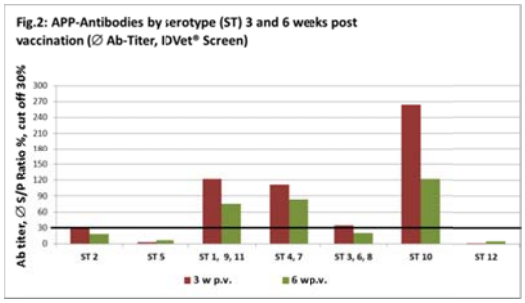
## Materials and Methods

The study was done on a farm unsuspicious for APP. In 2008 the herd was depopulated and repopulated with high health status animals. Since, the farm was monitored for different pathogens (incl. APP) on a regular basis, showing negative results every time. In September 2014, 20 gilts (140 – 160 days old) were individually marked and randomly assigned into two groups of ten. One group was vaccinated with 2 ml Porcilis® APP (Vaccine group) while the other group was not vaccinated (Control). Prior to vaccination all 20 gilts were tested for APP via serum and nasal swabs. Swabs were used to confirm absence of APP colonization of upper respiratory tract without seroconversion, according the APP-PCR protocol by Schaller[1]. Four weeks later, the vaccine group was re-vaccinated with 2 ml Porcilis® APP. Both groups were kept in the same compartment but in different pens. Serological tests for all samples were: IDEXX Apx IV ELISA, IDVet SCREEN (Serotype 1 – 12) ELISA(ID Vet, France) at and an in-house Apx II ELISA (IVD GmbH, Germany). ID Vet SCREEN and APP – PCR were done at the institute for animal health, Oldenburg, Germany, LUFA Northwest, Apx IV and APx II ELISA were done at IVD Gmbh, Hannover, Germany. Both laboratories perform these tests in routine diagnostics on regular basis.

## Results

All ELISA-Tests were negative in controls, as well as the APP-PCR tests for all gilts. Vaccinates were positive, doubtful and negative, with a reduced doubtful samples 6 weeks after vaccination. ID Vet Screen test was positive 3 and 6 weeks after vaccination, but with declining titers (Fig. 1) and same observation in serotype test (Fig 2).




## Conclusions and Discussion

This study confirms seroconversion of naïve gilts following Porcilis® APP vaccination as well as the importance of selecting the proper ELISA test in routine diagnostics. As expected, all animals were in the Apx IV ELISA. Vaccinated gilts were positive in the ID Vet ELISA, with different results in serotyping. The Apx II ELISA yielded doubtful and negative with the doubtful results negative in follow up testing. After vaccination with Porcilis® APP, according to the producers' guidelines, the ID Vet ELISA results were positive. APP vaccinated breeding pigs can be differentiated from infected animals by Apx IV ELISA. Since APP prevalence in pig-dense regions of NW Germany is up to 83%[3], accompanied by rising clinical outbreaks, there is a need for proper APP diagnostics and vaccination.

## References

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# Contribution Of Serasca Serology and Coprology To Study The Risk Factors of Internal Parasitism of Gilts in Industrial Swine Farms

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## Introduction

Pig farms are faced to varying degrees with nematode worm infestations. The Veterinary Faculty of Ghent University (Belgium) developed the SERASCA ELISA assay to assess *Ascaris suum* (As) in swine farms (1). Socavet used this tool and fecal examination to identify risk factors responsible for a high parasite burden in future breeders (gilts) from arrival in a farm until first farrowing.

## Materials and Methods

The study was performed in 6 Farrow-to-finish farms (Farms A1 to C2) in Brittany with about 200 sows each. Gilts in each farm were from 3 different multipliers (farms A, B and C). Thus, B2 is the second commercial farm buying its gilts from multiplier B. Each lot included 6-10 gilts and 6 lots of gilts were studied at two different periods (3 lots in quarantine and 3 in gestation/farrowing). Gilts were received in specific quarantine premises. After quarantine, method of breeding gilts varied: Pregnant gilts mixed or separated from the sow herd in gestation; Sows and gilts together, raised in separate batches, or mixed in a "dynamic" group that combines all batches. In each batch of each farm, a group of gilts was sampled (blood) upon arrival to and departure from quarantine and another group upon entering gestation and farrowing. Individual fecal samples were taken at the time of farrowing for fecal examinations. Individual sera were pooled in groups of ten max for the Serasca test. The test result is the average Optical Density Result (ODR):

| Average measured ODR | Infestation burden of *Ascaris Suum* |
|---|---|
| < 0.5 | ZERO or LOW |
| 0.5 to 0.79 | MODERATE |
| 0.8 and over | HIGH |

Seroconversion period is six to eight weeks. Fecal results were averaged in each group of individual counts, expressed in worm eggs per gram of feces. Five risk factors for internal parasitism of sows were identified as well as two health criteria of quarantined gilts (Table III). These 7 parameters were confirmed in each of the 6 farms and compared with serological and stool results.

## Results

Results are summarized in Tables I & II.
MD = Missing data

**Table I**: SERASCA serology of gilts & Fecals

| SAMPLING | BAND | FARM A1 | FARM A2 | FARM B1 | FARM B2 | FARM C1 | FARM C2 |
|---|---|---|---|---|---|---|---|
| Quarantine Entry | 1 | 0.69 | 0.55 | 0.38 | 0.28 | 0.23 | 0.21 |
| | 2 | 0.37 | 0.29 | 0.3 | 0.19 | 0.5 | 0.39 |
| | 3 | 0.35 | 0.11 | 0.25 | 0.23 | 0.34 | DM |
| Quarantine Exit | 1 | 0.33 | 0.61 | 0.72 | 0.54 | 0.56 | 0.8 |
| | 2 | 0.53 | 0.39 | 0.66 | 0.44 | 0.88 | 0.44 |
| | 3 | 0.33 | 0.62 | DM | 0.51 | 0.79 | DM |

| SAMPLING | BAND | FARM A1 | FARM A2 | FARM B1 | FARM B2 | FARM C1 | FARM C2 |
|---|---|---|---|---|---|---|---|
| Pregnancy Entry | 1 | 0.51 | 1.34 | 0.69 | 0.66 | 0.71 | 0.54 |
| | 2 | 0.73 | 1.31 | 0.33 | 0.45 | 1.12 | 0.69 |
| | 3 | 0.4 | DM | 0.61 | 0.63 | 0.73 | DM |
| Farrow. Entry | 1 | 0.68 | 0.52 | 0.33 | 0.44 | 0.57 | 0.29 |
| | 2 | 0.71 | 0.98 | 0.35 | 0.35 | 0.81 | 0.42 |
| | 3 | 0.46 | DM | DM | DM | 0.5 | DM |
| Farrow. Entry, Fecal Exam | | 23 eggs/g | 296 eggs/g | 0 eggs/g | 33 eggs/g | 115 eggs/g | 0 eggs/g |

Table II: Average Serasca & risk factors

| SAMPLING | FARM A1 | FARM A2 | FARM B1 | FARM B2 | FARM C1 | FARM C2 |
|---|---|---|---|---|---|---|
| Quarantine entry | 0.47 | 0.32 | 0.31 | 0.23 | 0.36 | 0.30 |
| Quarantine exit | 0.40 | 0.54 | 0.69 | 0.50 | 0.74 | 0.62 |
| Multiplier heavily contaminated? | no | no | no | no | no | no |
| Quarantine on straw or partial grating? | YES | no | YES | no | YES | YES |
| Deworming in quarantine? | yes | yes | yes | yes | NO | NO |
| Quarantine decontaminated between two bands? | NO | NO | NO | yes | NO | NO |
| Contact with sows or droppings? | YES | YES | no | YES | YES | no |
| Gilts coughing? | no | no | no | YES | YES | no |
| Fertility rate of gilts | 97% | 70% | 100 % | 85% | 90% | DM |

| SAMPLING | FARM A1 | FARM A2 | FARM B1 | FARM B2 | FARM C1 | FARM C2 |
|---|---|---|---|---|---|---|
| Pregnancy entry | 0.55 | 1.33 | 0.54 | 0.58 | 0.85 | 0.62 |
| Farrowing Entry | 0.62 | 0.75 | 0.34 | 0.40 | 0.63 | 0.36 |
| Fecal Examination Upon Farrowing | 23 eggs/g | 296 eggs/g | 0 eggs/g | 33 eggs/g | 115 eggs/g | 0 eggs/g |
| Pregnancy on straw or partial grating? | YES | No | No | No | No | YES |
| Regular deworming of sow herd? | Yes | NO | Yes | Yes | NO | Yes |

## Conclusions and Discussions

This study shows that quarantine management is a critical point of As infestation of gilts. Risk factors were present in all 6 farms at varying levels. Serasca appears to be a useful tool to assess As infection pressure in gilts during acclimation to the new farm and also assesses quality of existing preventive measures.

## Reference

Vlaminck J. and Coll, Veterinary Parasitology 189, (2012), 267-273.

# Comparison between liver spots and serological response in The SERASCA® test as a consequence of *Ascaris suum* infections in Danish finishing herds.

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## Introduction

The SERASCA® test[1,2] is a serological test that can be used to determine the exposure to both larval and adult *Ascaris suum* in pigs.

As antibodies persist for several months, a positive reaction reflects that pigs have been infected with *A. suum* during their fattening phase. This information is important, as diagnosis of *Ascaris suum* can be difficult due to the relatively short lifetime of white spots on the liver and the immune system's ability to control intestinal adult worms and shedding of eggs.

Ellegaard et al. showed in 2013[3] the prevalence of *Ascaris suum* (SERASCA® test) in conventional farms in Denmark.

Most Danish slaughterhouses perform a lot of registration of the carcass, among other liver spots. This kind of registration is underestimated[4], and it shows a lower prevalence compared to the real prevalence in slaughter pigs.

Liver spots will be present if the slaughter pigs had migrating larvae from *Ascaris suum* during the last 5-6 weeks before slaughter, but the liver spots will not be present, if the infection is earlier.

Since antibodies persist for longer time than liver spots, serological testing of blood from slaughter pigs is an effective alternative to examine herds. In particular, when prevalence of liver spots at slaughter is zero, but a history of early migrating larvae exists, resulting in liver spots that are no longer macroscopically detectable.

It is well known that infection with *Ascaris suum* has a negative impact on performance of pigs[5] and the response to some vaccinations[6].

The aim of this study was to use the SERASCA® test to determine the prevalence of positivity in batches of fattening pigs delivered for slaughter, and compare this information with the prevalence of liver spots registered at the slaughterhouse.

## Materials and Methods

Blood was sampled shortly after killing of pigs at the slaughter line in a large abattoir in Denmark. We don't know anything about anthelmintic treatment of those pigs, but we assume that it is very rare. None of the tested farms were organic farms. Individual farms were identified by their specific number and coded with a running number for future blinded handling and analysis of the samples by the investigator. A total of 172 sets of 10 blood samples were collected and screened with the SERASCA® test. The result of blood sampling was compared to the frequency of white spots registered on the farm within the next half year. We assumed that pigs were raised under similar housing and anthelmintic status condition.

## Results

A relationship was found between infection of *A. suum* by prevalence of white spots at slaughter over ½ year and the serological result by the SERASCA® test rom blood sampled at slaughter house. At one sampling, 30 % of serologically positive farms had no white spots at slaughter over half a year. On the other hand, 45 % of farms that were serologically negative were white spot positive on one or more occasions.

| | | **Liver spots in farm[a]** | |
|---|---|---|---|
| | | Positive | Negative |
| **Sero-logical[b]** | Positive | 44 | 18 |
| | Negative | 50 | 60 |

Table 1: Positive for liver spots is farms, where the slaughter house have detected at least one pig with liver spots. b) SERASCA® test: > 0,5 is positive..

## Conclusions and Discussion

The results suggest that a single serological test will give a false negative result at the farm level in 45 % of cases. This finding can be caused by differences in different batches, by very late infections or because of a very low reaction due to only low infection or few pigs positive in a farm. As a consequence, it is advised to combine white spots reported at slaughter and the SERASCA test to make the right diagnosis of *A. suum* infection level in a finishing farm.

## References

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# Herd performance and PRRS serological monitoring in farm after intervention through vaccination following an outbreak of PRRS type II in Thailand

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## Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS) is an important disease which can decrease economical performance (1). Both type I and Type II virus are found in pig farms in Thailand (2). PRRS serology has been used to monitor PRRSv in farms some time ago (1). Previous studies found that it is not necessary to select a vaccine that is homologous with the PRRSv isolate in the farm (3). The purpose of this study was to monitor pig performance and PRRS serology of herd after an outbreak and introducing an intervention.

## Materials and methods

This was a follow-up study in a 4000 sow farrow-to-finish farm in central part of Thailand. In beginning of 2013, this farm exhibited an abortion problem. PRRS US strain (Type II) was found in serum and fetuses from aborted sows suspecting that PRRS would be one cause of this problem. After that, Porcilis® PRRS was used once (according to label instructions) in gilt and sow herd combined with biosecurity improvements to solve and prevent the problem. To follow up this farm intervention, we monitored farm performance together with annual PRRS serological monitoring using ELISA

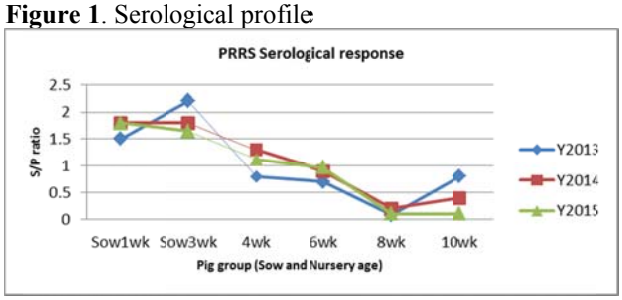
## Result

There were no serious problems in the farm breeding unit after initiating the interventions. Performance data showed that abortion rate and % loss before weaning were decreased and farrowing rate was increased in sows. Percent (%) loss in nursery was also decreased (Table 1). When we observed PRRSv serology profile in sows, we found an improved profile for pre-farrowing and before weaning group over the years. We also found a different trend in the nursery serological profile in that the piglets now remain serological negative after disappearance of MDA (fig 1).

**Table 1**. Sow and Nursery performance

| | Jan 13 | Jul 13 | Jan 14 | Jul 14 | Jan 15 | May 15 |
|---|---|---|---|---|---|---|
| farrowing rate | 77.72 | 73.3 | 74.2 | 77.9 | 81.86 | 80.4 |
| Abortion rate | 1.03 | 0.5 | 0.5 | 0.68 | 0.63 | 0.52 |
| %loss before wean | 6.0 | 5.3 | 4.5 | 1.1 | 5.1 | 2 |
| % loss Nursery | 2.10 | 5.30 | 2.18 | 1.39 | 1.43 | 1.25 |

**Figure 1**. Serological profile




## Discussion

The improvement of PRRSv serological profile might be indicative of the PRRSv status of pigs in farrowing & nursery herd when compared with farm performance. This study is also in agreement with previous studies on criteria to select a PRRS vaccine. Type I PRRS vaccine can also stabilize and prevent herd from Type II PRRS field challenge. Biosecurity and pig flow management still remain important practices to control this disease.

## Reference

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# Comparative study of serological response of one-shot and two-shots Swine Influenza vaccines

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## Introduction

Swine Influenza (SI) is one of the most important respiratory diseases in pigs [1]. In endemically infected farms, vaccination can play a crucial role in the prevention of the clinical impact of the infection [2]. Humoral response to vaccination is a key factor for developing the clinical protection in front of the infection of SIV [3]. Currently, inactivated both single and double-dose vaccines are commercially available in the Asian market. In this study we compared the humoral response of a single-dose commercial SI vaccine in front to a double-dose vaccine (GRIPORK®).

## Materials and methods

34 ten week old SI seronegative piglets were randomly distributed in 3 groups and vaccinated with either a 1-shot commercial vaccine at day (d) 0 (n=15) or with a 2-shots (GRIPORK®, Hipra) vaccine at d0 and d21 (n=15). At same time 4 pigs were kept unvaccinated and used as negative controls.

Humoral immunity was assessed during 55 day trial. Blood samples were taken from all pigs included in the study at d0, d21, d35 and d55. IDEXX influenza A Ab ELISA kit was used to measure vaccine humoral immunity. Percentage of seropositive pigs and average of serological titters (sample/negative control ratio) were compared between groups at the different sampling times.

## Result

At d35 of the study 100% of pigs vaccinated with GRIPORK® showed seroconversion and they were still all seropositive at d55. On the other hand, just 6.67% of pigs vaccinated with one-dose vaccine were positive just at d35 (see Table 1). Despite no significant differences were observed on average titer values between groups a clear increase of titres in GRIPORK® was observed from d35 (see figure 1).

***Table.1*** Percentage of positive pigs, IDEXX ELISA kit.

| | Day 0 | Day 21 | Day 35 | Day 55 |
|---|---|---|---|---|
| CONTOL | 0% | 0% | 0% | 0% |
| One dose | 0% | 6.67% | 0% | 0% |
| (GRIPORK®) | 0% | 6.67% | 100% | 100% |

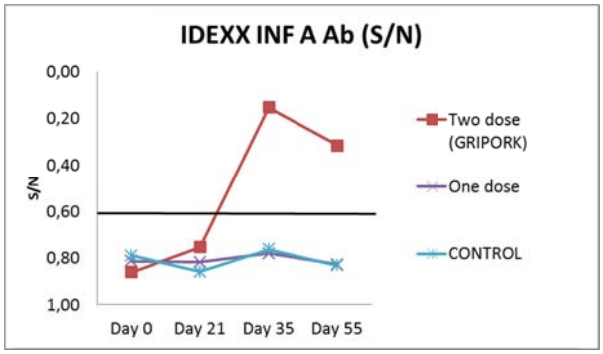


***Figure 1***. Average IDEXX sample/negative control ratio (S/N).Test cutoff: S/N ≥0.6 =negative; S/N <0.6 = positive

## Discussion

These results demonstrated a very poor and short seroconversion in SI seronegative pigs when vaccinated with 1-shot vaccine. Contrarily, 2 shots of GRIPORK® provided strong immunity to all vaccinated pigs from 2 weeks after completed vaccination program. Thus, in endemically infected regions such as Asia, vaccination with GRIPORK® could be a very useful tool for the prevention of SI in endemically infected farms.

## Reference

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# *In vitro* viability of vaccine attenuated PRRSv (UNISTRAIN® PRRS) when mixed with an inactivated Swine Influenza vaccine (GRIPORK®)

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## Introduction

In Asia, nowadays, pig producers need to protect their pigs and sows against several pathogens. Many vaccination programs have to be implemented in sows during the gestating or lactating period [1]. The possibility of reducing or administering vaccines simultaneously can potentially improve either welfare status or the labour efficiency [2]. Therefore, the objective of this trial was to estimate if the attenuated PRRS vaccine PRRSv (UNISTRAIN® PRRS) can keep its viability when it is reconstituted with an inactivated swine influenza vaccine (GRIPORK®).

## Materials and methods

Two 50-dose freeze dried tablet of UNISTRAIN® PRRS (strain VP-046 BIS. Hipra) vaccine (attenuated PRRS virus) were used. One was reconstituted with GRIPORK® (Hipra) (50-dose bottle; 100ml). The other one was reconstituted in 100ml aqueous commercial solvent (Hipra). After 0, 2, 3 and 4 hours post reconstitution at 25ºC, virus was titled measuring its cytopathic effect in CLON 8 cell line.

## Result

PRRS vaccine virus (UNISTRAIN® PRRS) when mixed with GRIPORK® maintained its *in vitro* viability with titers values equivalent or higher than the minimum effective concentration (MEC) of the product ($10^{3.5}$-$10^{5.5}$CCID$_{50}$) till 4 hours after reconstitution. The same results were obtained when mixed with the diluent (100ml) in commercial solvent (see table 1).

***Table.1.*** Results of titres per dose of PRRS vaccine virus.

| Time after reconstitution (h) | Vaccine virus titres per dose (2ml) (CCID50/dose) | |
|---|---|---|
| | UNISTRAIN®PRRS +Solvent | UNISTRAIN®PRRS +GRIPORK® |
| T0 | $10^{5}$ | $10^{4.93}$ |
| T1 | $10^{4.75}$ | $10^{4.81}$ |
| T2 | $10^{4.93}$ | $10^{4.06}$ |
| T3 | $10^{5.06.}$ | $10^{4.18}$ |
| T4 | $10^{4.64}$ | $10^{3.93}$ |

## Discussion

These results suggest there is no interference on PRRSv viability between vaccine components when UNISTRAIN® PRRS is reconstituted with GRIPORK®.
Moreover, UNISTRAIN® PRRS can keep its MEC till 4 hours after the reconstitution at room temperature. Bear in mind that field conditions can interfere in these results. Despite of further studies *in vivo* would be required in order to assure the safety and the immunogenic response of this vaccine mixing, it could be considered a potential vaccine combination in commercial farms.

## Referenes

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[2]. RUMA guidelines 2006, 10-11.

# Serological response of different commercial Swine Influenza vaccines.

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## Introduction

Swine Influenza (SI) is one of the most important respiratory diseases in pigs [1]. In endemically infected farms, vaccination can play a crucial role in the prevention of the clinical impact of the infection [2]. Humoral response to vaccination is a key factor for developing the clinical protection in front of the infection of SIV [3].
In this study we compared the humoral response of 3 different commercial SI vaccines available in Asian market.

## Materials and methods

49 ten week old SI seronegative piglets were randomly distributed in 4 groups and vaccinated with a commercial dose at day (d) 0 and d21 of the study: Vaccine A (n=15); GRIPORK® (Hipra) (n=15); Vaccine C (n=15) and control group (n=4) injected with PBS solution.
Humoral immunity was assessed during 55 days of trial. Blood samples were taken from all pigs included in the study at d0, d21, d35 and d55. IDEXX influenza A Ab ELISA kit was used to measure vaccine humoral immunity. Percentage of seropositive pigs and average of serological titters (sample/negative control ratio) were compared between groups at the different sampling times.

## Result

At d35 of the study 100% of pigs vaccinated with GRIPORK® showed seroconversion. On the other hand just approximately half of the pigs vaccinated with the 2 other commercial vaccines showed seroconversion at d35. Moreover at d55 all pigs vaccinated with GRIPORK® remained positive while percentage of positive pigs dramatically decreased for both vaccine A and C (see table 1). GRIPORK® also showed the highest titers ratios from d35 to d55, despite no significant difference was observed between groups (see figure 1).

***Table.1.*** Percentage of positive pigs, IDEXX ELISA kit.

| | Day 0 | Day 21 | Day 35 | Day 55 |
|---|---|---|---|---|
| CONTROL | 0% | 0% | 0% | 0% |
| Vaccine A | 0% | 0% | 53,30% | 0% |
| GRIPORK® | 0% | 6,67% | 100% | 100% |
| Vaccine C | 0% | 6,67% | 46,67% | 13,30% |

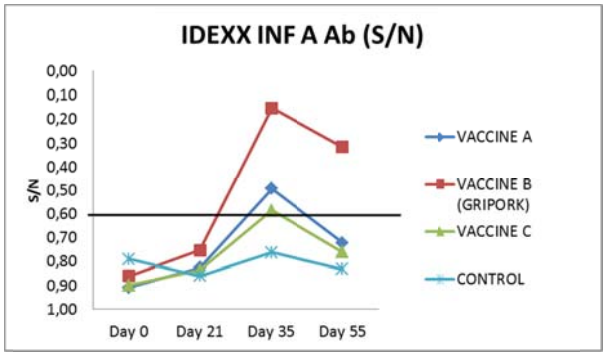


***Figure 1***. IDEXX sample/negative control ratio (S/N).Test cutoff: S/N ≥0.6 =negative; S/N <0.6 = positive.

## Discussion

In this study GRIPORK® showed the strongest and longest serological response compared to 2 other commercial vaccines, and being the only one providing complete seroconversion to the whole vaccinated population. Thus, vaccination with GRIPORK® could be a very useful tool for the prevention of SI in endemically infected farms.

## Reference

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[2]. Torremorell, M, (2014)
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# Comprehensive approach to control PRRS: A case study of the application of PRRS biosecurity assessment tool in Japan.

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## Introduction

The objective of this paper is to report a case study that we applied PRRS biosecurity assessment tool, along with diagnostic monitoring, in order to attempt control and potential elimination of PRRS virus in a Japanese pig farm.

## Materials and methods

To check the farm biosecurity revel, PRRS biosecurity assessment tool created by P-JET® was applied. We conducted the assessment of the farm. After the investigation of the farm based on this assessment, the farm manager and employees practiced specific measurements to improve the farm biosecurity, testing of gilts etc.

## Testing

For monitoring the PRRS virus circulation in the farm, serum samples were collected from sows and finishing pig, and tested for PRRS virus by PCR and ELISA. Those samples were collected at the same time when we carried out PRRS biosecurity assessment tool. We conducted monthly PCR testing of serum from suckling piglets since November 2013.

## Result

**Table 1**.Results of the scores from PRRS biosecurity assessment tool.

At these all rounds, serum from pigs were PCR-positive for PRRS virus. The virus was tested by RFLP and showed a cutting pattern of 1-3-1.

Monthly PCR testing for suckling piglets confirmed all negative since the first round (June 2014); therefore, the farm was classified as stage III (stable) at this point. Environmental swab testing was all negative for PRRS virus by PCR during the entire period of this study.

| item(/100points) | first round | second round | third round |
|---|---|---|---|
| overall points | 61.1 | 70.4 | 71.5 |
| External biosecurity | 52.4 | 52.0 | 55.2 |
| internal biosecurity | 78.4 | 83.7 | 86.2 |
| monitoring and education | 52.6 | 75.4 | 73.1 |

## Discussion

This case study indicated that comprehensive approach to control PRRS along with PRRS biosecurity assessment tool, diagnostic monitoring and environmental samplings can contribute to improve health management in the farm. The assessment tool also revealed that the communication among the farm staffs was one of the key component for the success.

## Reference

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# Clinical protection study of pseudorabies vaccine (AUSKIPRA® GN; Bartha k61 strain), in piglets infected with new Chinese PRV variant

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## Introduction

Since 2011, several Pseudorabies (PR) outbreaks have damaged Chinese swine industry [1].

The purpose of this experiment is to test the efficacy of pseudorabies vaccine AUSKIPRA®GN (HIPRA) against new pseudorabies virus variant (AH02).

## Materials and methods

Fifteen healthy and PRV free, from 4 to 6 weeks old piglets were randomly divided into 3 groups. Group A: inoculated intramuscularly (IM) with one dose (2ml) of AUSKIPRA®GN (Bartha k61) reconstituted with RED solvent (aqueous diluent) at D0 and challenged intranasaly (IN) with AH02 strain at D7 (n=5); Group B: inoculation IM with 2mL PBS at D0 and challenged IN with AH02 strain at D7 (n=5); Group C: inoculation IM with 2mL PBS at D0 and no challenge (n=5).

Clinical signs and body temperature of all animals were recorded from three days before vaccination until 14 days post challenge. At D14, piglets were euthanased and lung lesions were evaluated.

## Results

After challenge, morbidity and mortality were lower in groups A than in group B (*Table 1*).

***Table 1.*** *Percentage of morbility and mortality*

| Group | Morbility (%) | Mortality (%) |
|---|---|---|
| A | 40 | 0 |
| B | 100 | 60 |
| C | 0 | 0 |

Two piglets in group B died at D5 and one died at D6 post challenge.

Fever incidence and duration in group A were lower than in group B. (*Table 2*)

***Table 2.*** *Incidence and duration of fever.*

| Group | Fever(≥40.5℃) frequency | |
|---|---|---|
| | Number of pigs (a/b) | Time (days) |
| A | 2/5 | 1 to 2 |
| B | 5/5 | 2 to 8 |
| C | 0/5 | 0 |

Note: "a" indicates the number of piglets that showed fever. "b" indicates the number of piglets of the group.

All piglets in group B presented lung lesions with severe hemorrhage and congestion. None of group A and C piglets presented lung lesions (*Table 3*).

***Table 3.*** *Lung lesions score*

| Group | Lung lesion | |
|---|---|---|
| | - | + |
| A | 5 | 0 |
| B | 0 | 5 |
| C | 5 | 0 |

Note: - means no lesion; + means with lesions of severe hemorrhage and congestion

## Discussion

In this study AUSKIPRA®GN reconstituted with RED solvent reduce mortality, lung lesions and fever. Therefore, AUSKIPRA®GN can provide clinical protection in front of new PRV strains.

## Reference

[1] Yu X., et al. Emerg Infect Dis. 2014. 20:102–104.1]

# Vaccination with PRV vaccine (AUSKIPRA® GN; Bartha k61 strain), reduced virus shedding when challenge against Chinese PRV variant (AH02 strain)

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## Introduction

Since 2011 several Pseudorabies (PR) outbreaks have damaged Chinese swine industry[1].

In this study MLV PRV vaccine (AUSKIPRA®GN reconstituted in A3 oily diluent, HIPRA) was challenged with new PRV variant (AH02 strain) isolated from a farm suffering from acute outbreak of PRV in China.
Virus shedding on nasal swabs was studied in two independent experiments.

## Materials and methods

30 PRV seronegative 4 to 6 weeks old piglets were randomly divided into two independent experiments (n=15) and subdivided in three groups (table 1).

***Table.1***

| Group | Treatment | Dose | Challenge virus | Number |
|---|---|---|---|---|
| A | AUSKIPRA® GN | One dose, 2ml | AH02 strain | 5 |
| B | PBS | 2ml | AH02 strain | 5 |
| C | PBS | 2ml | - | 5 |

Piglets were treated intramuscularly at D0 and challenge 7 day later (D7). Nasal swabs of all piglets were collected daily for PRV titration by Karther method(D0 to D21).

## Results

No virus shedding was detected in any piglets, in any replicate in C groups. In the first experiment, all piglets from group B showed virus shedding in nasal swabs with titers $10^{1.125\sim2.365}$ $TCID_{50}$/0.1mL, from 1 to 2 days. In the second experiment, group B virus shedding was detected in 4 piglets with titers $10^{1.125\sim3.625}$ $TCID_{50}$/0.1mL lasting for 1 to 3 days. For group A, no virus shedding in piglet at the first replicate was detected. Only one piglet in A group in the second experiment showed; virus shedding titer of $10^{0.625}$ $TCID_{50}$/0.1mL, lasting for 3 days (Table 2)

***Table.2***

| Group | First experiment | | | Second experiment | | |
|---|---|---|---|---|---|---|
| | No. (a/b) | Titer (c) | Time (day) | No (a/b) | Titer (c) | Time (day) |
| A | 0/5 | / | / | 1/5 | $10^{0.625}$ | 3 |
| B | 5/5 | $10^{1.125}\sim10^{2.365}$ | 1～2 | 4/5 | $10^{1.125}\sim10^{3.625}$ | 1～3 |
| C | 0/5 | / | / | 0/5 | / | / |

Note: No. number of piglets. “a” indicates the number of piglets that virus shedding was detected in nasal swabs. “b” number of piglets of the group. “c” titer of virus expressed as $TCID_{50}$ in 0.1 mL

## Discussion

AUSKIPRA®GN vaccine reduce PRV shedding after AH02 strain challenge. Therefore, AUSKIPRA®GN can be a useful tool to control PRV infection in Chinese farms.

## Reference

[1] Yu X., et al. Emerg Infect Dis. 2014. 20:102–104.

# PRRS Live-Attenuated Vaccine Exerts Naught Effect on Efficacy of CSF Vaccine

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) and classical swine fever (CSF) are two major swine diseases; therefore the vaccination programs against these two pathogens were widely applied. PRRSV have been reported to modulate the immune response of host[1], increasing the risk of concurrent infection and failure of CSF vaccination[2]. To understand whether if the PRRSV live-attenuated vaccine interfered the efficacy of CSF vaccine, also the optimal time of vaccination, an animal experiment were implemented.

## Materials and methods

Twenty-three PRRSV-free, three week-old specific pathogen free pigs were randomly divided into five groups. Group A to D, each with five pigs, were inoculated with PRRSV live-attenuated vaccine (Ingelvac® PRRS MLV, Boehringer Ingelheim) at 3, 4, 5, and 6 week of age, respectively. Three pigs in group E were not inoculated and served as negative control. All groups were inoculated with CSFV vaccine (Attenuated LPC strain CSFV, AHRI) at 6 week of age. The blood samples were collected for PRRSV and CSFV antibody ELISA test (PRRS X3, CSFV Ab, IDEXX), as well as CSFV wild strain PCR test.

## Result

The level of antibody titres against CSFV are considerably consistent between each group, rises after the vaccination at 6 week of age (Fig.1), and maintain constant. PRRSV antibody also rises within 3 week after vaccination (Fig.2).

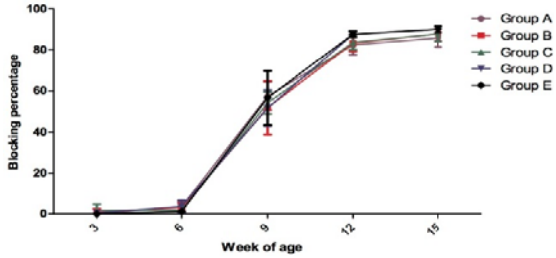


***Figure.1 Comparison of mean CSFV ELISA blocking percentage***

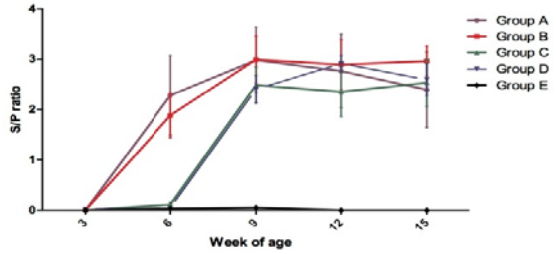


***Figure.2 Comparison of mean PRRSV ELISA S/P ratio***

## Discussion

Levels of antibody titres against PRRSV and CSFV were as expected. PRRSV antibodies rose within 3 weeks after vaccination in group A to D, while CSFV antibodies rose since 9 week of age and maintained a constant level. This result indicates that PRRSV live-attenuated vaccine will not interfere the efficacy of CSFV vaccine, in despite of different period between inoculations.

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[1] Chiou, M. T., Jeng, C. R., Chueh, L. L., Cheng, C. H., Pang, V. F. Vet Microbiol 2000: 71: 9-25.
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# Antiviral Activity of 42 Natural Compounds on Field Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Strain

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) is characterized by reproductive failure of sows and respiratory problems of nursery and growing pigs. PRRSV have been reported to modulate the immune response of host[1] and vaccination against PRRSV-infected pigs didn't provide a 100% protection. Natural compounds have been widely used to treat a variety of diseases and their antiviral activities have been studied. The aim of this study is to evaluate the antiviral ability of 50 compounds against PRRSV infection *in vitro*.

## Materials and methods

Cytotoxicity assay of the 42 compounds was measured by MTT assay[2]. Each compound was made by a 2-fold serially dilution. MARC-145 cells were seeded into 96-well plate at $5\times10^3$ cells/well and incubated 24 hours. Diluted samples were cultured with MARC-145 cells for 72 hours incubation, then MTT solution (100 µl/well) was added and incubated 4 hours. The supernatant was changed into DMSO before using ELISA microplate reader with a 570 nm. Antiviral assay of 42 compounds was evaluated as previously described[2]. The maximum non-cytotoxic concentration (MNTC) of each sample was mixed with $1TCID_{50}$µl/ml of PRRSV in a ratio of 1:1 and incubated 1 hour. Control groups were set up simultaneously. After incubation, the mixtures virus and controls were inoculated onto pre-seeded MARC-145 cells and incubated another 1 hour. Supernatant was substituted with each compound, with PBS washing 3 times. After 15 hours of incubation, the supernatants were applied for RNA extraction and then reverse transcription. The qPCR[3] was employed for PRRSV detection.

## Results

The 8 compounds showed high toxic on MARC-145 cells with the concentrations employed. The 42 compounds showed MNTC value ranging from 1/4 to 1/64 dilution. The cellular morphological changes were dose-dependent such as granulation, lyses. The 12 compounds had potent anti-PRRSV activity in a dose-dependent manner. Furthermore, *Thymus vulgaris* and *Nepeta cataria* significantly inhibited PRRSV infection higher than control group on MARC-145 cells ($P$<0.001) (Figure. 1).

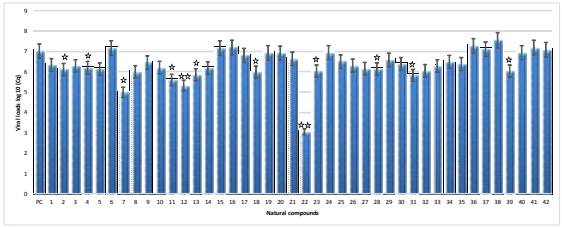

***Figure 1. The screening results of 42 compounds on field PRRSV strain in Taiwan by qPCR***

## Discussion

This study results indicated that 8 compounds showed high toxic on MARC-145 cells with the concentrations employed whereas 42 some compound had non-toxic on MARC-145 cells and also increased cell proliferation with antioxidant properties. *T. vulgaris* and *N. cataria* inhibit PRRSV infection on MARC-145 cells higher than other compound.

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# An effect of lysate Ultra-Corn® on the average daily weight gain (ADWG) improvement of nursery pigs under field condition in Korea

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**Introduction**

An ultrasonicated lysate of *Corynebacterium cutis* (Ultra-Corn®, Virbac, France) as an immunostimulating compound has been known to activate the cell-mediated immune response when detected by antigen presenting cells which are the first line defense cells of the host. By administration of Ultra-Corn® to nursery piglets at their weaning, the immune enhancement appeared to improve their health statue and uniformity at age of delivering. General situation of nursery pigs period in Korea is as follows; A secondary bacterial infection is occured usually 2 – 3 weeks after weaning with rapid loss of maternal immunity. Thus, usually clinical sign of respiratory follows 1-2 weeks and feed/water intake amount is decreased in this period. Being affected by this, ADWG and health statue shall be decreased negatively. In this study, we try to find any effect on ADWG for pigs during nursery period using Ultra-Corn® around weaning time.

**Materials and methods**

A 580 sows 2-sited GP swine farm was selected. This farm has 1 week batch management system and around 200-250 piglets were weaned normally and delivered around at age of 70 day. Each batch has a separate nursery compartment. We can distinguish by its own number(C1 – C7). 7 batches of weaned piglets were selected and divided into 2 groups according to their weaning weight; 3 batches of the experimental group (UC) which consisted of animals in lower body weight compared with normal control animals and 4 batches of the normal control group (CTL). The UC piglets which were in lower body weights than CTL piglets were given a single injection of Ultra-Corn® (1ml/pig) intramusculary at 4 days post weaning and the CTL group was maintained without Ultra-Corn® treatment. The number of delivered pigs, average weaning age and weight, average delivering age and weight were recorded as a base line data. Average daily weight gain(ADWG) of nursery pigs was evaluated and calculated.

**Result and Discussion**

Mean weaning weight of UC is 775g less than CTL and weaning age of UC is 3 days earlier than CTL. But the result of ADWG is 3g more in UC and uniformity of UC is also better than CTL at delivering time. As a result, UC had worse health status at weaning but we could see better results in UC about ADWG and uniformity.

***Table.1***

| | UC(Ultracorn) | Control |
|---|---|---|
| No. of delivered | 660 | 853 |
| Mean weaning age (days) | 25 | 28 |
| Mean weaning weight (KG) | 5.9 | 6.7 |
| Mean delivering weight(KG) | 20.2 | 20.5 |
| Mean delivering age(days) | 67.8 | 70.0 |
| ADG(KG) | 333.8 | 330.8 |

**Reference**

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# Effects of sows vaccination against *Mycoplasma hyopneumoniae* in suckling piglets

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## Introduction

*Mycoplasma hyopneumoniae* (MHP) is the causative agent of swine enzootic pneumonia and considered to play a primary role in the porcine respiratory disease complex commonly complicated by secondary infections with other bacteria. Our previous study showed that high rate of MHP in Taiwanese suckling pigs. Therefore, the objectives of the present study were to assess the effect of sow vaccination on detection rate and serologic status of their piglets.

## Materials and methods

Twenty-five sows (13 vaccinated and 12 unvaccinated) as well as four of their piglets were included in this study. Blood samples and nasal swabs from sows at 4 weeks pre-farrowing and 1 week post-farrowing and from piglets at 0, 1, 2, and 4 weeks of age were taken. Serum and nasals were tested using quantitative PCR (qPCR) to detect MHP DNA and by an ELISA test to detect antibodies of MHP.

## Results

Our results show sows vaccinated have a significantly higher percentage of seropositive (10/13, 76.9%) in 1 week after farrowing than non-vaccinated sows (5/12, 41.7%). In addition, the seropositive rate at 0 week old of piglets from vaccinated sows (44/50, 88.0%) is significantly higher than piglets from non-vaccinated sows (18/52, 34.6%) (Figure 1). The maternal antibodies last until 4 weeks of age in vaccinated group (Figure 1).

Overall, piglets from vaccinated sows had a significant lower ($P$<0.05) detection rate (25/198. 12.6%) of MHP in their serum than piglets from non-vaccinated sows (37/181, 20.4%) (Table 1). On the contrary, no statistical significant differences were found in the number of nasal qPCR positive piglets among different treatments ($P$ >0.05).

## Discussion and conclusion

Under the conditions described in this study, sow vaccination against MHP increased the percentage of seropositive sows and their piglets and reduced significantly the detection rate of MHP in piglet's serum.

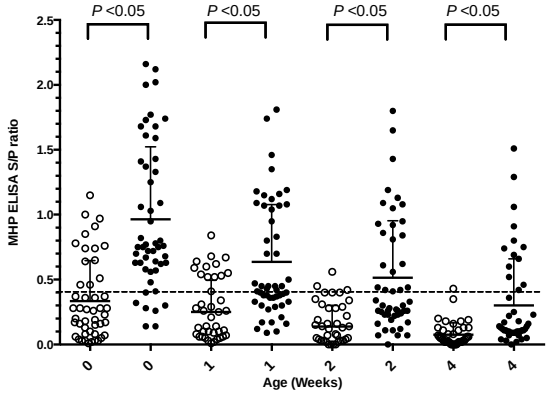


***Figure 1. MHP antibody status of piglets at 0, 1, 2, and 4 weeks of age from vaccinated (solid circle) and non-vaccinated sows (open circle).***

***Table 1. Detection rate of MHP DNA in serum of piglets among different treatments.***

| Age (weeks) | Vaccinated (%) | Non-vaccinated (%) | P value |
|---|---|---|---|
| 0 | 8/52 (15.4) | 13/48 (27.1) | |
| 1 | 3/50 (6) | 8/45 (17.8) | |
| 2 | 11/49 (22.4) | 11/45 (24.4) | |
| 4 | 3/47 (6.4) | 5/43 (11.6) | |
| Total | 25/198 (12.6) | 37/181 (20.4) | <0.05 |

## References

[1] Lin et al., Vet Microbiol 115:111-116, 2006.
[2] Fano et al., Can J Vet Res 71: 195-200, 2007.
[3] Sibila et al., Vet Microbiol 127:165-170, 2008.

# Efficacy and impact of current commercial porcine circovirus type 2 (PCV2) vaccines in dams and growing pigs

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## Introduction

Porcine circovirus type 2 (PCV2) is associated with a number of diseases and syndromes that are collectively referred to as porcine circovirus-associated diseases (PCVAD). Among them, postweaning multisystemic wasting syndrome (PMWS), porcine respiratory disease complex (PRDC) and reproductive failure are the most important [**1**].The introduction of sow vaccination has provided an effective means of controlling PCVD both in the post-weaning period and through to finishing. However in herds where the disease situation is established or immune transfer from sow to piglet is compromised it is necessary to vaccinate piglets. The purpose of this trial was to compare PCV2 vaccines given to dams or not in the farm with a high disease burden in the rearing herd.

## Materials and Methods

**Vaccination.** Sows will be either vaccinated or unvaccinated with the Merial (Circovac®) vaccine. Sows were equalized as far as possible across treatments for parity, size and litter history. One group were vaccinated in two doses; 5-6 weeks prior to farrowing date and at 2-3 weeks prior to farrowing, and the other group were not vaccinated .Then, two groups of the piglets (n=193) were vaccinated with the Boehringer Ingelheim (Ingelvac Circoflex®) vaccine. Tag number, weight and sex of each piglet were recorded at vaccination. All piglets were also vaccinated against pneumonia at this age.

**Serology.** All serum samples were tested for PCV2 antibody at the Animal Disease Diagnostic Center of National Chiayi University (R.O.C.). Measurement of antibody titers in serum was determined by an ORF2-based PCV2 ELISA kit (Biochek, Netherlands). Serum samples with a sample to positive ratio (S/P) equal to or greater than 0.5 were considered positive.

**Quantification of PCV2 in blood.** DNA extraction from serum samples was performed using the taco™ DNA/RNA Extraction Kit (GeneReach Biotechnology Corp., Taichung City, R.O.C.). DNA extracts were used to quantify PCV2 DNA copy numbers by real-time PCR. Quantitative (TaqMan) RT-PCR amplification of DNA was performed using the porcine circovirus type 2 (PCV2) Detection System (GeneReach Biotechnology Corp., Taichung City, R.O.C.).

**Growth performance.** The live weight of each pig was measured at 3, 20, and 24 weeks of age. The average daily weight gain (ADWG) (gram/pig/day) was analyzed over two time periods, (i) between 3 and 20 weeks of age and (ii) between 20 and 24 weeks of age. A *t*-test was applied to compare the ADWG results and the mortality rate.

## Results

**Anti-PCV2 IgG antibodies.** The vaccinated group may be effective controlling PCV2 infection in herd where active seroconversion occurs from 14 weeks of age. But, the unvaccinated group can only protect piglets against PCV2 challenge up to 9 weeks of age. Thus, active seroconversion occurs at 10 weeks of age.

**PCV2 DNA in sera.** Viremia of the unvaccinated group was observed at 10 weeks of age and there is a high proportion of PCV2-viremic pigs during finishing periods. However the vaccinated group was occurred at 14 weeks of age, but the proportion of PCV2-viremic pigs is lower than unvaccinated group.

**Growth performance.** During weeks 3 to 20 and 20 to 25, the ADWG of the vaccinated was significantly higher ($P < 0.05$) than unvaccinated groups. Mortality in both groups is no significant difference between the two groups.

## Discussions

The PCV2 vaccination in sows has been effective at controlling PCV2 infection in herds where PCV2 is horizontally transmitted early in life. So, the present data provide useful clinical information on how to select a proper PCV2 vaccine strategy for the control of PCV2-associated PRDC.

## References

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# Beneficial impact of vaccination with a PCV2 vaccine and a *Mycoplasma hyopneumoniae* bacterin in piglets under French field conditions

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## Introduction

PCV2 and *Mycoplasma hyopneumoniae (M hyo)*. are the etiologic agents of PCVD and enzootic pneumonia, respectively, and are considered to both play an important role in porcine respiratory disease complex (PRDC). In this study, two monovalent vaccines against these pathogens were administered to 4-week-old piglets under field conditions. Standard production performance and slaughter house observations were recorded.

## Material and methods

The trial was conducted on a 7-farrowing-batch, 420-sow farrow-to-finish farm located in France and not vaccinating against PCV2. Piglets use to be weaned at 4 weeks of age and kept in a continuous flow in the nursery and 2 fattening units. The herd was PRRSv-negative and faced degraded growth performance including lack of homogeneity at slaughter. Piglets of two batches (approximately 1200 piglets) were randomly allocated to two experimental groups balanced according to batch, sex and weight. Piglets in the PCV2-vaccinated group were injected with CIRCOVAC (Merial), 0.5 mL, IM and a *M hyo* vaccine (Stellamune Uno, Elanco), 2.0 mL, IM in two separated loci with a double-barrel syringe. Piglets in the "*M hyo* only" group were vaccinated according to the routine program, i.e. against *M hyo* only. All pigs were sent to slaughter at a target bodyweight of 94 kg. Group-based bodyweights were recorded at weaning and carcass weights were individually recorded at slaughter. The lightest pigs were sold before the end of the fattening period in the second batch whatever their treatment group. Lung checks (Madec's grid) were performed at the abattoir in approximately 180 pigs per group according to the same sampling schedule in each group. Statistical analysis were performed using Student t-test or Wilcoxon test for mean or median comparisons. Standard deviations were compared using F-test. Proportions were compared using Fisher's exact test.

## Results and Discussion

The production performance and lung check results at the abattoir are summarized in Table 1.

No adverse reaction to vaccination was observed in any of the pigs included in the study. Pigs vaccinated with CIRCOVAC in addition to the *M hyo* vaccine were slaughtered 2.7 days on average ($p<0.01$) before the ones vaccinated with the *M hyo* vaccine only and at a numerically higher bodyweight of 0.8 kg. Growth was subsequently significantly improved in the CIRCOVAC group by 19g/day ($p<0.01$). It yielded also a significant reduction of pigs sent to slaughter at age higher than 182 days ($p<0.01$), thus confirming the significant better homogeneity of the PCV2-vaccinated batch (95% CL for variance ratio: 0.54-0.89, $p<0.01$). No abnormality in mortality rate was reported during the course of the study, i.e. 1-2.5% in post-weaning and 3-5 % in fattening depending on the batch.

Table 1. Production performance and lung check results at the abattoir.

| | *M hyo* only | CIRCOVAC + *M hyo* | Difference |
|---|---|---|---|
| **Growth performance** | | | |
| Slaughter age (days) | 177.7 | 180.4 | -2.7* |
| Bodyweight (kg) | 93.45 | 94.25 | + 0.80 ns |
| Age 115 kg (days) | 174.2 | 177.6 | -3.4* |
| ADWG W-S (g/day) | 735 | 754 | +19* |
| #Pigs aged ≥182 days | 57% | 39% | -30%* |
| **Lung checks at the abattoir** | | | |
| % Non-damaged lungs (score <2) | 59% | 84% | +42%* |
| Average Score | 1.9 | 0.6 | -1.3* |
| % Severe pneumonia (score >5) | 8% | 1% | -86%* |
| % partial condemnation | 8.6% | 5.2% | -40% ns |

Growth performance are given only for batch one as the lightest pigs were sold in batch 2 before the completion of the study. * $p<0.01$; ns: not significant

At the abattoir, the lung lesion score was significantly improved in CIRCOVAC-vaccinated pigs thus demonstrating a better control of *M hyo* impact in the flow: a significant increase of non-damaged lungs ($p<0.01$) and a definite reduction of the median lung score ($p<0.01$) were observed. Severe pneumonia were also dramatically reduced in the CIRCOVAC - vaccinated pigs ($p<0.01$).

## Conclusion

CIRCOVAC vaccination in addition to the *M hyo* vaccination helped in the control of subclinical PCVD and PRCD. This resulted in a significant improvement of growth performance of the pigs.

®CIRCOVAC is a registered trademark of Merial.

# The Expression of Hepatocyte Growth Factor and MET Receptor Signaling of Proliferative and Necrotizing Pneumonia in Swine

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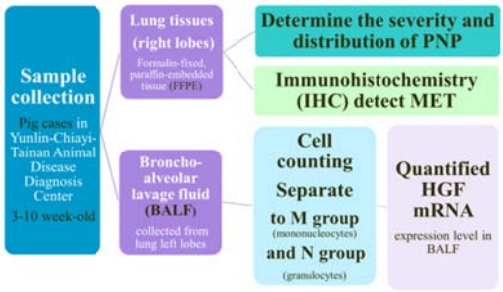
## Introduction

Proliferative and necrotizing pneumonia (PNP) is a form of interstitial pneumonia that occurs in 2-16 week-old pig herds, with a peak between 4 and 10 weeks. PNP characterized by hypertrophy and hyperplasia of type II pneumocytes and necrotic cells debris within alveolar spaces. Hepatocyte growth factor (HGF) is a pleiotrophic cytokine, capable of inducing responses on type II pneumocytes for growth-prompting activity via interaction with a single receptor, MET, in various species but not been reported in swine. The present study aimed to explore the causal role of HGF and MET signaling activation on type II pneumocytes proliferation of PNP in swine.

## Materials and methods




## Result

The results revealed that increased HGF expression level, as well as increased MET receptor activation (*Figure 1*), and proliferation in type II pneumocytes of PNP lesion score *(Figure 2*).

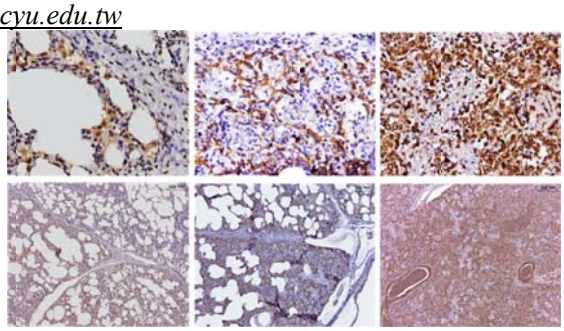


*Figure 1*

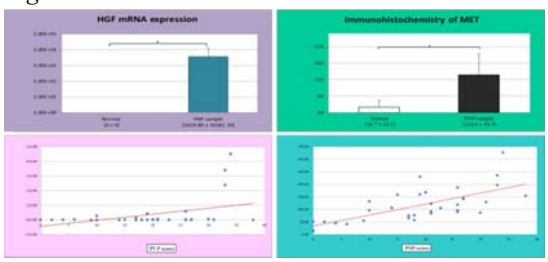



*Figure 2*

## Discussion

This study is the first report to demonstrate that HGF/MET signaling existents and is responsible for growth-promoting activity for type II pneumocytes in swine. Moreover, HGF mRNA expresses significantly in mononuclear inflammatory cells collected from BALF
in this work revealing there is a similar pattern between swine and mice [2], different to that is contributed from infiltrated neutrophils in human [1].

There was a correlation between lesion severity and the activation of HGF/MET signaling, being one of major pathway involved in PNP in swine. Furthermore, providing a possibility of novel therapeutic strategy against PNP formation through HGF/MET inhibition.

## Reference

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# A Field Study in the Philippines to Assess the Safety and Efficacy of a two-shot vaccination of piglets with Circoshield® vaccine Against Porcine Circovirus Associated Disease (PCVAD)

Dr. Renato T. Policarpio and Dr. Pamela A. Javier

## INTRODUCTION

Porcine Circovirus type 2 (PCV2), discovered in 1998 (Ellis J, Hassard L, Clark E, Harding J, Allan G, et al., 1998), is an emerging swine pathogen causing immense economic losses in the global swine industry (Meng XJ. 2012). During 1991 clinical outbreaks, the disease was described as having a poor growth rate in the growing stage, an increased mortality at the end of the weaning and at the beginning of the fattening stage. As more and more disease conditions such as reproductive disorder, enteric diseases and respiratory signs were linked to PCV2 infection, the disease was renamed porcine circovirus associated disease (PCVAD) in the United States and Porcine Circovirus Disease (PCVD) in Europe (Harding J. 1996). Currently, it has been suggested to call it PCV2-associated systemic disease or PCV2-SD (Segales, J., et al., 2005). Porcine Circovirus-Associated Disease (PCVAD) caused by Porcine Circovirus Type 2 (PCV2) has recently become a major disease affecting growing pigs worldwide (Allan, GM., Ellis, JA. et al., 2000).

## MATERIALS AND METHODS

Three (3) farrow-to-finish farms were selected for this study. These farms have a confirmed diagnosis of PMWS (PCV2 associated disease) as determined by the Philippine Animal Health Center (PAHC). Approximately, 360 suckling piglets per farm were admitted in this study. Each farm have 3 replicates of vaccinated and control group. Piglets included in this study were weighed, properly identified and randomly allocated to the study groups. The vaccinated group was injected with 1ml Circoshield® twice, initial vaccination was given at 3 weeks of age and booster vaccination at 5 weeks of age. The route of administration was deep intramuscular injection at the neck region.

## RESULTS AND DISCUSSION

The following data were gathered and compared for all groups:

The weaning weights of vaccinated group from Farm A, has no difference from the control group. However, weaning weights of both Farm B and Farm C of vaccinated group were statistically significant. Average daily gain (ADG) of vaccinated group as well as market weights were statistically significant compared with the control group.

The overall mortality rate as observed and recorded were numerically higher in the unvaccinated control group in all farms. All results in this study only indicate that growth rate is much better in the vaccinated group.

The overall mortality rate as observed and recorded were numerically higher in the unvaccinated control group in all farms

| Parameters | Farm A | | Farm B | | Farm C | |
|---|---|---|---|---|---|---|
| | Vaccinated | Control | Vaccinated | Control | Vaccinated | Control |
| Ave. weaning wt. (kg) | 7.20 | 7.20 | 7.28 | 7.19 | 7.36 | 7.28 |
| Ave. Daily Gain (g) | 563.30 | 544.51 | 562.62 | 555.11 | 566.20 | 562.92 |
| Market wt. (kg) | 92.83 | 89.98 | 93.42 | 92.84 | 92.85 | 91.57 |
| Morbidity (%) | 15.00% | 34.44 % | 14.44% | 32.77 % | 11.11% | 21.67 % |
| Mortality (%) | 10.00% | 17.78 % | 8.89% | 16.11 % | 7.22% | 13.33 % |

. All results in this study only indicate that growth rate is much better in the vaccinated group. These results validated the study that, some of the effects of PCV2 vaccination in pigs include greater ADG during the finishing period, lower finishing cull rates and lower probability of being lightweight at the time of marketing than in non-vaccinated pigs (Horlen KP. Dritz SS. et al. 2008, Fachinger V. Bischoff R. Jedidia SB. et al. 2008. Kixmoller M. Ritzmann M. et al. 2008). Removing or reducing the impact of PCV2 infection by vaccination may have left the pigs better prepared to ward off endemic infection (Neumann E, Simpson S, Wagner J, Karaconji B. 2009).

## CONCLUSION

There were no swelling and redness on the injection sites of pigs in both Vaccinated and Control groups. Absence of fever was also observed in all groups. Moreover, results showed a statistically significant effect of PCV2 vaccination on weaning weight, average daily gain (ADG) and market weight, on all Vaccinated groups of Farms A, B and C. Under the condition of this study, all these results suggested vaccination of Circoshield® vaccine against PCV2 was safe, and effective at improving growth performance, as well as reducing morbidity and mortality rate due to Porcine Circovirus Type 2. Serological test based on Enzyme-linked immunosorbent assay (ELISA) rendered positive results until 22 weeks to market. Therefore vaccination of Circoshield® against PCV2 should be considered to improve overall performance of fattening pigs.

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# Efficacy of a combined inactivated porcine reproductive and respiratory syndrome virus vaccine using North American and European strains in specific pathogen free pigs

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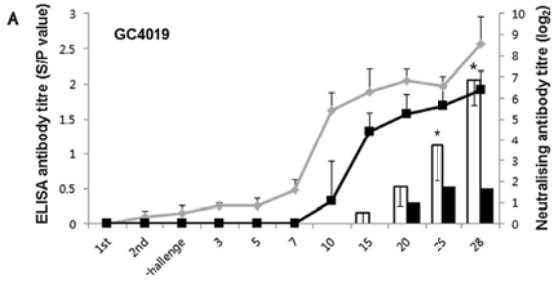
**Introduction**
A multi-strain vaccine consisting of antigenically distinct PRRSV strains would have greater protective effects against a wide-range of virus strains than a vaccine composed of a single strain. The aim of the present study was to evaluate the efficacy of a BEI-inactivated multi-strain PRRSV vaccine in pigs that were vaccinated and then challenged with individual virulent strains of PRRSV

**Materials and methods**
A total of 24 specific pathogen-free (SPF) hairless white Yucatan miniature pigs (6 weeks old) were randomly assigned to three vaccination groups (four pigs per group) and three mock vaccinated groups (four pigs per group). Pigs were injected intramuscularly (IM) twice with 2 mL of the inactivated combined vaccine (vaccinated groups) or 2 mL aluminium adjuvant (mock vaccinated groups), respectively. For challenge, each PRRSV strain (105.5 TCID50/mL) was administered intranasally to the vaccinated groups 2 weeks after the second vaccination.

**Result**
In comparison among nucleotide sequences of ORF5, type 1 PRRSV isolates WR-16 and -25 from the first case farm and isolates MANI-23 and -24 from the second case farm exhibited 86.3-86.6 % homology with European prototype PRRSV strain Lelystad (LV), while cluster I strains of Korean type 1 PRRSV showed higher homology (88.4-89.9 %) in the comparison to strain LV. In the phylogenetic analysis based on ORF5 gene, the isolates from the both case farms were closely related and belong to cluster I of Korean type PRRSV, although it seems that the isolates identified in the current study generated sub-cluster within the cluster I with other two Korean type 1 strains

*Figure 1*




**Discussion**
This study demonstrated that the vaccination with a BEI inactivated type 1/type 2 PRRSV combination vaccine had protective effects against infection by each virus strain in miniature pigs. In general, inactivated PRRSV vaccines confer weak immune responses in vaccinated animals and may not be able to protect against infection with heterologous strains. Therefore, studies on multistrain inactivated vaccines are worth pursuing.

**Reference**
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# Diseases Endemic to Asia

The 7th Asian Pig Veterinary Society Congress
October 25-27, 2015

# A novel oil adjuvant enhances the protection conferred by swine foot and mouth disease vaccines

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## Introduction

Water in oi in water adjuvants such as Montanide™ ISA 206 VG (ISA 206) are widely used for FMD swine vaccination. These oil emulsion technologies have replaced aluminium hydroxide adjuvants. They induce a strong short-term and long-term immune response which is mainly mediated by neutralizing antibodies (1), with low viscosity and low side reactions.

To improve cross-protective properties of FMD vaccines, one option is to develop new adjuvants that enhance cell mediated immune response. We have therefore selected the new adjuvant Montanide™ ISA 201 VG (ISA 201) which stimulates both humoral and cell mediated immune responses. Here we show that a swine FMD vaccine based on ISA 201 is safe, and induces higher immune response and protection against FMD than a reference vaccine based on ISA 206.

## Materials and Methods

9 groups of 5 pigs received respectively inactivated FMDV type O vaccine based on ISA 206, ISA 201 or non-adjuvanted at full dose (2ml), 1/3 dose or 1/9 dose. 5 control pigs were not vaccinated. Anti-FMDV antibody titers, IFNγ titers, and CD4+ and CD8+ T cells concentrations were measured at 0, 3, 7, 14, 21 and 28 dpv. All 50 pigs were challenged intramuscularly with 1000 $PID_{50}$ of FMDV type O at 28 dpv, and the $PD_{50}$ of each vaccine was calculated using Karber's method.

## Results

All vaccines were safe in swine. Antibody titers against FMDV were signicantly higher in the ISA 201 group compared to the reference ISA 206 group for full dose, 1/3 dose and 1/9 dose vaccinated animals at 14dpv, 21dpv and 28dpv. After full dose vaccination with ISA 201 vaccine, % of circulating CD4+ and CD8+ lymphocytes were enhanced compared to the ISA 206 group and the non adjuvanted vaccine group at 7dpv and 28dpv. Finally, full doses or 1/3 doses of both ISA 206 and ISA 201 vaccines were fully protective against FMDV type O challenge at 28dpv (5/5 pigs without any clinical signs), whereas non adjuvanted vaccine failed to protect the animals (protection rate 2/5). All non vaccinated control animals showed clinical signs. However, when the vaccines were used at 1/9 dose, only the vaccine based on ISA 201 was fully protective, showing that ISA 201 adjuvant improves the PD50 of the FMD vaccine compared to reference adjuvant ISA 206.

## Discussion

Montanide™ adjuvants that increase cell mediated immune response could extend the vaccinal protective shield against close variants such as local FMD virus strains while preserving the robustness, ease of injection and safety profile of FMD vaccines.

## References

[1] Barnard AL et al. 2005. Vaccine 23:1037-1047.

# SERO-PREVALANCE STATUS OF PSEUDORABIES IN MALAYSIA

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## Introduction

Pseudorabies (PR) or Aujeszky's disease is a major swine viral disease manifested by clinical signs and lesions that vary among different age groups[1]. In PR-endemic areas, breeding stock shall be vaccinated regularly and vaccination of all piglets is recommended[2]. It was reported on 1998 that PR was an endemic disease and despite vaccination, frequent sporadic outbreaks have been occurred in different parts of Malaysia[3]. Most of the farms in Malaysia only give PR vaccine to the breeding stock and usually there will be no PR vaccination done for piglets.

The objective of this study was to determine sero-prevalence induced by wild strain or vaccine strain and to study the epidemiology of PR in Malaysia.

## Materials and methods

In 2013, 13 farms were randomly selected from 6 different states of Malaysia. The selected farms must meet the criteria of either using gE deleted commercial vaccines or without using any commercial PR vaccines. Vaccination program of the farms involved was recorded. In each of the participating farms, 8 to 11 sows and 4 to 5 pigs at each 10, 13, 16 and 19 weeks of age were bled for blood samples. A total of 385 blood serum samples were collected to investigate the presence of gE antibodies of PR by IDEXX PRV/ADV gl Ab test.

## Result

It was shown that 6 farms from the 13 farms involved were infected or recently contacted with wild type PR virus and 11.43% of the pig samples from those farms were gE positive which was induced by the wild type virus.

Twenty eight percent (28.71%) of the sows tested gE positive. In general, the percentage of gE positive values was distinctly low before 16 weeks of age, and the positivity increased significantly at 19 weeks of age. (Table 1)

**Table 1: percentage of gE positive in different group of pigs**

| | Positive for gE antibody (%) |
|---|---|
| Sow herd | 28.70% |
| Pigs, 10 weeks of age | 1.59% |
| Pigs, 13 weeks of age | 1.56% |
| Pigs, 16 weeks of age | 0.00% |
| Pigs, 19 weeks of age | 7.81% |

## Discussion

All farms in this study were using only gE deleted vaccines. The gE deleted vaccines render an advantage of differentiating between the immune response of naturally infected pigs and those that have been vaccinated with gE deleted vaccines by using the enzyme-linked immunosorbent assay (ELISA) to detect the presence of antibody against the protein coded by the gE gene. Only the immune response toward the wild type PR virus will be able to give a positive result in the g1 Ab test.

However, there were 2 possibilities that will lead to the positivity of gE in pigs at 10 and 13 weeks of age. It could be due to the contact with the wild type PR virus or the presence of maternal derived antibody from gE positive sows. Piglets may acquire the antibody via the colostrum intake. It is said that maternal antibodies against the disease may persist up to 14 weeks of age[3].

Pigs with gE positive increase dramatically from 0% to 7.81% at 19 weeks of age, and this kind of trend implied the exposure to wild type PR virus in the grow-finish stage or reactivation from the latent infection of pseudorabies when the pigs were stressed in the farms.

## References

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2. Barbara E.Straw, et al (2006) Diseases of swine 9th edition. P429
3. Jasbir. Singh,. (1998) Epidemiology of Aujeszky's disease in Pigs in Malaysia.

# The serological characteristics of FMD vaccination

Yeong-iLL Wang[1], YunHo Choi[2], KyungHun Cho[3], YungKuk Kwon[4], IlHan Lee[5], HoChul Kong[6], PilSoo Jeong[7], SeungYoon Lee[8]

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## Introduction

The serological test is needed for monitoring whether FMD vaccination were done or not done at pig farm. There are some claims of farmers that those farmers didn't get +ve % result which they expect in South Korea. So we test the serology sow and before/after FMD vaccination with FMD O SP ELISA kit.

## Material and Method

Five sows of two different farms and their progeny were selected for this test. The blood samples were collected from each farm and sent them to diagnostic laboratories(Gyeonggido BukBu veterinary service).

Table1. Sampling and vaccination

| | sows | 30DO | 60DO | 90DO | 120DO | 150DO |
|---|---|---|---|---|---|---|
| # heads | 5 | 42 | 42 | 42 | 42 | 40 |
| Vx | * | - | Vx | - | - | - |

* Sow: vaccinate 3weeks before farrowing
* Vx: FMD vaccination: Aftopor (Merial)

ELISA test were done with PrioCHECK FMDV type O ELISA kit (Prionics, Swiss).

## Result

ELISA titer(PI value) was increased at 120 days old pigs when the two months after vaccination (Fig1).

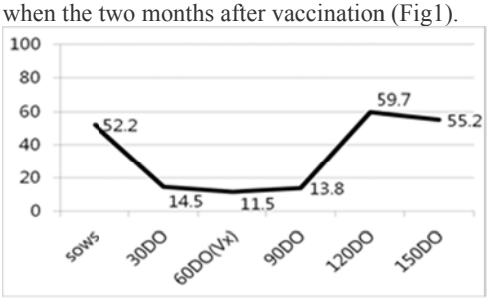


Fig1. Serological pattern of FMD vaccination (ELISA titer, PrioCHECK FMDV type O ELISA kit, Prionics, Swiss)

Positive ratio is around 60% two months after vaccination(Fig2). PI(percentage of inhibition) value was same or over to 50 is regarded as positive(+ve). PI value of pig less than 10 at 60 days old is increased one month after vaccination but PI value of pig around 6.4 at 60 days old is decreased (Fig3),

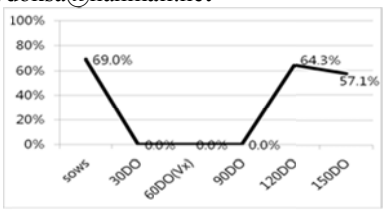


Fig2. +ve % pattern of FMD vaccination

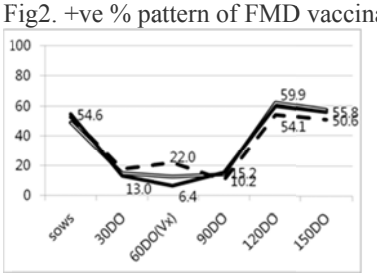


Fig 3.

## Discussion

For screening the farm didn't do FMD vaccination by ELISA test is limited by sensitivity of the test and by some factors including storage vaccine, health status of animals, and others.

In this study, there are quite a big variation of ELISA titer and 50-60% +ve ratio could be reached after vaccination, not 100%. And, FMD ELISA titer could be increased two month after vaccination(Table1).

Table1. ELISA titer (PI value)

| | sows | 30DO | 60DO | 90DO | 120DO | 150DO |
|---|---|---|---|---|---|---|
| Mean ± STD | 52.2 ± 10.8 | 14.5 ± 7.9 | 11.5 ± 6.4 | 13.8 ± 7.0 | 59.7 ± 20.0 | 55.2 ± 23.2 |

There might be MDA(maternal derived antibody) interference in between 60days old(vaccination) and 90days old(Fig3, Table2).

Table2. ELISA titer between 60days old and 90days old pigs.

| # pigs | 60DO | | 90DO (PI) | Δ |
|---|---|---|---|---|
| | GROUP (PI) | (PI) | | |
| 19 | <10 | 6.4 | 15.2 | +8.8 |
| 15 | 10-20 | 12.4 | 13.9 | +1.5 |
| 8 | 20-30 | 22.0 | 10.2 | -11.8 |

## Reference

1. J Vet Serv, 2013, 36(1), 15-21

# The analysis results of PRRS ARC project

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## Introduction

PRRS is also major pathogen cause serious economic impact on pig farms in Korea. So, Korea Swine Association and HanByeol Farm Tech(private pig consulting company) cooperate for doing PRRS ARC project in PoCheon area. In doing PRRS ARC project in PoCheon, we analyzed the performance data before and after of PRRS ARC project.

## Materials and Methods

PRRS control strategies is like these. Evaluation and improvement of biosecurity, serological monitoring and environment control(ventilation) are the strategies for the negative and stable site. And for positive site we applied 'Load-Close-Homogenized' process additionally. For unknown site we inform what we are doing.

There are twelve sites in PoCheon area. We followed 'the standard PRRS ARC process' which was made by HanByeol Farm Tech' based on US process. All data used in this paper collected by swine veterinarians who involved in PRRS ARC project.

## Results

The performance data were as below.

Table1. The performance data of sites in PoCheon area (nursery production, pig inventory)

| | sows* | nursery | total |
|---|---|---|---|
| Before | 2,407 | 7,987 | 10,394 |
| After | 2,431 | 8,408 | 10,839 |
| Δ | +24 | +421 | +445 |

* three sites

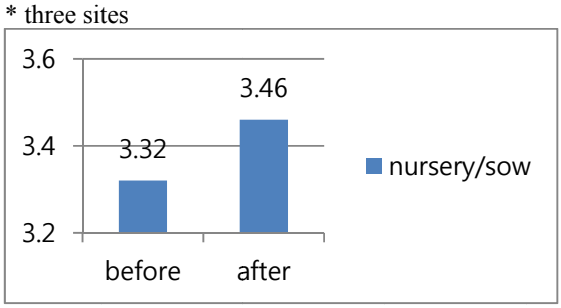


Fig1. The performance of PRRS ARC project (nursery production-three sites)

Table2. The performance data of sites in PoCheon area (fattener production, pig inventory)

| | Sows* | nursery | grower | finisher | total |
|---|---|---|---|---|---|
| Before | 711 | 2,780 | 1,794 | 1,830 | 7,117 |
| After | 693 | 2,623 | 2,015 | 2,018 | 7,349 |
| Δ | -18 | -157 | +221 | +188 | +234 |

* four sites

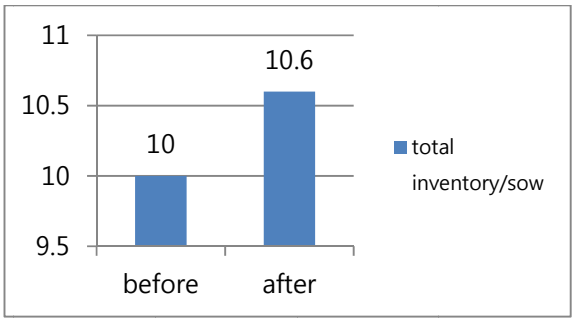


Fig2. The performance of PRRS ARC project (fattener production-three sites)

## Discussion

There is some need to evaluate of the PRRS ARC project. In PoCheon area is selected for evaluation. The performance was improved both at nursery production sites and farrow to finish sites.

For this kind of evaluation, there is no data of computerized program, so we checked the number of pig at each site when this PRRS ARC project started and finished.

The performance was improved 0.14% of nursery per sow at nursery production sites and 0.6% of total inventory per sow at farrow to finisher sites.

We thought the PRRS ARC project could improve the performance of sites. Even though it can be nursery production sites and also farrow to finish sites.

Reference
1. APVS2013 Vietnam. Application of SEBS (site evaluation of biosecurity system) for PRRS ARC in Korea
2. APVS2013 Vietnam. PRRS ARC process in Hapchun (Danji: High pig dense area)
3. APVS2013 Vietnam. New approach for PRRS status classification
4. APVS2013 Vietnam. Control strategies of bacterial pathogens in Danji (high pig dense area)
5. APVS2013 Vietnam. PRRS virus circulation within Danji (high-pig dense area)

# The analysis results of PRRS ARC project

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4,5,6HanByol Farm Tech(Swine consulting Group), Seoul, Korea, 143-701

**Introduction**

PRRS is also major pathogen cause serious economic impact on pig farms in Korea. So, Korea Swine Association and HanByeol Farm Tech(private pig consulting company) and KOVCOG Consulting(Swine Consulting Group) cooperate for doing PRRS ARC project in HapCheon area. In doing PRRS ARC project in HapCheon, we analyzed the performance data before and after of PRRS ARC project.

**Materials and Methods**

PRRS control strategies is like these. Evaluation and improvement of biosecurity, serological monitoring and environment control(ventilation) are the strategies for the negative and stable site. And for positive site we applied 'Load-Close-Homogenized' process additionally. For unknown site we inform what we are doing.

There are twelve sites in HapCheon area. We followed 'the standard PRRS ARC process' which was made by HanByeol Farm Tech' based on US process. All data used in this paper collected by swine veterinarians who involved in PRRS ARC project.

**Results**

The performance data were as below.

Table1. The performance data of sites in HapCheon area (fattener production, pig inventory)

| | Sows* | nursery | grower | finisher | total |
|---|---|---|---|---|---|
| Before | 1437 | 2,214 | 4,296 | 6,093 | 14,192 |
| After | 1318 | 2,129 | 4,957 | 6,387 | 15,037 |
| Δ | -119 | -85 | +661 | +294 | +845 |

* four sites

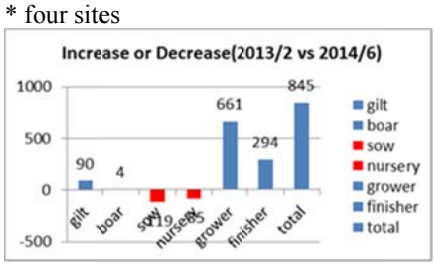


Fig1. Performance data before/after of PRRS ARC project (pig inventory)

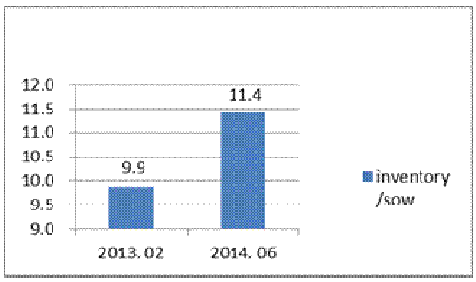


Fig2. Performance data before/after of PRRS ARC project (total pigs/sow)

**Discussion**

There is some need to show some evidence of the PRRS ARC project for continuing PRRS ARC project. The performance of all sites was improved in HapCheon area after PRRS ARC project.

For this kind of evaluation, we checked the number of pig at each site when this PRRS ARC project started and finished.

The performance was improved 15.2% based on pig inventory of sites.

We thought the PRRS ARC project is very useful to improve the performance of pig farm even in the pig dense area.

**Reference**

1. APVS2013 Vietnam. Application of SEBS (site evaluation of biosecurity system) for PRRS ARC in Korea
2. APVS2013 Vietnam. PRRS ARC process in HapCheon (Danji: High pig dense area)
3. APVS2013 Vietnam. New approach for PRRS status classification
4. APVS2013 Vietnam. Control strategies of bacterial pathogens in Danji (high pig dense area)
5. APVS2013 Vietnam. PRRS virus circulation within Danji (high-pig dense area)

# The environment check for PED virus in farrowing house

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## Introduction

PED(Porcine Epidemic Diarrhea) is a major concern of Korean pig industry. It is thought that PED problems caused 7-8% loss of P/S/Y(wean piglets/sow/year) during 2013/2014 winter[1].

There were some cases PED re-outbreaks one or two month after 'feedback' procedure. It may cause by lack of sow immunity and by re-infection from the environment.

In this study, we check 'PED' virus the environment at farrowing house, there is no PED clinical signs one months after 'feedback' procedure.

## Material and Method

Sterilized distilled water, Sweeper paper, vinyl gloves, zipper bag and conical tube are used for PED virus sampling. The samples were sent to Optipham(Diagnotic Lab.) for PED PCR test.

1. Place 10ml of sterilized distilled water (SDW) in a bag with sweeper paper.
2. Massage the bag so that the sweeper will absorb the SDW.
3. With gloved hand remove the sweeper carepully.
4. Wipe the target area.
5. Put the sweeper into the bag.
6. Squeeze SDW from sweeper inside the bag and then pour SDW into the conical tube for submission.
7. Submit the tube in a cooler on ice to the diagnostic lab for PCR test.

Fig1. Sweeper paper

Fig2. Squeezed SDW

## Result

PED PCR results in farrowing house are as below (Table1).

Table1. PED PCR results in farrowing house.

| | Farm A | Farm B | % +ve |
|---|---|---|---|
| Empty pen (cleaned) | 0/2 | 1/2 | 25% |
| Pen (diarrhea) | 3/3 | 1/3 | 66.6% |
| Pen (no-diarrhea) | 2/3 | 0/3 | 33.3% |
| Pig board (herding) | 1/1 | 1/1 | 100% |
| Cart (piglets) | 1/1 | NA* | 100% |
| Boots (employ) | NA* | 0/1 | 0% |

*Not available

## Discussion

In this study, we checked the how much % contaminated one month after 'feedback' procedure. The data shows that the environment were significantly contaminated with PED virus even after diarrhea was gone by 'feedback' procedure.


Fig3. Feedback procedure

This environment check method is easy and simple, so it can be done by stock person. And the results can show us whether 'cleaning --> disinfection --> drying' is sufficient or not. And it also can be used as the barometer of the PED virus eradication level inside the pig farm.

## Reference

1. http://www.hyunchuk.co.kr/news/7357

# The serological comparison of infection and vaccination of PED.

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macrophage0@hanmail.net

## Introduction

PED(Porcine Epidemic Diarrhea) is a major concern of Korean pig industry. It is thought that PED problems caused 7-8% loss of P/S/Y(wean piglets/sow/year) during 2013/2014 winter[1].

There were many cases PED outbreaks in the herds which were vaccinated with PED vaccine include killed and/or live vaccine (I.M. and/or P.O). So there are many questions whether PED vaccination can protect pigs from PED infection or not and it can reduce the loss caused by PED problems or not.

So we collect the blood samples of PED vaccinated farms and PED outbreak(feedback) farms and analyzed the data.

## Material and Method

Four farms were selected for this study. Two farms have PED outbreak history within three months and the other two farms have no PED outbreak and only PED vaccination history.(Table1) All samples are tested by PED ELISA test kit (Bionote, Korea)

Table1. Farms and # pigs for serological monitoring

| Farm | PED | sow | weaner | 40 DO | 60 DO | 110 DO | 150 DO |
|---|---|---|---|---|---|---|---|
| A | +ve | 4 | 2 | 4 | 4 | 5 | 5 |
| B | +ve | 5 | 5 | 5 | 5 | 5 | 5 |
| C | -ve, Vx* | 8 | 4 | 4 | 4 | 3 | 3 |
| D | -ve, Vx** | 5 | 5 | 5 | 5 | 5 | 5 |

* Vx: Live-vx 2 times(I.M)

* Vx: Live-vx 2 times(I.M) and Killed-vx 2times(I.M.)

## Result

ELISA titer(IgG and IgA) was shown as Table2.

## Discussion

To reduce the loss suckling piglets by PED infection, we interest to make neutralizing antibody of colostrum/milk of sows by vaccination and feedback procedure.

ELISA IgG titer of sow are quite different between around 1.09 of sows vaccinated and around 2.12 of sows infected. We can suppose that IgG titer of sows can be used to evaluate for immunity of PED of the sow herds. For example, near 2.0 IgG titer might be reduce some of piglets loss by PED infection.

Table2. ELISA titer (Mean±STD)

| PED | Ig | sow | weaner | 40 DO | 60 DO | 110 DO | 150 DO |
|---|---|---|---|---|---|---|---|
| +ve | IgG | **2.12 ± 0.83** | 1.15 ± 0.90 | 0.71 ± 0.25 | 1.10 ± 0.37 | 1.10 ± 0.29 | 1.44 ± 0.56 |
| | IgA | 0.77 ± 0.44 | 0.37 ± 0.53 | 0.28 ± 0.30 | 0.56 ± 0.43 | 0.81 ± 0.56 | 0.76 ± 0.26 |
| -ve, Vx | IgG | **1.09 ± 0.21** | 0.42 ± 0.06 | 1.11 ± 0.52 | 1.01 ± 0.32 | 1.64 ± 0.58 | 1.31 ± 0.19 |
| | IgA | 0.03 ± 0.30 | 0.03 ± 0.01 | 0.96 ± 1.13 | 1.04 ± 1.14 | 0.99 ± 1.11 | 0.64 ± 0.26 |

The immunity by infection (feedback) was stronger than the immunity by vaccination (Live-vx, Oral-vx, Killed-vx).

There is good correlation between IgG titer and IgA titer. Correlation coefficient (r) is 0.6613. (Fig1)

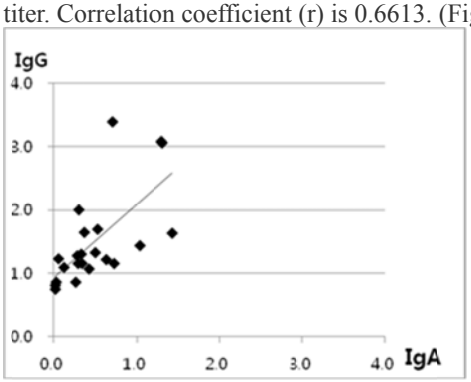


Fig1. Correlation between IgG and IgA titer. (ELISA kit, Bionote, Korea)

We're studying to know the relation between colostrum IgA and serum IgA and IgG.

Because there are some difficult to sample the colostrum of sow, we think serological approach to evaluate the sow immunity can be possible based on this study.

## Reference

1. http://www.hyunchuk.co.kr/news/7357

# The value of isolation barn for gilt introduction

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[1,2,6] *HanByol Farm Tech(Swine Consulting Group), Gyeonggi-do, Korea, 463-785*
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## Introduction

PRRS is also major pathogen cause serious economic impact on pig farms in Korea. So, Korea Swine Association and HanByeol Farm Tech(private pig consulting company) and KOVCOG Consulting(Swine Consulting Group) cooperate for doing PRRS ARC project in HapCheon area. In doing PRRS ARC project in HapCheon, we evaluate the value of isolation barn for new gilt introduction.

## Materials and Methods

PRRS control strategies is like these. Evaluation and improvement of biosecurity, serological monitoring and environment control(ventilation) are the strategies for the negative and stable site. And for positive site we applied 'Load-Close-Homogenized' process additionally. For unknown site we inform what we are doing.

After 6-7 months 'Herd Closure', farmers introduced new gilts which were PRRS negative source to various place of their farm.

We collect the data of culled/death number of gilts based on where the new gilts introduced.

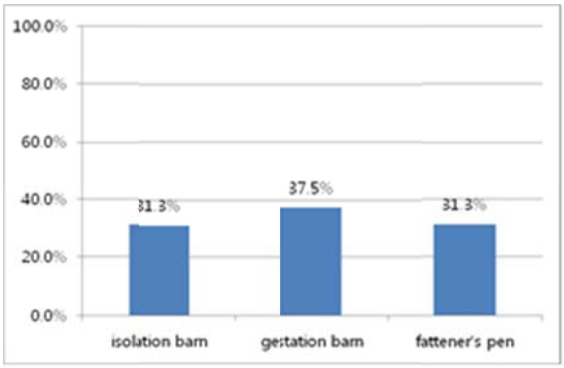


Fig1. the percentage rate of place for gilt of HapCheon area.

## Results

The difference of culled and death rate of gilts are quite a big (Fig2)

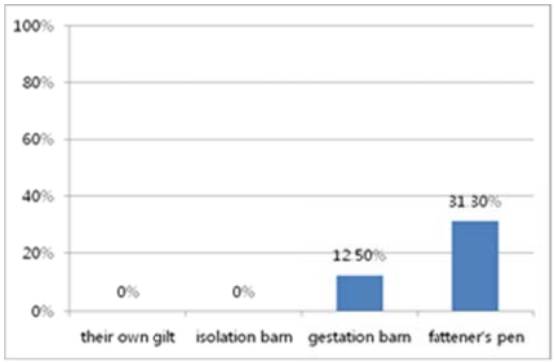


Fig2. the percentage rate of culled/dead gilts

## Discussion

There was no culled and death rate of gilt introduced at isolation barn and the their own gilt. But the gilts introduced to gestation barn and fattener's pen showed 12.5% and 31.3% culled and death rate. It means very important that the place for gilt which newly introduced.

Fig3. Isolation barn for new gilt - small herd

We've educated farmers to built the isolation barn for introducing new gilt even after PRRS stabilization.

## Reference

1. Holtkamp DJ, Polson DD, Torremorell M, et al. Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. *J Swine Health Prod.* 2011;19(1):44-56.

2. APVS2013 Vietnam. New approach for PRRS status classification

# New approach for PRRS status classification

JiYong Park [1], JaeHo Lee [2], YoungCheul Moon [3], HoCheol Kong[4], PilSoo Jeong[5], SeungYoon Lee [6]
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[4,5,6] *HanByol Farm Tech(Swine Consulting Group), Seoul, Korea, 143-701*
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## Introduction

PRRS status of sow herds is classified based on PCR test of weaners in US. But we experience high variation of the PCR test results from some laboratories in Korea. That means there may be some risk to make decision for making the strategies for PRRS control the site and/or the area. So, we tried to make new classification tool using both PCR and ELISA test.

## Materials and Methods

Thirty blood samples were collected from each farm of nineteen farms in Danji. Five samples were collected each group which are weaners, forty days old of age, sixty days old of age, hundred days old of age and hundred thirty days of age and sows (Table 1).

Table1. Sampling and test way

| | sows | weaners | 40days | 60days | 100days | 130days |
|---|---|---|---|---|---|---|
| heads | 5 | 5 | 5 | 5 | 5 | 5 |
| PCR | - | O | - | - | - | - |
| ELISA | O | - | O | O | O | O |

The twenty five samples were tested with ELISA kit(IDEXX ELISA kit 3XR) and five samples of weaners were tested with PCR.

Classification of sow herds adjusted with ELISA test results of forty and sixty days of old pigs as below (Table2).

**Table 2. New classification of PRRS status of sow herds**

| Classification | Markers of farms |
|---|---|
| **Unknown:** Unknown infection status of PRRS.Farm inspection had not, or do not know the results. | ■ (black) |
| **Positive(unstable):** PRRS infection symptoms. From PRRS virus PCR results.Produced positive piglets. | ■ (red) |
| **Positive(stable):** Have no symptoms from the breeding herd. Negative-weanrs production. 90 days the last point the virus has vanished from postweaning. *40 days old or 60 days old pigs all -ve on ELISA test | ■ (green) |
| Negative: All pigs were ELISA and PCR negative. | ■ (blue) |

* in Korea

## Results

The results are as below (Table 3).

**Table 3. Comparison of PRRS status by two classification tools**

Area1

| | Former one | New |
|---|---|---|
| Basic test | PCR(weaners) | PCR+ELISA |
| -ve/total | 7/7 | 4/7 |
| % of stable | 100% | 57.1% |

Area2

| | Former one | New |
|---|---|---|
| Basic test | PCR(weaners) | PCR+ELISA |
| -ve/total | 9/10 | 5/10 |
| % of stable | 90% | 50% |

Area3

| | Former one | New |
|---|---|---|
| Basic test | PCR(weaners) | PCR+ELISA |
| -ve/total | 11/17 | 4/17 |
| % of stable | 64.7% | 23.5% |

## Discussion

It can be a disaster if the unstable herd may be classified as stable herd for PRRS control of the site and the area. Some intervene methods for control PRRS can be removed based on the classification. The PCR test shows some variation, so practitioners in Korea usually rely on the result of ELISA test (IDEXX kit). Two classification show over ten times % difference of PRRS stable herds. It is more difficult to get stable status for the site with new classification which we developed. In our condition, new classification tool might be safer than the former one.

## Reference

1. Holtkamp DJ, Polson DD, Torremorell M, et al. Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. *J Swine Health Prod.* 2011;19(1):44-56.

# Impact of PRRS vaccination with Ingelvac PRRS MLV to PRRS negative and positive growing pigs

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## Introduction

In Korea, PRRS vaccine has been used in sows for several years and many farms have achieved the stabilization status in breeding herds. In most farms however, after weaning pigs get infected with wild-type PRRSv negatively affecting the growth performance.
The purpose of this study was to investigate growth performance impact by vaccinating growing pigs from PRRS negative and positive sow herd source with Ingelvac® PRRS MLV.

## Materials and methods

The study was done in a grower to finisher farm which has 1600 heads capacity, the pigs of which come from 2-site sow farms which have about 400-sows respectively. One farm was PRRS negative and the other is positive status with PRRS type 1. Two (2) batches of 80-day old pigs from negative sow farms and 1 batch from positive farm were involved in the study. The type 1 PRRS virus was detected at the PRRS positive sow farm before.

Table.1 Intervention by groups

| | Source farm | Vaccination |
|---|---|---|
| Group 1 | PRRS negative | Non-vx |
| Group 2 | PRRS negative | Vx |
| Group 3 | PRRS positive | Vx |

All the 3 groups were reared in almost similar barns. Historically the pigs showed late seroconversion at 110-140 days and respiratory symptoms after 2-3 weeks after the movement.
Within a week after introduction to the grow to finisher barn, all the pigs of group 2 and 3 were vaccinated by 2ml of Ingelvac PRRS MLV given intramuscularly (Table 1).
Growing performances were analyzed for each groups.

## Result

The mortality rate of each group was 1.9%, 1.4% and 2.9% respectively. It was relatively higher in group 3 than other groups. Average market weight was 107, 112.6 and 111.5. Days to market was 190.7, 182.7 and 180.7. FCR was 3.19, 3.235 and 3.245. FCR of group 1 was a little bit lower than other groups, and no big differences between group 2 and 3. ADG was 0.678, 0.817 and 0.775. ADG of group 2 was higher than other groups. Feed cost per weight gain(kg) was 1,629, 1,478 and 1,491(Table 2). The cost was calculated automatically by the computer program and means overall economic value.

Table.2 Summary of the study results

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| No pigs | 427 | 370 | 376 |
| Days of age | 74.3 | 87.9 | 80.9 |
| Ave weight(kg) | 27.5 | 34.8 | 32.8 |
| Mortality rate | 1.87% | 1.35% | 2.93% |
| Ave market weight(kg) | 107 | 112.6 | 111.5 |
| Days to market | 190.7 | 182.7 | 180.7 |
| FCR | 3.19 | 3.24 | 3.25 |
| ADG | 0.678 | 0.817 | 0.775 |
| Feed cost/weight gain(kg)* | 1,629 | 1,478 | 1,491 |

* *KRW*

## Discussion

In this study, it was concluded that even though pigs was from the PRRS positive sow farms, PRRS vaccination makes better performances than non vaccination group. This is consistent with other researchers' findings[1,2,3].

## Reference

[1] Kolb et al. 2015, AASV proceedings, 167
[2] Patterson et al, 2014, IPVS proceedings, 308
[3] Spronk et al, 2009, Leman conference

# The reduction of post weaning mortality of using Ingelvac® PRRS MLV in Korean swine farm

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## Introduction

For over the 10 years, the Korean swine industry has h ad significant economic losses due to PRRS virus and PCV2-induced respiratory symptoms, reproductive pr oblems, wasting and sudden death. During those periods, Ingelvac® PRRS MLV dedicated as an important tool to control PRRSV in breeding herds in Korea. Currently, most of Korean swine farmers vaccinate their sows periodically. So they've achieved reproductive performance much better than before. However, piglet infection by PRRSV and consequent secondary bacterial infection still remains a big problem especially after weaning. PRRSV-infected pi gs usually show poor growth performance and are hig hly susceptible to bacteria and other virus leading to death[1]

In this study, we evaluated the efficacy of Ingelvac® PRRS MLV by measuring mortality rate in nursery house.

## Materials and Methods

This study was conducted on a 2,500 sow level farm which has 2-site system. Around 70 days of age piglets are moved to the grow-finisher farm. Around 1,200 piglets of 28 days of age are weaned every week. This farm is well managed, and production performance was fairly good. Especially nursery mortality was less than 1% since 2013. The poor growing piglets are culled in the farrowing house. However on September 2014, type 2 PRRSV was detected at 7 week old piglets by serum monitoring through PCR test. And sero-conversion was observed from 9 week old piglets. On October 2014, piglets started to show neurological signs and subsequently lead to death.

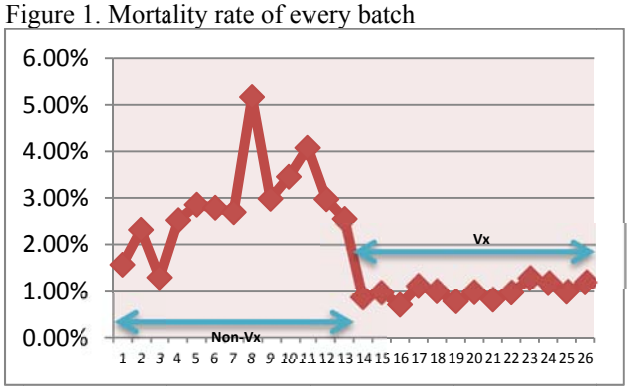
In order to resolve this problem, the farmer decided to vaccinate 3 week old piglets with Ingelvac® PRRS MLV as a method of 3FLEX®. This farm already used FLEXcombo®.

After that we surveyed mortality of 13 batches (PRRS vaccinated group), and compared former 13 batches (control group).

## Results

When we surveyed mortality rate every batch, there was big difference between PRRS vaccinated group and the control group. Before vaccination mortality rate was distributed from 1.29% to 5.17%. But after vaccination this range was quite narrowed from 0.71% to 1.29% (Figure 1).

Figure 1. Mortality rate of every batch




And we surveyed detailed performance in nursery house. From Aug to Oct 2014, 15,800 pigs were weaned. Among them 449 pigs were dead in nursery. So in this period mortality rate was 2.84%. From Nov '14 to Jan '15, 14,507 pigs were weaned. Among them 143 were dead in nursery. So in this period mortality rate decreased to 0.99% (Table 1).

Table 1. Summary of the results in terms of mortality rate of control and vaccinates

| | Control (Aug-Oct'14) | Vaccinates (Nov'14-Jan'15) |
|---|---|---|
| No of batches | 13 | 13 |
| No of Weaned pigs | 15,800 | 14,507 |
| No of dead pigs | 449 | 143 |
| Mortality (%) | 2.84 | 0.99 |

## Conclusion

If we conducted this trial at same season, the difference of the mortality between 2 groups will be much higher.

In this trial, we got some results. First, PRRS virus and secondary bacterial infection can be controlled by PRRS piglet vaccination. Second, Ingelvac® PRRS MLV has a cross protection against PRRS EU strain. Finally we could achieve better growing performances of pigs after PRRS vaccination at PRRS positive farm.

## References

1 Lunney JK et al. Virus Res. 2010;154:1-6.

# Case report: Growing performance improvement after using 3FLEX® (Ingelvac CircoFLEX® and Ingelvac MycoFLEX® and Ingelvac® PRRS MLV) at 3weeks old piglets in a Korean swine farm

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSv) causes respiratory disease in nursery and grow-finisher pigs and reproductive failure in sows and boars[1]. PRRSv-infected pigs usually suffer from poor growth performance and are highly susceptible to co- or secondary bacterial and other viral infections[2]. Especially, PRDC (Porcine Respiratory Disease Complex) is major problem in Korean swine industry due to the presence of the three main pathogens (PRRSv, PCV2, Mycoplasma).
In this study we evaluated the efficacy of 3FLEX® (Ingelvac CircoFLEX®, Ingelvac MycoFLEX® and Ingelvac® PRRS MLV) in a Korean swine farm.

## Materials and Methods

This study was conducted in a commercial 1,000 sow farm with multi-site rearing systems and different contracted finisher farms. The farm had already been used FLEXcombo® (Ingelvac CircoFLEX® and Ingelvac MycoFLEX®) at 3 weeks old piglets but still had problems with PRDC in finishing pigs due to PRRSv infection.
Therefore we decided to execute a control program using 3FLEX® at weaning. In June 2013, vaccination was performed with 3FLEX® (2ml) at 3 weeks old piglets. This farm already performed mass vaccination in the breeding herd with Ingelvac® PRRS MLV. Strict needle management as one needle for individual sows was implemented. The performance data was analyzed before and after 3FLEX vaccination period.

## Results

There was an improvement in performance in the herd (table 1). The average slaughter rate (pigs vaccinated minus losses minus culls) was 5.17% higher in the 3FLEX® vaccinated group (88.96 % vs 94.13 %) and in addition FCR was reduced by 0.12 and ADG was increased by 0.018 (0.726 vs 0.744) in the 3FLEX® group.

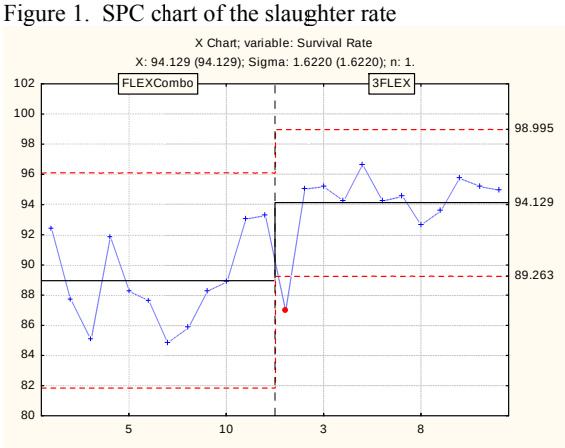
We also evaluated economic benefits based on BECAL (Boehringer Ingelheim Economic CALculator). Using this program, an economic evaluation of the vaccination resulted in a ROI of 5.27:1. Also, after piglet vaccination with 3FLEX® at 21 days of age, the respiratory clinical signs gradually disappeared and we could reduce antibiotics at grow-finisher farms.

Table1. Effect of 3FLEX vaccination on pig performance

| Parameters (wean to finish) | 2013 (FLEXcombo) | 2014 (3FLEX) | Diff. |
|---|---|---|---|
| Slaughter rate(%) | 88.96 | 94.13 | 5.17 |
| FCR | 2.99 | 2.87 | 0.12 |
| ADG(kg) | 0.726 | 0.744 | 0.018 |
| MSY(head)[1] | 20.0 | 23.5 | 3.5 |

[1] Pigs marketed per sow per year

Figure 1. SPC chart of the slaughter rate




## Discussion and Conclusions

According to the results of this case report, performance has been increased after vaccination of 3 weeks old piglets with 3FLEX®. In addition it reduces the workload for the vaccination at the farm. Considering these results, this 3FLEX vaccination program can be a good method to other farms that have PRDC problems in the grow-finish period.

## References

1. Zimmerman, J., et al. (2006). Disease of swine. p. 387-417
2. Lunney JK et al. Virus Res. 2010;154:1-6.

# Quantification of relative realtime RT-PCR of porcine epidemic diarrhea virus using UV-C irradiation

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## Introduction

Recently, porcine epidemic diarrhea virus (PEDV) was detected in the USA. PEDV have continued to cause ongoing disease challenges for pork producers. In order to prevent economic losses from PED, many pork producers use UV-C light as a sterilizer. The purpose of this experiment was to measure levels of UV-C induced RNA damage by using the property that damaged RNA of PEDV can inhibit PCR.

## Materials and methods

The PEDV strain, P-5v, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV-C lamp (Enputech, Korea) with wavelength output at 254nm, 150μW•sec/$Cm^2$, for 0.5, 1, 2, 4, 8 and 16 min, respectively. RT-PCR amplified a 90bp of PEDV membrane protein (M) gene region(337-426) (Table. 1) and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. To evaluate effects of UV induced RNA damage of PEDV, regression normalization(a linear trendline correction) was used.

**Table 1. Oligonucleotide primer used test**

| Primer | Primer Seq. | Product Size |
|---|---|---|
| **PEDV-f** | 5`AATCCTGAAACTGACGCGCT3` | 90bp |
| **PEDV-r** | 5`TAGCGTTACACCAGTTGGGTC3` | |

## Results

The inactivation ratio of PEDV was showed relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999) (Fig. 1).

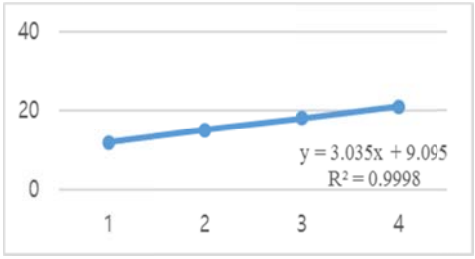


Fig. 1. Linear trendline correction of standard curve of serially diluted control samples. Y axis is Ct value and X acis is –Log (dilution ratio)-3.

The inactivation of PEDV by UV-C light was showed dose dependent (Table. 2).

Table 2. The results of Ct value and inactivation ratio of PEDV by exposed time using ReTi-PCR

| Exposure Time (min) | Ct value | Inactivation ratio (%) |
|---|---|---|
| **0.5** | 18.24 | 89.89 |
| **1** | 18.57 | 92.13 |
| **2** | 19.22 | 95.19 |
| **4** | 21.45 | 99.11 |
| **8** | 22.04 | 99.43 |
| **16** | 22.90 | 99.71 |

## Discussion

The UV-C light was highly effective to inactivation of PEDV. But substantial viral inactivation occurred after exposure of 4 min. Increasing wavelength output of the UV lamp can be an effective way to reduce the time of exposure. The data reported in this experiment suggest that PEDV is killed by UVC light when it has absorbed the required amount of radiant energy in the lethal range. It is thought that these results can be a useful data for sterilizing PEDV.

## Reference

[1] Ayman AM. et al. 2008. New Microbiologica 31; 47-55
[2] Dea S. et al. 2000. Arch Virol 145; 659-688

# Quantification of lethal effects against porcine respiratory and reproductive syndrome virus(PRRSV) using UV-C irradiation

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## Introduction

Porcine respiratory and reproductive syndrome virus (PRRSV) as RNA virus causes devastating swine diseases with massive economic losses to the swine industry worldwide. In order to prevent economic losses from PRRS, many swine producers use UV-C light as a sterilizer for the workers, equipment, surface of farm units etc. The aim of the experiment was to measure levels of UV-C induced RNA damage by utilizing the property that damaged RNA of PRRSV can inhibit PCR.

## Materials and methods

PRRSV strain, ATCC VR2332, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150μW•sec/$Cm^2$, for 0.5, 1, 2, 4, 8, 16, and 32 min, respectively. RT-PCR amplified a 100bp region (1154-1253) (Table. 1) and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), Using regression normalization (a linear trendline correction).

**Table 1. Oligonucleotide primers used test**

| Primer | Primer Seq. | Product Size |
|---|---|---|
| **PRRSV-f** | 5'ACGGACCTATCGTCGTACAG3' | 100bp |
| **PRRSV-r** | 5'AGGAGGTCCTCAAACCCAGA3' | |

## Results

The inactivation ratio of PRRSV was showed relatively quantified results from ReTi-PCR. The Ct-value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999). The inactivation of PRRSV by UV-C light was dose-dependent (Table 2). Table 3 showed inactivation ratio of PRRSV using a linear trendline correction.

**Table 2. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR**

| Exposure Time (min) | Ct value | Inactivation ratio(%) |
|---|---|---|
| 0.5 | 23.92 | 61.68 |
| 1 | 25,5 | 86.9 |
| 2 | 26.54 | 93.53 |
| 4 | 28.14 | 97.82 |
| 8 | 29.31 | 99.01 |
| 16 | 29.86 | 99.32 |
| 32 | 31.43 | 99.77 |

**Table 3. Calculated inactivation ratio of PRRSV**

| Virus | 1D* | 2D | 3D |
|---|---|---|---|
| PRRSV | 10.72 | 72 | 638.2 |

*D=1$log_{10}$, unit is mJ/$cm^2$ (Erwin *et al*., 2004)
* Inactivation ratio
1D=95.0% , 2D= 99.0%, 3D=99.9%

## Discussion

In Table 2&3, PRRSV was sensitive to UV light irradiation. The UV-C light is very effective and environment friendly for disinfection in pig farm units. It is thought that these results can be a useful data for sterilizing of PRRSV.

## Reference

[1] Scott D. et al. 2011. J Vetmic 150; 96-99
[2] Brouwer J. et al. 1994. Vet Q 16; 95-100

# Quantification of lethal effects against classical swine fever virus (CSFV) by UV-C irradiation

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## Introduction

Classical swine fever virus(CSFV) as RNA virus is a highly contagious disease of pigs. It is classified as List A-grade disease determined by OIE. Because of its extremely high mortality with severe symptoms, UV light is used by many pork producers. However, it is very little known about actual effects of UV-C induced RNA damage. The aim of this experiment was to measure levels of UV-C induced RNA damage of CSFV.

## Materials and methods

CSFV, LOM strain, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV-C lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150μW•sec/cm$^2$, for 1, 2 and 4 min, respectively. RT-PCR amplified a 99bp region(7824-7922), oligonucleotides CSFV-f (5'ACT ATC AAG GAA AAA GCC AAA CAG3') and CSFV-r (5'CGA ACA AGG GGG TCA GGT3'), and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), using regression normalization (a linear trend line correction).

## Results

The inactivation ratio of CSFV was showed relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999) (Fig. 1). The inactivation of CSFV by UV-C light exposure time was dose-dependent(Table1&2).

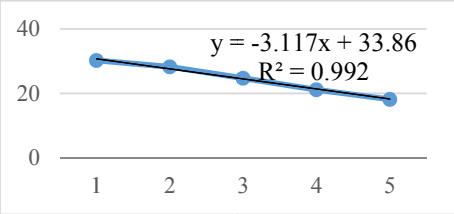


Fig. 1. Standard curv of serially diluted control samples Y axis is Ct value and X axis is Log(dilution ratio)+8.

| Exposure Time (min) | Ct value | Inactivation ratio (%) |
|---|---|---|
| **0** | 25.74938 | 0 |
| **1** | 26.5385 | 44.17158 |
| **2** | 27.25156 | 67.03028 |
| **4** | 30.4959 | 96.99835 |

**Table 1. The results of Ct value and inactivation ratio of CSFV by exposed time using ReTi-PCR**

**Table 2. Calculated inactivation ratio of CSFV**

| Virus | 1D* | 2D | 3D |
|---|---|---|---|
| CSF | 23.89894 | 101.3937 | 430.1734 |

*D=1log$_{10}$, unit is mJ/cm$^2$ (Erwin *et al*., 2004).
*Inactivation ratio:
1D=95.0% , 2D=99.0%, 3D=99.9%.

## Discussion

The UV-C light is effective to inactivate CSFV. As described in Table 1, it is required the longer than 4min to certainly inactivate CSFV. The UV light is convenient to use, as well as environment-friendly for disinfection of CSFV in pig farm units. These data can be thought to be useful for sterilizing of CSFV.

## Reference

[1] Hoffmann B et al. 2005. J.Virol.Methods 130; 36-44
[2] Erwin D et al. 2004. Appl Environ Microbiol 70; 4538-4543

# The effects of newPED-X® on suckling pigs against PEDV infection

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## Introduction

Porcine epidemic diarrhea (PED) had been recognized as only a sporadic disease for many years in South Korea. However, recently as of late 2013, the virus explosively spread nation-wide and contributed to the tremendous economic losses all over the country. The virus, which may have been introduced from the US [1], is highly pathogenic and genetically differs from pre-existing PEDV strains in Korea and this may be the reason PED vaccines currently available in the domestic market are less effective. We have recently developed a latest US-originated, inactivated PED vaccine (newPED-X), and in this study, we analyzed its protective efficacy by challenging piglets following sow vaccination.

## Materials and methods

**Groups and vaccinations:** 5 sows that were expected to give birth at the same day were chosen from a PEDV-free farm. At the farm, 3 sows (vaccinated groups; sow1, 2, and 3) were vaccinated with 2ml of newPED-X at 5 and 2 weeks before parturition and the others (control groups; sow4 and 5) were un-vaccinated.

**Housing:** After parturition, 5 sows and their 3 day-old piglets (8 piglets per sow) were moved to a experimental facility and allocated into 5 different pens.

**Challenges:** Each 8 suckling pigs were challenged with US-strain PEDV at 5 days of age and their daily gains and clinical scores were recorded for later assessment.

## Result

**Average rate of gain (ARG, %)**: The ARG of piglets in the vaccinated group turned positive from 7 DPI (Fig. 1), however the control group remained negative until the last pigs died (the numbers above or below the error bars indicate the number of death at corresponding DPI)

**Clinical significance score (CSS):** Starting from 2 DPI, CSS of the control group stayed significantly higher (Fig. 2) than of the vaccination group; the scores of dead piglets (score 4) were calculated into CSS only once at the time (DPI) of death.

***Figure 1.*** *Average Daily Rate of gain(%)*

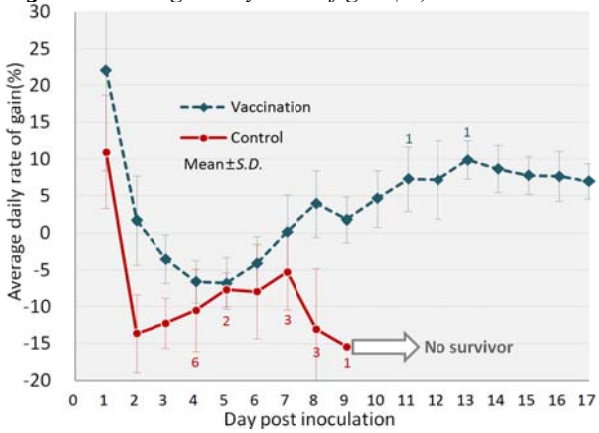
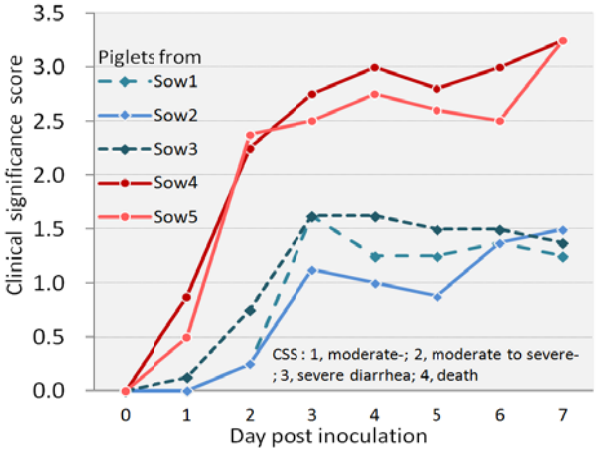



***Figure 2.*** *CSS after PEDV challenge*




## Discussion

The results show that the newPED-X vaccine dramati-cally reduces clinical signs and improves daily gains in PEDV infected suckling pigs. This suggests that the vaccine can protect piglets very effectively against US-strain PEDV infection.

## Reference

[1] Lee, S.H. et al., Emerging Infectious Diseases, Vol.20, No.7. 1223-1226

# PRRS virus situation of Korea ARC area

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## Introduction

PRRS is still a disease that have the greatest economic losses in the swine industry. PRRS ARC is a good tool to control the PRRS in the region. From 2013 until 2014 in Korea, ther are three regional PRRS ARC team(Pochon, Habcheon, Masan). We evaluate the map of each area before and after the PRRS status.

## Material and Method

Mapping is based on the PCR and serological test (IDEXX PRRS 3XR kit). Blood samples were collected 30 samples at the beginning of the ARC research activities, additional sampling was done every six months. The test was done by Optipharm for serological test and PCR and sequencing were done by QIA.

Classification was followed by KR standard as below Table1.

Table 2. New classification of PRRS status of sow herds

| Classification | Markers of farms |
|---|---|
| **Unknown:** Unknown infection status of PRRS.Farm inspection had not, or do not know the results. | ■ (black) |
| **Positive(unstable):** PRRS infection symptoms. From PRRS virus PCR results.Produced positive piglets. | ■ (red) |
| **Positive(stable):** Have no symptoms from the breeding herd. Negative-weanrs production. 90 days the last point the virus has vanished from postweaning. *40 days old or 60 days old pigs all -ve on ELISA test | ■ (green) |
| Negative: All pigs were ELISA and PCR negative. | ■ (blue) |

* in Korea

## Result

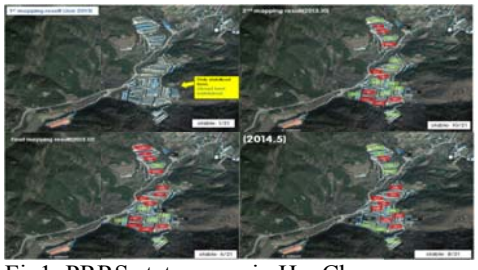


Fig1. PRRS status map in HapCheon area

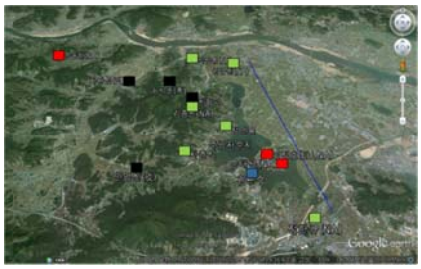

Fig2. PRRS status map in ChangWon area.

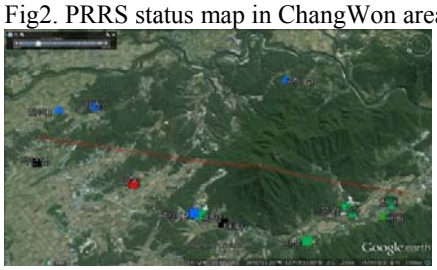

Fig3. PRRS status map in PoCheon area.

## Discussion

The PRRS status map of three area showed that the risk of introduction of PRRS virus from their neighbor farm. There are several PRRS negative farms which were located nearby PRRS positive unstable farm.

More than 70% area is mountain in Korea. The area where pig farms are located is mountain and very nearby mountain. So it seems there are some factors wind direction, wind velocity, and load location.

We hope to study more about how mountain influence PRRS spreading between farms in Korea.

## Reference

1. REPORT OF THE OIE AD HOC GROUP ON PORCINE REPRODUCTIVE RESPIRATORY SYNDROME Paris, 9 - 11 June 2008
2. APVS2013 Vietnam. Application of SEBS (site evaluation of biosecurity system) for PRRS ARC in Korea
3. APVS2013 Vietnam. PRRS ARC process in HapCheon (Danji: High pig dense area)
4. APVS2013 Vietnam. New approach for PRRS status classification
5. APVS2013 Vietnam. Control strategies of bacterial pathogens in Danji (high pig dense area)
6. APVS2013 Vietnam. PRRS virus circulation within Danji (high-pig dense area)

# Quantification of Foot-and-mouth disease killed vaccine using Real-time PCR

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## Introduction

Foot-and-mouth disease (FMD) is a highly infectious viral disease causing enormous economic losses in livestock industry and vaccination is generally considered as a best strategy for the prevention of FMD. Commercial FMD vaccine available in South Korea indicates their antigen contents by 50% protective dose method (PD50). However, the unit calculated by vaccine potency tests is relative concept, because the vaccine strain of FMD exhibits various protective potencies depending on FMD viral strains. Therefore, developing methods for the quantification of vaccine antigen contents is necessary to comparing between different FMD vaccines. In this experiment, several quantification methods for virus contents in vaccine are evaluated on the basis of real-time PCR amplification method.

## Materials and methods

Vaccine A, commercially available in South Korea and vaccine B were used for the comparison. All the vaccines are inactivated vaccine, produced in double oil emulsion formulation. For the phase separation of the vaccine solution into oil and water phase, instability inducing methods such as freezing-thawing method and other organic solvent treatment were used for sample preparation.[1] Vaccines were frozen at -30℃ and -70℃ for 3 hours and subsequently thawed at room temperature for 2 hours. Isopropyl alcohol and ethyl alcohol are mixed with vaccine for phase separation. After separation, viral RNA was extracted (Viral gene spin, Intron, Korea) in water and oil phase. cDNA was synthesized (SuPrimeScript RT premix, GeNet Bio, Korea) and real-time PCR (Chromo 4, Bio-rad, USA) was conducted.

## Result

Vaccine A oil phase frozen in -70℃ and vaccine B water phase frozen in -70℃ exhibit higher efficiency than other methods. (Table. 1)

***Table 1. Ct value and PCR amplification efficiency***

| Vaccine | Method | | C(t) | Efficiency |
|---|---|---|---|---|
| A | Refrigerator | | 12.54 | 58.05 % |
| | -30℃ | Oil | 14.16 | 57.40 % |
| | | Water | 15.05 | 63.72 % |
| | -70℃ | Oil | 14.81 | 85.56 % |
| | | Water | 17.14 | 71.91 % |
| | Isopropyl alcohol | Oil | 16.54 | 48.32 % |
| | | Water | 13.77 | 71.00 % |
| | ethyl alcohol | Oil | 17.68 | 75.39 % |
| | | Water | 15.69 | 69.32 % |
| B | Refrigerator | | 14.28 | 61.06 % |
| | -30℃ | Oil | 17.71 | 60.81 % |
| | | Water | 16.24 | 52.02 % |
| | -70℃ | Oil | 21.76 | 49.66 % |
| | | Water | 14.11 | 90.40 % |
| | Isopropyl alcohol | Oil | 28.07 | 37.82 % |
| | | Water | 21.68 | 62.33 % |
| | ethyl alcohol | Oil | 29.82 | 38.26 % |
| | | Water | 23.00 | 48.80 % |

## Discussion

Real time PCR amplification was efficiently used for the relative quantification of FMDV antigen in killed vaccine. Considering the fact that standard curve must be based on the amplification efficiency of 90 % to 110 % for the exact quantification. Using freezing-thawing method at -70℃ is considered to be the best approach for the recovery of the FMDV viral genome for the real-time PCR.

## Reference

[1]Rojas E. C. et al. 2007. Langmuir. Jun 19;23(13):6911-7.

# The comparison of two different Foot-and-Mouth disease vaccine efficacy in South Korea

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## Introduction

Foot-and-Mouth disease (FMD) is ongoing problem since first outbreak in 2000. Vaccination has been used for the control of FMD in Korea since 2010/2011 outbreak. There was only one commercially available FMD vaccine (A) comprising 3 different inactivated virus strains (O1 manisa, A Iran 05, Asia1) has been released in the filed. In this study, the efficacy of the new FMD vaccine provided by B manufacturer was evaluated by comparison with that of the existing FMD vaccine A.

## Materials and methods

Thirty of 8 weeks old pigs were tested in the farm condition, six of 8 weeks old pigs were tested in the laboratory condition, respectively. In field condition, twenty piglets were vaccinated with vaccine A and the others were vaccinated vaccine B. Otherwise, all of six piglets were vaccinated with vaccine B in laboratory condition. They were injected vaccine through I.M route in about 40mm behind of the ear. Blood samples were taken before and after vaccination in both conditions. Blood sampling after vaccination was done every 2 weeks for 6weeks in field condition and every 5 days for 40days in laboratory condition. Quantitative real-time PCR (qPCR) of both vaccine A and B was used for the estimation of antigen concentration [1]. The titer of antibody against FMDV in the collected blood samples were measured by anti-FMD O type antibody ELISA Kit. Percentage inhibition (PI) value was calculated according to manufacturer's manual and regarded as positive when it is over 50.

## Result

The C(t) value of quantitative PCR for vaccine B antigen is a little bit higher than that of A (*Table.1*). The positive rate and PI value between vaccine A and B showed difference when the antibody titer reached at peak. In pigs vaccinated with vaccine B PI value and positive rate showed the highest value at 3~4weeks post vaccination and thereafter decreased gradually. Otherwise, the positive rate of vaccine A vaccination pigs steadily increased until 6weeks after vaccination. (*Fig.1*)

***Table.1 The C(t)value was obtained by qPCR using FMDV killed vaccines***

| | A | B |
|---|---|---|
| C(t) value | 12.21 | 17.48 |

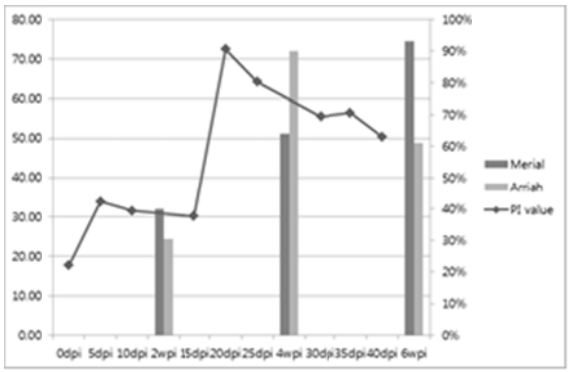


*Figure .1 PI value and positive rate.*

## Discussion

The differences were detected between vaccine A and B in the result of qPCR and ELISA test.
It can be assumed to be different antigenic volume in vaccines through qPCR result. And ELISA test results show difference in immune response. Many factors, such as vaccine formulation, manufacturing process or shipping and storage condition, can be influenced vaccination efficacy in field and actual variation result was observed in this study.

## Reference

[1] Niedbalski, W. Kesy, A., Bull Vet Inst Pulawy 54, 3-7, 2010.

**Analysis of the correlation between SN test and ELISA responses after vaccination with an inactivated EU-typed PRRS vaccine**

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory disease in young pigs. In general, most neonatal infections can be prevented by passive lactogenic immunity obtained from the sow. The objective of this study was to compare two serological assessments, serum neutralization (SN) test and enzyme-linked immunosorbent assay (ELISA) in sows and their piglets after vaccination with an inactivated EU-typed PRRS vaccine, under Korean field conditions.

## Materials and methods

The study was carried out in an EU-typed PRRSV-positive 420-sow farm located in Dang-jin city, South Korea. Eight randomly chosen sows were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titres of all sera were analyzed using an indirect ELISA[1] (IDEXX Laboratories. Inc., Westbrook, USA). SN test was implemented according to Jusa[2] with PRRSV Lelystad strain, and MARC-145 cells. Data analyses were performed with SPSS statistics 21 (IBM Corp., USA) and Excel 2013 (Microsoft, USA).

## Results

Results are shown in Table 1 and Fig. 1&2.

Table 1. Coefficient of correlation between ELISA and SN test.

| Day (sow) | -63[a] | -42 | 0 | 26 |
|---|---|---|---|---|
| Coefficient of correlation | 0.262 | 0.517 | 0.810 | 0.371 |
| Day (piglet) | 7 | 14 | 26[b] | |
| Coefficient of correlation | 0.384 | 0.345 | 0.247 | |

a: Day -63 (1st vaccination), Day -42 (2nd vaccination), Day 0 (parturition) and Day 26 (weaning).
b: Day 26 (weaning)

The results of ELISA and SN test in sows looked more correlated to each other on the farrowing day.

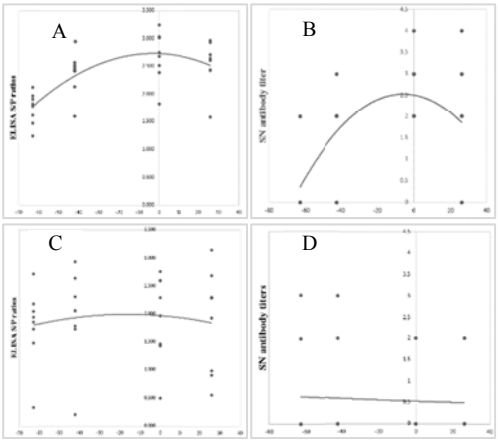


Figure 1. ELISA (A), (C) and the SN test (B), (D) antibody responses in V and NV sows. The dots show distribution of serologic responses, and the line on each graph represents the trend curve fitting.

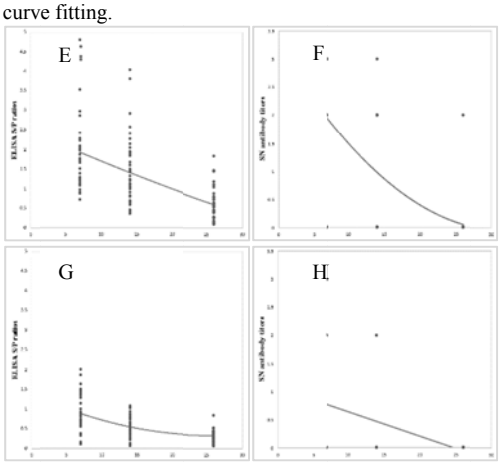


Figure 2. ELISA (E), (G) and SN test (F), (H) antibody titres of piglets born from V and NV sows. Dots and lines: see Fig. 1.

## Discussion

Correlation between the two tests appeared rather low, except for sows on the farrowing day. This may be due to the fact that ELISA results are expressed in S/P ratios although SN test responses are expressed in Log titres. Despite this lack of linear correlation, the calculated lines representing the fitting curves seem to follow the same trends.

## Reference

[1]Yoon K.J., J. Vet. Diagn. Invest., 1995, 7: 305-312
[2]Jusa E.R., J. Vet. Med. Sci., 1996, 58: 749-753

**ELISA antibody response after vaccination with an inactivated EU-typed PRRS vaccine in a Korean farm**

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a RNA virus that causes devastating swine diseases with massive economic losses to the swine industry worldwide. It causes reproductive failure in sows and respiratory tract disease in young pigs. The objective of this study was to assess the serological response in sows after vaccination with an inactivated EU-typed PRRS vaccine, and the transfer of antibodies in their piglets by enzyme-linked immunosorbent assay (ELISA) under Korean field conditions.

## Materials and methods

The study was carried out in an EU-typed PRRSV positive 420-sow farm located in Dang-jin city, South Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titres were analyzed in all sera using an indirect ELISA[1, 2] (IDEXX Laboratories. Inc., Westbrook, USA). T-test and Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) were used for statistical significance.

Results

Table 1. Average PRRSV-specific ELISA antibody titres of sows and piglets

| Sows | Day -63[a] | Day -42 | Day 0 | Day 26 |
|---|---|---|---|---|
| V | 1.74[b] (±0.28[c]) | 2.38 (±0.36) | 2.68 (±0.31) | 2.53 (±0.30) |
| C | 1.78 (±0.47) | 1.96 (±0.57) | 1.95 (±0.54) | 1.84 (±0.64) |
| *P* value | 0.872 | 0.213 | 0.037 | 0.086 |

| Piglets | Day 7[d] | Day 14 | Day 26 |
|---|---|---|---|
| V | 1.92(±0.33) | 1.40(±0.27) | 0.60(±0.13) |
| C | 0.82(±0.14) | 0.51(±0.08) | 0.31(±0.05) |
| *P* value | 0.000 | 0.000 | 0.000 |

a: Day of sow blood sampling (1st vaccination, 2nd vaccination, farrowing and weaning).
b: Average S/P ratio of each group.
c: Confidence interval(CI) in confidence level(CL) of 95%.
d: Day of piglet blood sampling (1-week-old, 2-week-old and weaning).

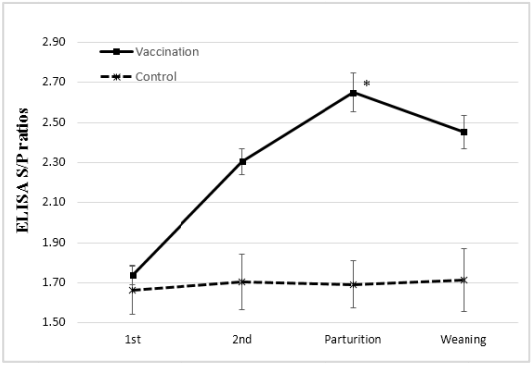


Figure 1. PRRSV-ELISA antibody titres in V and NV sows

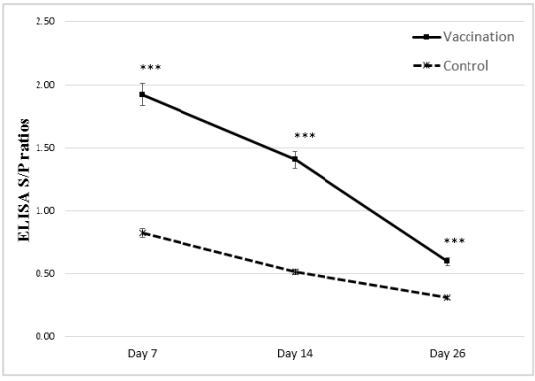


Figure 2. PRRSV-ELISA antibody titres in piglets born from V and NV sows

## Discussion

Antibody titres in PROGRESSIS vaccinated sows clearly increased and were statistically significantly higher than those of the control group at farrowing. Although antibody titres of piglets born from vaccinated and control sows decreased over the experimental period, there were significantly higher antibody titres in piglets born from vaccinated dams at each sampling time. There was a good transfer of ELISA antibodies to piglets born from vaccinated sows.

## Reference

**[1]** Albina E, Ann. Rech. Vet. 1992, 23: 167-176
[2] Yoon K.J., J. Vet. Diagn. Invest. 1995, 7: 305-312

# Serum neutralization (SN) antibody response in sows and transfer to piglets after sow vaccination with an inactivated EU-typed PRRS vaccine in a Korean farm

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disease in young pigs. In general, most neonatal infections can be prevented by the right passive colostrum and lactogenic immunity from the sow. The aim of this study was to assess the sow sero-neutralizing response and the antibody transfer to piglets after sow vaccination with an inactivated EU-typed PRRS vaccine, in Korean field conditions.

## Materials and methods

The study was carried out in a 600-sow EU-typed PRRSV negative farm located in Bo-ryeong city, South Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. The Lelystad PRRSV strain and MARC-145 monolayer cells cultures were used to run the SN test[1,2]. Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) was used for statistical significance.

## Results

Table 1. Results of average PRRSV-specific antibody titres of the sows and piglets by SN test.

| Sows | Day -63[a] | Day -42 | Day 0 | Day 26 |
|---|---|---|---|---|
| V | 0[b] (±0[c]) | 1.13 (±0.81) | 2.63 (±0.49) | 1.88 (±0.81) |
| C | 0.25 (±0.46) | 0 (±0) | 0.25 (±0.46) | 0 (±0) |
| *P* value | 0.167 | 0.105 | 0.001 | 0.010 |
| Piglet | Day 7[a] | Day 14 | Day 26 | |
| V | 2.62[b](±0.26[c]) | 1.59(±0.36) | 0.32(±0.24) | |
| C | 0.32(±0.24) | 0(±0) | 0(±0) | |
| *P* value | 0.000 | 0.000 | 0.011 | |

a: Day of sow blood sampling (1st vaccination, 2nd vaccination, farrowing and weaning).
b: Average $\log_2$ neutralizing antibody titre of each group.
c: Confidence interval(CI) in confidence level(CL) of 95%.
d: Day of piglet blood sampling(1-wk-old, 2-wk-old and weaning).

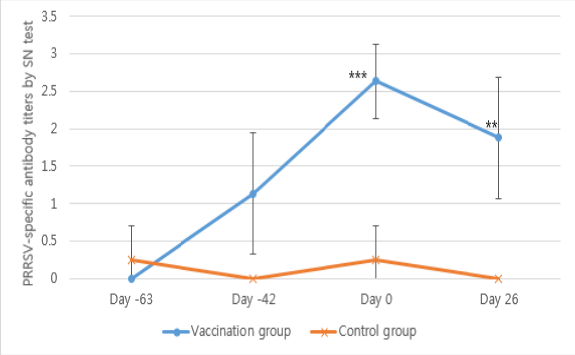


Figure 1. PRRSV-SN antibody titres of V and NV sows

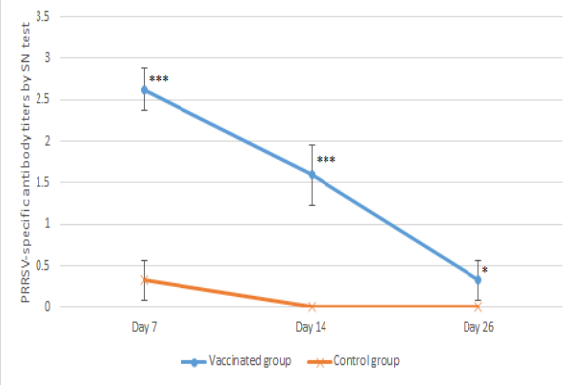


Figure 2. PRRSV-SN antibody titres of piglets from V and NV sows

## Discussion

SN titres of NV sows remain negative shows that there was not any contamination during the trial period. The SN titres of V sows clearly increased after two shots of vaccine and were significantly higher (D0, D26). The SN titres of piglets of the V group were significantly higher from control group at all stage of this study. It suggests maternal SN PRRS antibodies from sows were well intaken by their piglets. These results show that sow vaccination with PROGRESSIS improves the sows SN humoral immunity and can help to prevent infections during pregnancy and during the suckling period in baby piglets.

## Reference

[1] Jusa E.R., J. Vet. Med. Sci. 1996, 58: 749-753
[2] Yoon I.J., J. Vet. Diagn. Invest. 1994, 6: 289-292

# Comparison between antibody titres after vaccination with an European-type porcine reproductive and respiratory syndrome (EU-typed PRRS) vaccine using ELISA and serum neutralization (SN) test

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) causes a lot of economic losses in the swine industry. PRRS virus causes reproductive failure in sows and respiratory tract disease in young pigs. PRRS antibody in serum can be detected by ELISA, but ELISA antibodies do not mean ability of the pig to defend against PRRSV. So other assays should be run to evaluate the ability of the pig to defend himself against PRRSV. The objective of this study was to compare the results of PRRS antibody evaluation using an ELISA[1], a SN test[2] and an IFN-γ ELISA under field conditions in Korea.

## Materials and methods

The study was carried out in an EU-typed PRRSV negative 600-sow farm located in Bo-ryeong city, South Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titres of all sera were analyzed using an indirect ELISA[1] (IDEXX Laboratories. Inc., Westbrook, USA) and an SN test[2], with PRRSV Lelystad strain, and MARC-145 cells. A commercial IFN-γ ELISA kit (Quantikine® porcine IFN-γ ELISA, R&D Systems, Inc., USA) was used for IFN-γ titration in all the serum.

## Results

The correlation coefficients between SN titres, ELISA S/P ratios and IFN-γ dosage are shown in Table 1 for sows and in Table 2 for piglets. Moderate correlation is shown between SN test and ELISA in sows and piglets. Low correlation is shown between SN test and IFN-γ in sows and piglets. A slightly higher correlation was found between ELISA and IFN-γ in sows but a low correlation was shown in piglets.

Table 1. Coefficient of correlation between ELISA, SN test and IFN-γ in sows

| Sows | Coefficient of correlation(R) |
|---|---|
| SN test – ELISA | 0.527 |
| SN test - IFN-γ | 0.337 |
| ELISA – IFN-γ | 0.677 |

Table 2. Coefficient of correlation between ELISA, SN test and IFN-γ in piglets

| Piglets | Coefficient of correlation(R) |
|---|---|
| SN test – ELISA | 0.536 |
| SN test - IFN-γ | 0.331 |
| ELISA – IFN-γ | 0.242 |

## Discussion

In this study, correlation between ELISA and SN test was moderate. IFN-γ ELISA showed low correlation with other methods. SN antibodies titres and IFN-γ are linked to the protection of pigs against PRRS but after infection, it has been shown[3], along with ELISA titres, that these three markers of immunity have different behaviours and kinetics consequence of their quite different mechanisms of induction. The rather weak correlation between ELISA, SN antibodies and IFN-γ, may be explained by different kinetics of induction during the period of sampling covering the establishment of a complex immune response early after the first contact with the vaccine in naïve animals.

## Reference

[1] Albina, E., Ann. Rech. Vet. 23, 167-176
[2] Jusa Enuh Raharjo, J. Vet. Med. Sci. 1996 ; 58(8), 749-753
[3] Lopez O.J. and Osorio F.A. 2004, Vet Immunol Immunopath, 102:155-163

# ELISA response after vaccination with an inactivated European-type porcine reproductive and respiratory syndrome (PRRS) vaccine in negative animals, under Korean conditions

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## Introduction

Porcine reproductive and respiratory syndrome (PRRS) causes reproductive failure in sows and respiratory tract disease in young pigs. The objective of this study was to assess the ELISA response after a sow vaccination with an inactivated EU-typed PRRS vaccine and the transfer of ELISA antibodies to their progeny, in field Korean conditions.

## Materials and methods

The study was carried out in an EU-typed PRRSV negative 600-sow farm located in Bo-ryeong city South-Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titers of all sera were analyzed using an indirect ELISA[1, 2] (IDEXX Laboratories. Inc., Westbrook, USA). T-test and Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) were used for statistical significance.

## Results

The results of antibody titers of sows are shown in Table 1 and Figure 1 and that of piglets are shown in Table 2 and Figure 2.

Table 1. Result of average PRRSV-specific antibody titers of sows and piglets by ELISA

| Sows | Day -63[a] | Day -42 | Day 0 | Day 26 |
|---|---|---|---|---|
| V | 0.12[b] (±0.08[c]) | 0.67 (±0.46) | 0.81 (±0.56) | 0.75 (±0.52) |
| C | 0.16 (±0.12) | 0.17 (±0.12) | 0.17 (±0.12) | 0.19 (±0.13) |
| *P* value | 0.382 | 0.009 | 0.005 | 0.000 |

| Piglets | Day 7[d] | Day 14 | Day 26 |
|---|---|---|---|
| V | 1.08(±0.21) | 1.01(±0.20) | 0.60(±0.10) |
| C | 0.20(±0.03) | 0.22(±0.04) | 0.19(±0.04) |
| *P* value | 0.000 | 0.000 | 0.000 |

a: Time of sows' blood sampling (1st vaccination, 2nd vaccination, parturition and weaning).
b: Average S/P ratio of each group.
c: Confidence interval(CI) in confidence level(CL) of 95%.
d: Time of piglets' blood sampling (1-wk-old, 2-week-old and weaning).

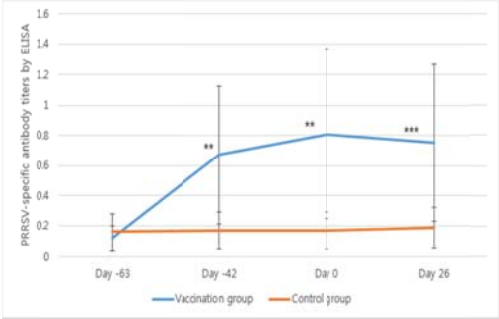


Figure 1. PRRSV-specific antibody titers of V and NV sows

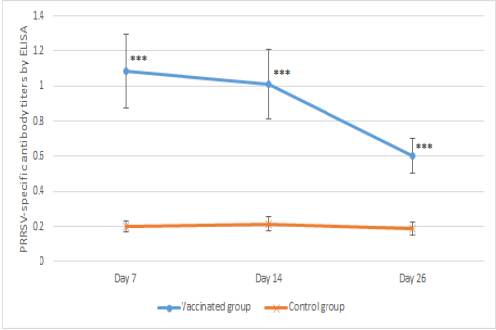


Figure 2. ELISA PRRSV-specific antibody titers of piglets from V and NV sows

## Discussion and conclusion

ELISA titers of vaccinated sows significantly increased although the ones of the control sows remained low (indicating no field contamination during the observation period). ELISA titers of piglets of the vaccinated group were significantly higher than those of the control group at every sampling point and decreased over time as expected.

In this study, pre-farrowing sow vaccination with PROGRESSIS was shown to induce high level of ELISA antibodies in PRRS negative sows, that are well transferred to their piglets.

## Reference

[1] Yoon, K. J., J. Vet. Diagn. Invest. 7, 305-312

[2] Albina, E., Ann. Reah. Vet. 23, 167-176

# The results of type I PRRS vaccination (Unistrain®, Hipra) for sow herd

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## Introduction

Type I PRRS infected cases are quite popular in Korea nowadays. Major clinical signs of Type I PRRS are low pregnant rate and hypogalactia /agalactia in Korea. We applied type I PRRS live vaccine to sow herd which was infected wild type I PRRS virus and monitored pregnant rate(%).

## Materials and methods

We selected the PRRS-unstable farm which has 1,700 sows in Korea. This farm was PRRS-unstable and we applied herd closure and type II PRRS vaccine from Jan. to Dec. 2013. As a result this farm got PRRS-stabilized. But, this farm was infected by type I PRRSV on May 2014. And we applied type I PRRS vaccine(Unistrain®, Hipra) to sow herd without herd closure because this farm was finished herd closure just before 5 months ago. Mass vaccination was applied at every three months.
The data about pregnant rate was analyzed.

*Figure 1 Clinical signs at farrowing pen*

## Result

After infection of type I PRRSV on May 2014, the mean pregnant rate was decreased as 88.1%. And we applied type I PRRS vaccine(Unistrain®, Hipra) during 10 months from May. 2014 to Feb. 2015 without herd closure. We checked that this farm was PRRS-stable on Mar. 2015. After PRRS stabilization, mean pregnant rate was 93.2% during 3 months.

The pregnant rate(%) are as below.

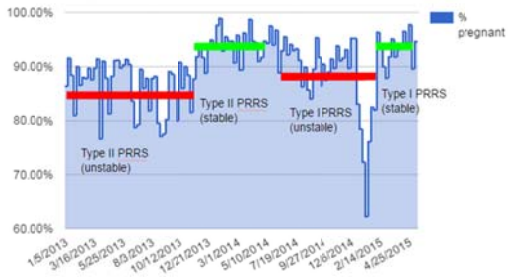
*Table.1 The pregnant rate(%)*

| PRRS status | Unstable[1)] | Stable[2)] | Unstable[3)] | Stable[4)] |
|---|---|---|---|---|
| Period | Jan2013 ~Dec2013 | Jan2014~Apr2014 | May2014~Feb2015 | Mar2015~May2015 |
| Control measure | Type II vax With herd closure | Type II vax | Type I vax Without herd closure | Type I vax |
| Pregnant rate(%) | 86.6 | 94.2 | 88.1 | 93.2 |

*Figure 2 Pregnant rate(%) of this herd*

## Discussion

In this farm, since type I PRRS virus(wild type) was introduced just 5 months after finishing herd closure, we applied the type I PRRS vaccine(Unistrain®,




Hipra) without herd closure. As a result, the sow herd produced PRRS –ve weaners and the pregnant rate(%) recovered since Mar. 2015. Therefore, the results show us that type I PRRS vaccination without herd closure can be a option for recovering the pregnant rate(%) to normal production level.

## Reference

1. Scott Dee, et al. (1996) Swine Health and Production, Control of porcine reproductive and respiratory syndrome (PRRS) virus

**Comparison of pathological features of F18 encoding enterotoxigenc *E. coli* and enterotoxigenic *E. coli*/shigatoxin producing *E. coli* in orally infected post-weaned pigs**

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**Introduction** : *E. coli* strains encoding F18 fimbrial gene are major sources of edema disease (ED) and post-weaning diarrhea (PWD) in pigs. Generally, shigatoxin-producing *E.coli* (STEC) expressing F18ab fimbriae is associated with ED whereas enterotoxigenic *E.coli* (ETEC) that expresses F18ac fimbriae is associated with PWD. However, virulence trait and pathogenesis of ETEC/STEC that expresses F18 fimbriae are questionable. Therefore, the aim of this study is to identify the pathological features of ETEC and ETEC/STEC encoding F18ab and F18ac gene in post-weaned pigs.

**Materials and Methods** : Three-week-old fifteen pigs were divided into 3 group: G1 (F18ab variant), G2 (F18ac variant) and control (G3). Pigs of the G1 and the G2 were orally inoculated with $10^{10}$colony forming units (CFU) of each subtype of $F18^{+}$ strain. General health status and feces were checked daily and occurrence of F18 subtype was evaluated by real-time PCR. All pigs were euthanized and necropsy was performed. Organs were fixed with 10% neutral buffered formalin, embedded, stained with hematoxylin and eosin for histopathology. Electron microscopy and TUNEL assay were also performed to examine the pathological changes in the blood vessels.

**Results** : Survival rate was higher ($p < 0.01$) in the G2 than the G1. Body weight losses and diarrhea occurred in G1 and G2 pigs. In fecal score, G1 was higher in G1, G2 rather than G3. Histopathologically, the lesions were mainly observed in the small intestine, compared to other organs. In the G1, necrosis with haemorrhage was also detected in the mucosa in jejunum and ileum. Nuclear fragmentation in tunica media of arteriole in ileum was observed in G1. The mucosa and epithelia in G2 appeared intact. By the electron microscopy, *E. coli* were closely attached on the microvilli of the small intestine in G1 and G2.

**Conclusions :** On the basis of clinical signs and pathological changes, the results of this study suggest that F18ab is associated with edema but F18ac ETEC/STEC cause diarrhea in post-weaned pigs. It is suggested that virulence trait of the F18ac encoding STEC/ETEC strain used in this study is similar to ETEC rather than STEC.

**References :**

1. **Nagy B, Fekete PZ.** Enterotoxigenic *E.coli* in farm animals. Vet Res 1999, 30(2-3): 259-284.
2. **Smith HW, Halls S.** The production of oedema disease and diarrhea in weaned pigs by the oral administration of *Escherichia coli*: factors that influence the course of the experimental disease. J Med Microbiol 1968, 1(1): 45-59.

**Corresponding author:* Jae-Won Byun *D.V.M., Ph.D., E-mail: jaewon8911@korea.kr*

# Detection of *Brachyspira* spp.and pathologic lesions in the farm

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*[2]Research planning division, Animal and Plant Quarantine Agency, Anyang, Gyeonggi-do, Korea*

**Introduction;** Swine dysentery (SD) caused by *Brachyspira (B.) hyodysenteriae* is a severe mucohemorrhagic enteric disorder causing mortality, lowering weight gain and economic losses [1]. Although, SD is the main problem in the farms, several species have been associated with diarrhea in growing and finisher pigs. Thus, the aim in this study was to investigate the frequency of *Brachyspira spp.* in continuous farms and pathologic changes in affected pigs.

**Materials and Methods;** The 100 fecal swabs showing cement-like appearance were obtained according to age which was divided into post-weaned (30 to 50 days), grower (60 to 90days), and finisher (above 100 days). Necropsy was performed in the pigs suspected in brachyspiral infection and all organs including colon were routinely processed following the standard method. Bacterial isolation was done by anaerobic culture using blood and selective agar (BAM-SR) at 37ºC for 72 hr. The colony showing hemolysis was transferred into blood agar. The DNA was extracted using QIAamp DNA stool kit (Qiagen) for swab and boiling for colony, respectively. PCR was performed to amplify the NADH oxidase (*nox*) gene [2]. Twenty microliters of reaction volume were composed of 2 μL of template DNA, 10 μL of 2 × Emerald Mastermix (Takara, Japan), 1 μL of each primer (10 μM). Reaction profiles were composed of an initial cycle of 95℃ for 5 min, followed by 35 cycles of 95℃, 30 s, 60℃, 15 s, and 72℃ 20s. All the PCR products were visualized in 2% agarose gel stained with Ethidium bromide and subjected to commercial sequencing (Macrogen, Korea).

**Results;** Grossly, SD was associated with gelatinous edema in spiral colon. There is a petechial hemorrhage in the lumen. On the other hands, most of the cases were unlikely to discriminate the lesion. Microscopically, there is hyperplasia of goblet cells and erosion in lamina propria attached with fibrin in the colon in SD cases. However, brachyspiral infection showed less severe lesion rather than SD and was mostly associated with *B. murdochii*.

***Conclusions*;** The objective of this study was to investigate the frequency of *Brachyspira* spp. in continuously reared farm and pathologic changes in affected pigs. Although the pathologic lesion was consistent with other reports, *B. murdochii* was the predominant in Korean farm. Accordingly, animal experiment for *B. murdochii* needs to be done to understand the pathogenesis of brachyspiral infection.

***References***

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2. Chander P, Primus A, Oliveira S, Gebhart CJ. Phenotypic and molecular characterization of a novel strongly hemolytic *Brachyspira* species, provisionally designated "*Brachyspira hampsonii* ", JVDI 2012: **24**: 903–910.

# Field study to assess SUISENG® efficacy in three South Korea farms

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## Introduction

Neonatal diarrhoea is an important and devastating disease to swine producer responsible of a substantial economic impact in farms worldwide [1].

**Figure 1. Mean weight at weaning and mean daily gain.**

In general, most neonatal infections can be prevented by passive calostral and lactogenic immunity obtained by vaccination of the sow. Neonatal diarrhoeas induced by *E.coli* are commonly prevented through sow vaccination that are booster vaccinated 2-3 weeks before farrowing [2].

SUISENG® contains purified adhesion factors (F4ab, F4ac, F5 and F6) and the heat labile toxin (LT) of *Escherichia coli*, the β toxin of *Clostridium perfringens* type C and the α toxin of *Clostridium novyi*.

The aim of this study is to assess the efficacy of SUISENG® in field conditions, in three commercial farms located in South Korea.

**Figure 2.Diarrhoea index during lactation.**

## Materials and methods

The study was carried out in three South Korean farms. The experimental groups consisted of 30 piglets of vaccinated group and 20 piglets of control group in each farm (5 piglets/sow). The main concern in the farrowing units was neonatal diarrhoea appeared in piglets after 3 day of life. Crushed piglets, starvation and diarrhoea were the main causes of death.

The Colibacillosis diagnosis of the diarrhoea was carried out by a multiplex PCR. This PCR is able to detect different Coli adhesins factors linked with virulence (F4, F6, F5) and toxin β and α produced by different types of *Cl. Perfringens*[3]. Sampling was performed using fecal samples from piglets showing acute signs of the diarrhoea. F4, F5 and F6 adhesins were detected from the samples.

Vaccination using SUISENG® was implemented and the vaccination program used was, one dose 6 weeks before farrowing and a revaccination 3 weeks later. In order to assess the field efficacy of SUISENG® data of weaning weight, daily gain, and piglets with diarrhoea during lactation were reported.

## Result

The different parameters assessed are shown in the next figures (Figure 1 and 2). Diarrhoea in piglets was assess by a fecal consistency score: soft (1), diarrhoea (2) and watery diarrhoea (3). Weights at weaning and daily gain are the average of all piglets include in the trial. Statistical differences between vaccinated and control group were observed in all the parameters evaluated. ($p<0,05$, t-test for independent samples).

| | | Weight weaning | Daily gain |
|---|---|---|---|
| Farm A | SUISENG | 6,24 | 188,07 |
| | Control | 5,91 | 167,15 |
| Farm B | SUISENG | 7,01 | 218,97 |
| | Control | 6,88 | 213,37 |
| Farm C | SUISENG | 6,16 | 211,53 |
| | Control | 5,08 | 168,77 |

## Discussion

Based on the data presented, SUISENG® vaccination program implemented in a farm affected by a chronic

| | | 1 week | 2 week | Weaning |
|---|---|---|---|---|
| Farm A | SUISENG | 7 (1) | 8 (1) | 3 (1) |
| | Control | 5 (1); 2 (2); 1 (3) | 2 (1); 7 (2) | 6 (1); 1 (2) |
| Farm B | SUISENG | 7 (1) | 8 (1); 1 (2) | 1 (1) |
| | Control | 3 (1); 7 (2) | 8 (1); 5 (2) | 7 (1); 1 (2) |
| Farm C | SUISENG | 7 (1) | 11 (1) | 4 (1) |
| | Control | 5 (1); 6 (2) | 6 (1); 7 (2) | 6 (1); 2 (2) |

Colibacillosis showed better results than those of control group in all farms, so vaccination was able to prevent the negative effects of E. coli infection in suckling piglets; thus improving number of piglets with diarrhoea, and consequently improving the weaning weight.

## Reference

1. Dean-Nystrom, *et al.* 2001. Proceedings of the AASV meeting 2011. 223-224.

2.Gyles & Fairbrother. Dis. of swine, 10th edn. 731-733.

# Assesment of SUISENG® safety under field conditions in three South Korea farms

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## Introduction

SUISENG® is an innovative vaccine against neonatal diarrhoea associated with *E.coli* and *Clostridium perfringens* type C. It is also indicated in the control of sudden death in sows associated with *Clostridium Novyi*.

SUISENG® recommended vaccination program includes two doses in gilts prior to breeding and an initial program of two doses in sows (6 and 3 weeks before farrowing) and thereafter, one dose 3 weeks before each farrowing.

**Table 1. Average reproductive parameters on each farm.**

SUISENG® has a new generation adjuvant, HIPRAMUNE® G, a state-of-the-art aqueous adjuvant developed by HIPRA based on ginseng saponines, ginsenosides, with known immunological properties.

The objective of this study is to assess the safety of SUISENG® vaccine under field conditions.

## Materials and methods

The study was carried out in three South Korean farms with around 10% of mortality, and diarrhoea occurrence between 10 and 30%, during lactation. The farms were selected with *Clostridium perfringens* and *E.coli* pre-diagnosis using fecal samples from piglets with signs of diarrhoea.

Farm A: 300 sows in southeastern South Korea, detection of *E.coli* F4, F5 and CpA.. Diarrhoea occurrence around 18%.

Farm B: 420 sows in southern Seoul, detection of *E.coli* F4, F5 and F6. Diarrhoea occurrence 30% of sucking piglets.

Farm C: 600 sow farm in western South Korea, detection of *E.coli* F4, F5 and F6 with a diarrhoea occurrence of 10% of sucking piglets.

We recovered data from 6 vaccinated sows on each farm, after vaccination and among lactation period, of adverse reactions (congestion/erythema, hemorrhage, edema, swelling, suppuration, granuloma and necrosis), clinical signs (fever, anorexia, depression, pain and dyspnea) and reproductive parameters.

## Result

After vaccination with SUISENG®, there was no adverse reaction and clinical signs. Additionally, there were no differences between vaccinated and non-vaccinated group. Reproductive parameters in farm A showed clear distinction between vaccination group and control group. In farm B and C, reproductive parameters of vaccination group were a little better than those of the control group.

| FARM | GROUP | TB | LB | MM | ST | PR | DE | WE |
|---|---|---|---|---|---|---|---|---|
| A | SUISENG | 14.2 | 13.0 | 0 | 1.2 | 0 | 0 | 0,7 |
| | Control | 10.5 | 8.5 | 0 | 2 | 0 | 0 | 0 |
| B | SUISENG | 10.7 | 10.3 | 0 | 0.2 | 0 | 0 | 0.2 |
| | Control | 10 | 9.5 | 0 | 0 | 0.5 | 0 | 0 |
| C | SUISENG | 11.2 | 10.5 | 0 | 0.3 | 0 | 0 | 0.3 |
| | Control | 10.5 | 8.5 | 0.5 | 0.75 | 0 | 0 | 0.75 |

(TB, total born; LB, live born; MM, mummified; ST,stillbirth; PR, premature; DE, deformed; WE, weak).

## Discussion

Based on the data presented, there was no difference between vaccination group and control group in terms of adverse reactions, clinical signs and reproductive parameters, so is consider SUISENG® as a safety vaccine under field conditions. Moreover SUISENG® group improved the total born and the live born piglets. Therefore the use of a friendly adjuvant from ginseng included in SUISENG® reduce the adverse reactions and clinical signs after vaccination and shows better performance in reproductive parameters than a non-vaccinated group.

# FIELD TRIAL TO EVALUATE THE SAFETY AND EFFICACY OF PROVAC-TP VACCINE AGAINST TGE AND PED IN PIG IN VIETNAM

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**Introduction**

TGE (Transmissible Gastro-Enteritis) and PED (Porcine Epidemic Diarrhea) have caused a big loss in pig production in Vietnam. In some cases, mortality of piglet was over 80%. The disease spread from farm to farm depended on the distance, farm scales and the management measure. Many prevention methods have been applied including auto-vaccine with variant results. The good vaccine really need to prevent or lessen the symptom the infection of this disease. In order to evaluate the safety and efficacy of PRO-VAC TP vaccine under field conditions, the field trial was carried out. These results are the important criterion, basis for permission to provide this vaccine for the market to prevent or lessen the infectious diarrhea diseases caused by TGEV and PEDV in pigs.

**Materials and methods**

- Product tested: PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea
- Animal: 50 pregnant sows for trial have never immunized any TGE and PED vaccines devided by three groups, group one for control with 10 sows non vaccination, group two for safety evaluation with 10 sows applied double recommendation dose and group three with 30 sows vaccinate as recommenadion dose.

**Result**

*Safety criteria*: There was no death pigs, no abnormally clinical signs caused by vaccine during experimental period. There was no adverse reactions in the first two hours and during 28 days after vaccination. There was no local reactions at injection position during the experimental period. Weight gain ability of vaccinated pigs was not influenced by vaccine. These results demonstrated that PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea, which was used to prevent the infectious diarrhea diseases caused by TGEV and PEDV, was safe when vaccination for experimental sows. After administration of a double recommendation dose, all of the vaccinated pigs had normal feeding and no any adverse reactions.

*Efficacy criteria*: After injection of the trial vaccine, all of sows were health and normal feeding. Immune response were measured on sows (colostrum and serum samples after delivery) and piglets (serum samples).

- *The result of colostrum antibody in sows*: Antibody against to TGE in colostrum: 95% (19 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. Antibody against to PED antibody in colostrum: 80% (16 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.
- *The result of serum antibody level in sows after delivery*: The antibody against to TGE in serum: 90% (18 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. The antibody against to PED in serum: 90% (18 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.
- *The result of serum antibody in piglets after to be delivered*: TGE antibody in serum: 80% (16 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. PED antibody in serum: 85% (17 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.

**Conclusion**

- PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea, which was used to prevent the infectious diarrhea diseases caused by TGEV and PEDV in pigs, was safe when vaccination for experimental pigs.
- When vaccinate PRO-VAC TP vaccine for experimental pigs, pigs had the immunization to against the infectious diarrhea diseases caused by TGEV and PEDV.

**Acknowledgement**

- This experiment was carried out at Phon Thinh Breeding farm, Thanh Long Co. Ltd located at Cu Yen ward, Luong Son district, Hoa Binh province, Vietnam.
- This experiment was supervised by National Center for Quality Control of Veterinary Medical Products, located at 30/78 Giai Phong street, Dong Da district, Hanoi city, Vietnam.
- All samples were tested at National Center for Quality Control of Veterinary Medical Products.

# Evaluation of serological effect of SUISENG® against preweaning diarrhea on field trial

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## Introduction

*Escherichia coli*(*E. coli*) and *Clostridium perfringens* type C(CpC) are the most important causes of diarrhea in piglets. In order to prevent economic losses from *E. coli* and CpC, many swine producers use inactivated vaccine in sows. SUISENG®(Hipra, Spain) contains purified adhesion factors (F4ab, F4ac, F5 and F6), the heat labile toxin (LT) of *E. coli* and toxoid of CpC. The aim of this study is to assess the serological efficacy of SUISENG® on field conditions in Korea.

## Materials and methods

The study was carried out in Korean farm with 600 sows showed 15% diarrhea occurrence and around 10% mortality during lactation. The experimental groups consisted of 6 vaccinated sows and 4 control sows (30 piglets of vaccination group and 20 piglets of control group). Sera from sows, vaccinated with SUISENG® and with a placebo, and their preweaners were used in this work. Tested by using in-house ELISA provided from Hipra HQ. Mann-Whitney test and T-test($p<0.05$) of SPSS statistics 20 (IBM Corp., USA) were used for statistical significance.

## Results

The mean of antibody titer against each antigens (IRPC) of the ELISA are represented in the tables 1 and 2. Tables showed clear distinction between vaccination group and control group. Sows and their piglets of vaccination group were showed higher antibody titer against all fimbrial antigens and toxin.
Statistical differences between vaccinated and control group were observed.

**Tables 1. Antibody titer against all fimbrial antigens and toxins in sows(mean IRPC) on each group**

| | 2nd shoot | | Farrowing | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 57.3 | 46.2 | 71.3 | 12.4 |
| F4ac | 45.9 | 40.3 | 64.2 | 36.5 |
| F5 | 45.1 | 50.0 | 72.0 | 34.7 |
| F6 | 37.3 | 11.0 | 44.3 | 8.7 |
| LT | 12.0 | 10.1 | 21.7 | 11.5 |
| CpC | 56.5 | 14.3 | 62.2 | 12.4 |

**Tables 2. Antibody titer against all fimbrial antigens and toxins in piglets(mean IRPC) on each group**

| | 2-3 days | | 1 week | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 51.8 | 15.4 | 39.0 | 14.7 |
| F4ac | 50.4 | 19.2 | 44.2 | 20.2 |
| F5 | 44.4 | 24.5 | 40.2 | 21.5 |
| F6 | 42.6 | 10.4 | 36.1 | 8.3 |
| LT | 21.5 | 11.9 | 23.4 | 11.0 |
| CpC | 47.5 | 13.2 | 47.6 | 11.2 |

## Discussion

There was statistical significance in F4ab, F4ac, F6 and CpC antibody titers of farrowing sows. And there was significant difference in antibody titers against all antigens and toxins of 1week-old-piglets. The results clearly demonstrate that SUISENG® induces the production of specific antibodies the LT toxin and fimbrial antigens of *E. coli* and toxoid of CpC.

## Reference

[1] Fairbrother J. et al, 2005, Animal Health Research Reviews 6(1); 17-39
[2] Springer S. et al, 1999, FEMS Immunology and Medical Microbiology 24; 333-336
[3] Dean-Nystrom E. et al, 2002, American Society for Microbiology 70(5); 2414-2418

# Assessment of SUISENG® efficacy under field conditions in Korean farm

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## Introduction

Recently, *Escherichia coli*(*E. coli*) and *Clostridium perfringens* type C(CpC)-associated diarrhea in preweaner causes massive economic losses to the swine industry worldwide. In order to prevent economic losses from E.coli and CpC, many swine producers use inactivated vaccine.
Commonly, diarrheas induced by these pathogens are prevented through sow vaccination that are booster vaccinated 2 times before farrowing. The aim of this study is to assess the serological efficacy of SUISENG®(Hipra, Spain) in commercial farm located in Korea.

## Materials and methods

The study was carried out in a farm with 300 sows in Korea showed 15% occurrence of diarrhea and around 10% mortality during lactation. The experimental groups consisted of 6 vaccinated sows and 4 control sows (30 piglets of vaccination group and 20 piglets of control group). Sera from sows, vaccinated with SUISENG® and with a placebo, and their preweaners were used in this work. Tested by using in-house ELISA provided from Hipra HQ. Mann-Whitney test and T-test($p<0.05$) of SPSS statistics 20 (IBM Corp., USA) were used for statistical significance.

## Results

The mean of antibody titer against each antigens (IRPC) of the ELISA are represented in the Tables 1 and 2. Tables showed clear distinction between vaccination group and control group. Sows and their piglets of vaccination group were showed higher antibody titer against all fimbrial antigens and toxin. Statistical differences between vaccinated and control group were observed.

**Tables 1. Antibody titer against all fimbrial antigens and toxins in sows(mean IRPC) on each group**

| | 2nd shoot | | Farrowing | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 50.6 | 39.0 | 51.8 | 29.7 |
| F4ac | 63.4 | 41.5 | 72.3 | 29.9 |
| F5 | 26.5 | 47.6 | 62.2 | 19.9 |
| F6 | 30.2 | 10.4 | 60.3 | 13.5 |
| LT | 24.0 | 11.1 | 16.6 | 3.8 |
| CpC | 69.9 | 47.6 | 50.5 | 22.9 |

**Tables 2. Antibody titer against all fimbrial antigens and toxins in preweaners(mean IRPC) on each group**

| | 2-3 days | | 1 week | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 54.4 | 41.6 | 58.5 | 32.8 |
| F4ac | 85.7 | 42.0 | 48.4 | 41.1 |
| F5 | 32.2 | 36.4 | 43.2 | 13.4 |
| F6 | 64.3 | 29.2 | 48.4 | 18.1 |
| LT | 33.3 | 17.8 | 20.8 | 7.8 |
| CpC | 74.2 | 50.8 | 60.6 | 21.1 |

## Discussion

There was statistical significance in F4ac, F5.antibody titers of farrowing sows. And there was significant difference in antibody titers against CpC, F4ab, F5, F6 and LT of 1week-old-piglets. The results clearly demonstrate that SUISENG® induces the production of specific antibodies the LT toxin and fimbrial antigens of *E. coli* and toxoid of CpC.

## Reference

[1] Fairbrother J. et al, 2005, Animal Health Research Reviews 6(1); 17-39
[2] Springer S. et al, 1999, FEMS Immunology and Medical Microbiology 24; 333-336
[3] Dean-Nystrom E. et al, 2002, American Society for Microbiology Vol.70, No.5; 2414-2418

# Effects of dietary supplementation of bacteriophages in treatment of infection in post-weaning pigs challenged with enterotoxigenic *Escherichia coli* K88 and K99

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## Introduction

The post-weaning diarrhea or colibacillosis is a most costly disease causing substantial mortality as well as growth retardation in swine production.

Bacteriophages or phages have recently received reemerging attention as alternatives to antibiotics because of several merits as feed additives including their high stability within the feed and digestive tract as well as their high specificity of transfection. The present study was therefore initiated to evaluate the efficacy of dietary phages on treatment of colibacillosis induced by an oral challenge of ETEC K88 and K99 in post-weaning pigs.

## Materials and methods

Eighteen 35-d-old post-weaning pigs were allotted to three groups, after which two groups were orally challenged with $3.0 \times 10^8$ cfu of each of ETEC K88 and K99. The unchallenged group and one challenged group were fed a typical nursery diet (Control and Chal/Basal, respectively) while the remaining challenged group received the same diet supplemented with $1.0 \times 10^9$ cfu of each of ETEC K88- and K99-specific phages per kg (Chal/Phage). All animals were killed after a 7-d feeding trial and subjected to necropsy.

## Results

The results of body temperature of challenged pigs are shown in Figure 1 and fecal consistency score of challenged pigs are shown in Figure 2.

The ETEC K88 and K99 were detected in all feces samples obtained on d 1, 3, and 7 only in the Chal/Basal and Chal/Phage groups. The log cfu values of ETEC K88 per g feces on d 1 and 3 and per g tissue in the ileum and cecum at necropsy were less in the Chal/Phage group vs. the Chal/Basal group whereas in ETEC K99, neither the fecal excretion nor intestinal adhesion was influenced by the phage therapy.

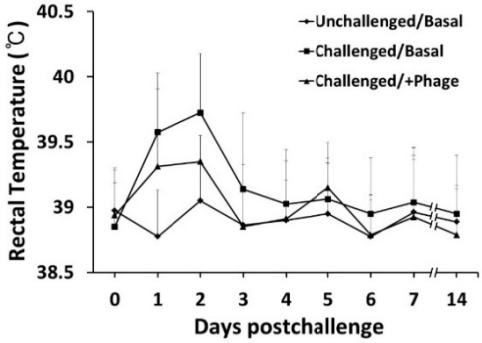


**Figure 3. Body temperature of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88 and K99.**

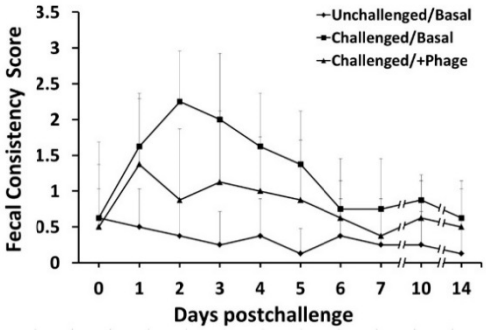


**Figure 4. Fecal consistency score (right) of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88. (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea).**

## Discussion

The effect of the phage therapy appears to be significant in the ETEC K88 infection, but not in the ETEC K99 infection, in terms of suppression of intestinal adhesion and fecal excretion of the pathogens. Future studies are therefore needed to be focused on the effects of the ETEC K88-specific phage on the pathophysiological measures to further evaluate the phage as a therapeutic or prophylactic agent against porcine colibacillosis.

## Reference

[1] Runnels, PL, Infect. Immun. 28, 298-300

[2] Joerger, RD, Poult. Sci. 82, 640-647

# Effects of lipid-encapsulated zinc oxide supplementation on colibacillosis, growth and intestinal morphology in weaned piglets challenged with enterotoxigenic *Escherichia coli* K88

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## Introduction

Enterotoxigenic *Escherichia coli* (ETEC), which frequently causes post-weaning diarrhea, proliferates in the small intestine and also is shed into feces after weaning of the piglets. Dietary supplementation of 1500–3000 ppm zinc oxide (ZnO) has been widely used to prevent porcine colibacillosis. Shield Zn® is a proprietary ZnO product which is encapsulated (coated) with lipid to allow the active component to reach the intestine without being ionized in the stomach. The present study was therefore initiated to investigate the effects of dietary supplementation of 100 ppm of the coated ZnO relative to those of 2500 as well as 100 ppm of native ZnO in weaned piglets with colibacillosis induced by the ETEC K88 challenge.

## Materials and methods

Thirty-two 35-day-old weaned piglets were orally challenged with $3 \times 10^{10}$ colony forming units of ETEC K88 while eight piglets received no challenge (control). Each eight challenged piglets received a diet containing 100 ppm ZnO (low ZnO), 2500 ppm ZnO (high ZnO) or 100 ppm of lipid (10%)-coated ZnO (coated ZnO) for 7 days; control pigs received the low
ZnO diet. Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, villus height in the jejunum and ileum, fecal consistency score, serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum were measured by the coated ZnO, high ZnO and low ZnO groups.

## Results

The results of body temperature of challenged pigs are shown in Figure 1 and fecal consistency score of challenged pigs are shown in Figure 2.

Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, and villus height in the jejunum and ileum, which decreased due to the challenge, were equally greater in the coated ZnO and high ZnO groups versus low ZnO group. Serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum, which increased due to the challenge, were equally low in the coated ZnO and high ZnO groups versus low ZnO.

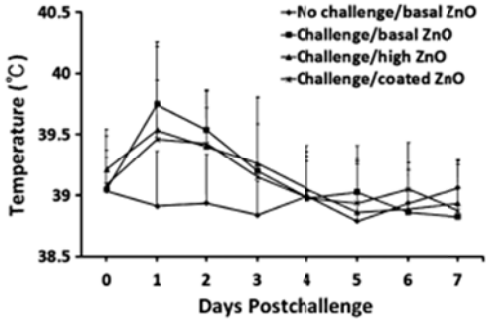


**Figure 5. Body temperature of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88.**

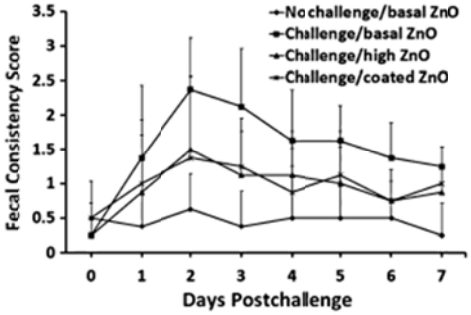


**Figure 6. Fecal consistency score (right) of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88. (0, normal;1, soft feces; 2, mild diarrhea; 3, severe diarrhea).**

## Discussion

Dietary supplementation of 100 ppm of lipid-coated ZnO (72 ppm Zn) can effectively alleviate colibacillosis caused by an oral challenge with ETEC
K88 in weaned piglets. Moreover, the effect of 100 ppm of the coated ZnO on colibacillosis was equal to that of 2500 ppm of native ZnO (2000 ppm Zn) in almost all the measures associated with colibacillosis examined in the present study.

## Reference

[1] Bosi, P, J. Ani. Sci. 82, 1764-1772
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**Lack of interference of a *Mycoplasma hyopneumoniae (M hyo)* bacterin on an inactivated antigen fraction from a PCV2 vaccine when combined in the same syringe in 3-week-old piglet: a challenge efficacy study**

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## Introduction

Vaccine mixing is common practice in the field aiming to reduce the number of injections. It contributes to the reduction of labor costs and risks, animal stress, pathogen transmission and meat price devaluation due to broken needles. PCV2 and *M hyo* are the etiologic agents of PCVDs, enzootic pneumonia respectively and are considered to play an important role in PRDC. In this study, two monovalent vaccines against these pathogens licensed for piglets 3 weeks of age or older were combined. The protection against a PCV2 challenge 5 weeks post-vaccination was assessed on virological and histological criteria.

## Material and methods

Forty healthy 3-week-old CDCD, PCV2-seronegative pigs were allocated into 2 groups using litter as a randomization The *M hyo* vaccine is a ready-for-use adjuvanted bacterin and the PCV2 vaccine is presented in two vials, one containing the antigen solution and the other one the same adjuvant. On D0, one group of 20 piglets was vaccinated IM in the neck with 2.0 mL of an extemporaneous preparation combining the PCV2 antigen solution in the *M hyo*. vaccine (1 dose: 1 dose). The other group was administered Saline (Placebo). Five weeks post-vaccination, all pigs were challenged with a PCV2 solution by both IM and intranasal routes.

Blood samples were collected in all pigs on D-1, D21, D28, D35, D37, D42, D44, D49, D56 and D63 and sent to the ISU VDL for PCV2 ELISA and viremia quantification using PCV2 Real-Time PCR (threshold value: CT=35).

Four weeks following PCV2 exposure, all piglets were humanely euthanized. A mesenteric, a tracheobronchiolar and a sub-iliac lymph node as well as tonsil and spleen were sampled for slide preparation: inflammation assessed by histiocytic replacement and lymphoid depletion were evaluated based upon histopathology. PCV2 colonization was scored by immunohistochemistry. The incidence of viremia, histopathological lesions and lymphoid tissue colonization were statistically compared using Fischer's exact test.

## Results and Discussion

One pig from the vaccination group was found dead before challenge. Cause of death was unrelated to vaccination. Seroconversion in vaccinated animals and absence of PCV2 infection before challenge in the Placebo group were confirmed serologically.

**Table 1.** Frequency of post-challenge positive rtPCR for PCV2 and p-value of the incidence comparison.

| Day | Group | Negative | Positive | p-value |
|---|---|---|---|---|
| D37 | Vaccinated | 12 | 7 | p<0.0001 |
| | Placebo | 0 | 20 | |
| D42 | Vaccinated | 14 | 5 | p<0.0001 |
| | Placebo | 0 | 20 | |
| D44 | Vaccinated | 11 | 8 | p<0.0001 |
| | Placebo | 0 | 20 | |
| D49 | Vaccinated | 16 | 3 | p<0.0001 |
| | Placebo | 0 | 20 | |
| D56 | Vaccinated | 15 | 4 | p<0.0001 |
| | Placebo | 0 | 20 | |
| D63 | Vaccinated | 19 | 0 | p<0.0001 |
| | Placebo | 0 | 20 | |
| overall | Vaccinated | 8 | 11 | p=0.01 |
| | Placebo | 0 | 20 | |

**Table 2.** Prevalence of PCV2 colonization and histopathological lesions following challenge.

| Parameter | Group | Negative | Positive | p-value |
|---|---|---|---|---|
| Virus Colonization | Vaccinated | 16 | 3 | p<0.01 |
| | Placebo | 7 | 13 | |
| Inflam--mation | Vaccinated | 19 | 0 | p=0.23 |
| | Placebo | 17 | 3 | |
| Lymphoid Depletion | Vaccinated | 19 | 0 | p=0.23 |
| | Placebo | 17 | 3 | |

At necropsy, the prevalence of virus colonization of all lymphoid tissues in the vaccinated group was significantly lower than that of the Placebo group (p<0.0001). The stage at which the disease was captured was not advanced enough to cause significant histiocytic replacement and lymphoid depletion in the Placebo group so a vaccine effect for these variables could not adequately be assessed.

## Discussion and conclusion

Under the conditions of the study, these results demonstrated the efficacy of the combination of a whole PCV2 antigen (CIRCOVAC®) and a *M hyo*. vaccine (SPRINTVAC®) in the reduction of PCV2 colonization of all lymphoid tissues and viremia in pigs vaccinated as piglets and experimentally challenged with PCV2 at 8 weeks of age.

# Tylvalosin tartrate-medicated feed inhibits the replication of highly pathogenic porcine reproductive and respiratory syndrome virus in vivo

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## Introduction

An atypical and highly pathogenic porcine reproductive and respiratory syndrome (PRRS) variant (HP-PRRS) has occurred in China and Southeast Asia. This disease was characterized by a high fever of above 41˚C, anorexia, red discoloration of the ears (blue ear) and high mortality in pigs of all ages. Recently, it has reported that macrolide antibiotics may have an anti-viral effect on PRRSV [1, 2]. We previously demonstrated that the macrolide antibiotic, tylvalosin tartrate, had anti-viral activity against HP-PRRSV replication *in vitro* [3]. In the present study, the macrolide antibiotic was tested for anti-viral activity against HP-PRRSV replication *in vivo*.

## Materials and methods

Fifteen 4-week-old specific pathogen-free pigs were used in the experimental infection. Six pigs (group 1) and 3 pigs (group 3) were fed with 200 ppm of Aivlosin® plus 10 (1% premix of tylvalosin tartrate, ECO Animal Health Inc.) a day per pig for one week before the viral inoculation and during the experiment period. Group 1 and another 6 pigs (group 2) were intranasally inoculated with $10^5$ $TCID_{50}$/pig Vietnamese HP-PRRSV isolate 2010 (100186-614 strain), while group 3 was used as uninfected controls. All pigs were monitored daily for clinical signs. Blood and oral fluids were sequentially collected until 12 days post-inoculation (dpi). When all pigs were necropsied at 12 dpi, gross findings were assessed and a bacterial examination was carried out. The amount of virus in the serum and tissues were measured by quantitative real time RT-PCR. Serum samples were tested for PRRSV antibodies using the commercial ELISA kit (IDEXX).

## Results

All pigs in groups 1 and 2 exhibited high fever (40-41.9 ˚C), anorexia and dyspnea. During the experiment period, only one pig in group 2 died at 11 dpi. Moreover, one pig in each group 1 and 2 were moribund at 12 dpi and *E.coli* was isolated from these pigs at necropsy. Hemorrhages and consolidation of lung and blood spots in kidney were also observed. Other pigs in infected group 1 and 2 had pneumonia and enlargement of various lymph nodes. The amount of PRRSV RNA in serum (Figure 1), oral fluid and tissues in group 1 was significant less than those of group 2. However, the S/P ratio of ELISA was no difference in two groups. No clinical signs and lesions were observed in control animals (Group 3).

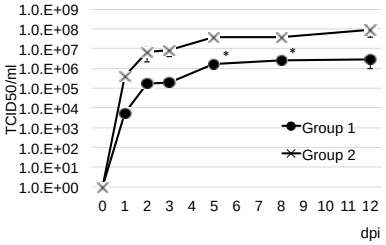


**Figure 1.** The amount of PRRSV RNA in serum of pigs in groups 1 and 2.
Asterisks indicate statistically significant differences within main effect ($p < 0.05$).

## Discussion

The clinical signs were no difference between the untreated pigs and pigs fed with tylvalosin tartrate, inoculated with HP-PRRSV. However, the replication of HP-PRRSV was reduced in pigs fed with Aivlosin®, tylvalosin tartrate, compared with the inoculated animals without untreatment. The previous study suggested that tylvalosin tartrate had anti-viral activity against HP-PRRSV replication in MARC145 cell line and porcine alveolar macrophage [3]. Therefore, the replication of HP-PRRSV may be inhibited in macrophage of pigs fed with tylvalosin tartrate.

**Acknowledgements**

This work was partly supported by research project for improving food safety and animal health of the Ministry of Agriculture, Forestry and Fisheries of Japan.

**Reference**

[1] Benfield. et al. (2002). Proc. AASV Meeting, 87-91.
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# Surveillance of PEDV and TGEV in Taiwan during January 2014 to May 2015

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## Introduction

Porcine epidemic diarrhoea (PED) and transmissible gastroenteritis (TGE) are two major swine diarrhoea diseases distributed worldwide, caused enormous economic loss. A major PED outbreak occurred in late 2013 in Taiwan, raised the alertness to these diseases. Since PED and TGE cannot be distinguished and diagnosed based on the clinical signs, a multiplex real-time PCR technique was applied for the examination.

## Materials and methods

Contents of ileum were collected from clinical cases registered in the Animal Disease Diagnosis Center in National Pingtung University of Science and Technology. Total nucleic acids were extracted with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche®), and immediately reverse transcripted with PrimeScript™ RT Reagent Kit (TaKaRa®) into cDNA. The primers and probes (Table.1) used in multiplex real-time PCR were designed for distinguishing PEDV and TGEV. Reaction mixture with a final volume of 10μL containing 1μL of cDNA, 5μL of probe master mix, 0.2μL of each primer, 0.1μL of each probe and 3μL of DEPC treated water. Hundred-fold serial dilution of the cloned plasmid were applied with the concentration of $1x10^8$ to $1x10^4$ copies of both PEDV and TGEV as standard control.

## Results

From January 2014 to May 2015, 547 out of 879 cases were examined as PEDV-positive, and 17 out of 480 cases were examined as TGEV-positive, the positive rate was 62.23% and 3.54%, respectively.

| Pathogen | Name | Sequences (5'-3') |
|---|---|---|
| PEDV | 133F | TTGGCTGCTGGGCTATGG |
| | 133R | TGAAAAGGTACTGCGTTCCC |
| | PED TM Probe | HEX-AGCCAGTGGTAAGTCAGTGCAAGAAGAA-BHQ |
| TGEV | P211 | CACTAGATCCAGACGTTAGCTC |
| | P276 | CCGAGGAATTACTGGTCATCGCG |
| | Alpha1-CoV Probe | FAM-GGAGGTACAAGCAACCC-ZNA-3-BHQ |

***Table 1. Sequences of primers and probes used.***

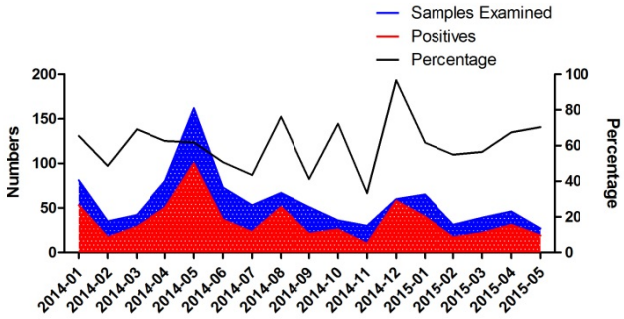


***Figure 1. Positive examined of PEDV***

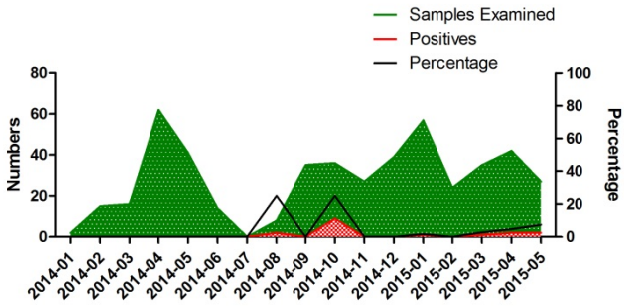


***Figure 2. Positive examined of TGEV***

## Discussion

From the beginning of 2014, the positive rate of PEDV examination raised to a high peak till May 2014, which is consistent to the outbreak occurred in late 2013 in Taiwan. The positive rate of PEDV examination keeps dropping to a low level before November 2014, and rise again after December 2014, while the season turns into winter in Taiwan. Meanwhile the positive rate of TGEV maintained low throughout the period.

## Reference

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[2] Song, D., Park, B., Virus Genes 2012:44(2):167-175.

# Comparison of two PCV2 vaccines administered at weaning in a South-Korean commercial farm

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## Introduction

Several commercial PCV2 vaccines are available for piglet in Korea. CIRCOVAC® (Merial), is an inactivated PCV2 vaccine registered for piglets for the prevention of PCVDs. The purpose of this trial was to compare the efficacy of CIRCOVAC and Circoflex® (Boehringer Ingelheim) vaccination in piglets in a commercial farm.

## Materials and methods

The study was carried out on a 1000-sow farm, located in Gyeonggi, South-Korea. Piglets born from 26 sows housed in the same farrowing unit were selected: each litter was ear-tagged and randomly split into two groups of 5 piglets: the first group was vaccinated at weaning (3 weeks of age) with CIRCOVAC and the second group was vaccinated with Circoflex at the same age. Each vaccine was administered according to the recommendations of the manufacturers to a total of 130 piglets per vaccine. The pigs were housed commingled in the same building in the nursery then 100 pigs per group were raised separated in different pens for FCR monitoring. Each pig was individually weighted at weaning, 7 and 20 weeks post-weaning. A cohort of twenty five piglets per group was individually blood sampled at 0, 2, 4, 6, 8, ,10, 12, 16 and 20 weeks post-injection (WPI). Total PCV2 antibody (Ab) level and PCV2 DNA in sera were assessed by respectively ELISA (SERELISA® PCV2 Ab Mono Blocking ELISA kit) and rtPCR technique. Statistical inferences were performed using standard tests and assumptions, using a $p<0.05$ as statistical significance threshold.

## Results

The serological and virological results are given in Figures 1 & 2.

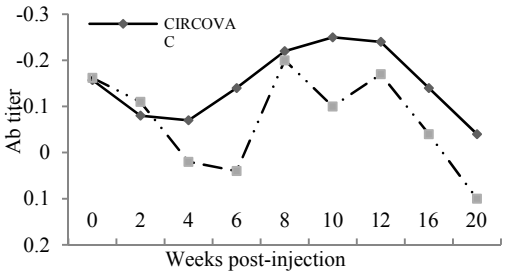


Figure 1. Ab titers up to 20 weeks post-vaccination

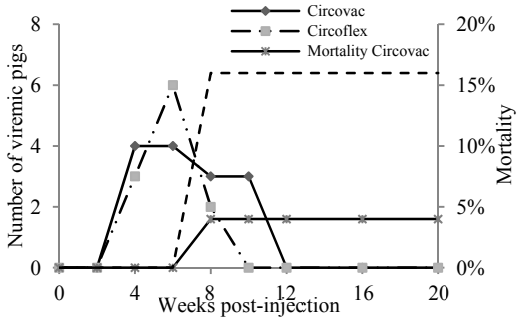


Figure 2. Mortality and virological results (out of 25 pigs / group)

Following weaning, PCV2 Ab titres declined for 4 to 6 weeks then increased rapidly and peaked 8 WPI (Circoflex) or 10 WPI (CIRCOVAC). Ab titres then steadily decreased during late fattening in both groups. Ab titres were significantly higher in pigs vaccinated with CIRCOVAC on 5/6 dates following seroconversion as compared to Ab titres in Circoflex-vaccinated pigs. Despite numerically higher in the group vaccinated with Circoflex, the number of vireamic pigs and viral DNA loads were not significantly different between groups.

Table 2. Growth, mortality and feed conversion records.

| Parameter | CIRCOVAC | CIRCOFLEX |
|---|---|---|
| BW Weaning (kg) | 6.9 | 6.7 |
| BW End of Nursery (kg) | 24.1 | 22.7 |
| BW Slaughter (kg) | 95.9 | 96 |
| Mortality Nursery | 3.8% | 1.5% |
| Mortality Fattening | 6.0% | 9.0% |
| Total Survival | 90.4% | 89.6% |
| Total Starting Weight (kg) | 2413 | 2273 |
| Total Exit Weight (kg) | 10597 | 10214 |
| Total Feed Volumes (kg) | 23315 | 22814 |
| FCR | 2.85 | 2.87 |
| Age at 113 kg (days) | 194.4 | 194 |

No significant difference between groups was found regarding growth, mortality and feed conversion performance (Table 1).

## Conclusion

Under the conditions of the study, both vaccines successfully helped to control the PCV2 circulation until slaughter. In this context the efficacy of the two vaccines was similar regarding production performances, namely mortality, growth and feed conversion.

# Evaluation of post-vaccination seroconversion to Erysipelas antigen with two commercial ELISA kits: a comparative trial

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## Introduction

Erysipelas serology is a controversial issue because of the lack of correspondence between the results obtained in current commercial ELISA kits and protection (1). However, farmers and practitioners frequently use these tests as a vaccination compliance tool. Sometimes, young animals (gilts) having received a basic vaccination yield negative serological results when tested. Therefore, the present trial was devised to study the serological response to PARVORUVAX® in two commercial ELISAs.

## Material and methods

Twenty five cross-bred gilts were selected from a farm in which seroconversion to Erysipelas had not been detected by a commercial ELISA kit after basic vaccination with an Erysipelas vaccine. The animals included in this study were vaccinated twice 3 weeks apart with PARVORUVAX according to the manufacturer instructions. Blood samples were taken on each vaccination day and one month following the booster injection. The samples were centrifuged and three aliquots of each kept under freezing conditions. At the end of the sampling period three different labs were sent a panel of all samples collected for Erysipelas antibodies titration using their own indirect ELISA: CIVTEST SUIS SE/MR (HIPRA), or an in-house test based on the former one or INGEZIM Mal Rojo, 11.MR.K1 (INGENASA). The labs were blinded to expected serological status of the samples. The OD results were classified as negative, positive or doubtful according to the manufacturer/lab instructions.

## Results

The trial results are summarized in Table 1.

Table 1. Serological results in gilts vaccinated with PARVORUVAX according to different ELISA techniques.

| Day of protocol | Result | ELISA kit | | |
|---|---|---|---|---|
| | | CIVTEST | In-house test | INGEZIM |
| Primo-vaccination * | + | 0 | 0 | 0 |
| | ? | 0 | 1 | 0 |
| | - | 23 | 22 | 23 |
| Booster | + | 0 | 0 | 12 |
| | ? | 7 | 8 | 6 |
| | - | 18 | 17 | 7 |
| One month post-vaccination | + | 2 | 1 | 17 |
| | ? | 17 | 19 | 6 |
| | - | 6 | 5 | 2 |

+: positive; ?: doubtful; -:negative;*23/25 animals were sampled

All the samples collected before primovaccination yielded negative results except one doubtful result when tested by the in-house ELISA, thus cleary indicating both an absence of previous contact with *E rhusiopathiae* and the absence of residual maternal derived antibodies. The in-house test and CIVTEST resulted in a very low positivity rates both following primovaccination and booster injection. These trends, consequently demonstrated the inadequacy of the kit to detect the onset of immunity in gilts vaccinated once or twice with PARVORUVAX. On the contrary, when using the INGENZIM Mal Rojo, 11.MR.K1 kit, a clear serological response was evidenced in about half the animals vaccinated once (48%) and in most (68%) of the gilts vaccinated twice.

## Discussion

The high correspondence between the in-house test and the CIVTEST was a clear confirmation that both tests were based on the same reagents. Both tests yield unsatisfying results for the monitoring of Erysipelas serological response elicited by PARVORUVAX. In contrast, the INGENZIM test for Erysipelas was much more reliable as vaccination compliance detection tool. Indeed, it was able to detect seroconversion even in half of the animals having received a single vaccination. Although it is well known that commercial ELISAs are not suitable as a means of assessing the degree of protection conferred by vaccination (1) they can eventually be useful for vaccination compliance purposes. Even in such an scenario it is important to bear in mind that there might be substantial differences between commercial kits in this respect.

## Conclusion

In the conditions of this trial, a low concordance and huge differences in the ability to detect seroconversion against Erysipelas after vaccination was observed between commercial ELISAs. It should be taken into consideration in case of observing seronegative results in young vaccinated animals, particularly when trying to evaluate vaccination compliance.

## References

1. Gimenez-Lirola *et al*. 2012. J Microb Meth, 91:191-197.

# Efficacy of an inactivated PCV2 vaccine against a subclinical PCV2 infection under Vietnamese field conditions

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## Introduction

Despite Porcine Circovirus type 2 (PCV2) vaccines have helped to reduce clinical signs of PCVDs, the virus is still causing great economic losses in subclinical infection scenarios. Therefore, the use of PCV2 vaccines is focused on improving the production parameters in pig farming. The aim of this study was to assess the benefit of PCV2 vaccination with CIRCOVAC® at weaning (Merial, Lyon, France) in a Vietnamese commercial operation.

## Material and methods

The study was carried out in a commercial farrow-to-finish farm located in Southeastern Vietnam. Thousand 3-week-old piglets were individually tagged for the study. Fifty piglets were randomly allocated according to their bodyweight at weaning to each of 2 treatment groups: one vaccinated against PCV2 with CIRCOVAC, 0.5mL, IM in the neck and the other group unvaccinated. Individual bodyweights were recorded at weaning, 9 weeks of age, as well as just before slaughter (5 months of age). Mortality rates, wasting rate at the end of the nursery phase and health status remarks were recorded. The total consumption of each treatment group was recorded to calculate feed conversion ratios (FCR). Each pig was bled before vaccination and 4 then 6 weeks post vaccination. for PCV2 total antibody titration (SERELISA® PCV2 Ab Mono Blocking, Synbiotics Europe). Feces samples were also collected an assayed for PCV2 detection by a commercial ELISA test (SERELISA® PCV2 Ag Capture, Synbiotics Europe). Statistical comparisons were performed using Student t-test and Fisher's Exact test.

## Results

Vaccination yielded a significant increase of anti-PCV2 antibody levels at least from 4 weeks post-vaccination ($p<0.001$) and induced a definite ($p<0.01$) decrease the proportion of pigs excreting virus particles at the end of nursery phase (Table 1). The clinical signs and the production performance are summarized in Table 2.

Severe clinical forms of PCVDs were not observed in any of the experimental pigs. Mortality rate and culling rate were also low in both groups thus validating the subclinical PCV2 case.

Table 1. Serological and virological results.

| | Control | CIRCOVAC | p-value |
|---|---|---|---|
| *S/N ratios of total PCV2 antibodies in sera* | | | |
| Weaning | 0.195 ± 0..087 | 0.184 ± 0.075 | p>0.05 |
| 4 weeks pv | 0.245 ± 0..149 | 0.134 ± 0.029 | ***p<0.001*** |
| 6 weeks pv | 0.284 ± 0..122 | 0.127 ± 0.029 | ***p<0.001*** |
| *Seropositivity rate* | | | |
| Weaning | 54% | 50% | p>0.05 |
| 4 weeks pv | 32% | 88% | ***p<0.001*** |
| 6 weeks pv | 21% | 92% | ***p<0.001*** |
| PCV2 positives in feaces | 34% | 8% | ***p<0.01*** |

pv=post-vaccination

Table 2. Growth, mortality and feed conversion records.

| Parameter | Control | CIRCOVAC | p-value |
|---|---|---|---|
| BW Weaning (kg) | 7.54 ± 0.73 | 7.53 ± 0.74 | *p>0.05* |
| BW End of Nursery (kg) | 20.2 ± 3.5 | 21.9 ± 3.4 | ***p<0.01*** |
| BW Slaughter (kg) | 83.2 ± 5.51 | 85.7 ± 5.46 | ***p<0.01*** |
| Mortality Nursery* | 16% | 8% | *p>0.05* |
| Number of wasting pigs 6 weeks post-vaccination | 5/47 | 2/48 | *p>0.05* |
| Number of pigs with severe wasting 6 weeks post-vaccination | 3/47 | 1/48 | *p>0.05* |
| Mortality Fattening | 0% | 0% | *p>0.05* |
| ADWG Nursery (g/day) | 301.5 ± 80.4 | 342.3 ± 70.6 | ***p<0.001*** |
| ADWG Fattening (g/day) | 689.9 ± 44.3 | 703.9 ± 41.2 | ***p<0.05*** |
| ADWG Weaning-Slaughter (g/day) | 494 ± 179 | 579 ± 116 | ***p<0.05*** |
| FCR Nursery | 1.6 | 1.4 | NA |
| FCR Fattening | 2.9 | 2.5 | NA |

BW=bodyweight; *includes the number of wasting pigs 6 weeks post-vaccination; NA: not applicable

In this context, growth was significantly improved both during the nursery phase ($p<0.001$) and fattening ($p<0.05$). Slaughter weight was consequently significantly improved (+2.5 kg, $p<0.01$). A definite improvement of feed conversion efficiency by at least 10% was observed as well in the vaccinated group as compared to the unvaccinated group.

## Conclusion

Under this subclinical PCVD scenario, CIRCOVAC vaccination in 3-week-old piglets was effective at inducing an immune response in piglets and reducing the number of contaminated pigs. This yield a significant improvement of production performance.

®CIRCOVAC is a registered trademark of MERIAL.

# THE EFFECT OF AIVLOSIN® IN PRRS FIRST OUTBREAK FARM OF THE REGION RECOVERING FROM FMD DISEASE

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## Introduction

All pigs and cattle in Koyu region of Miyazaki Prefecture were culled in FMD outbreak 2010. It restarted from November 2010 and now recovered up to 70% of before. For the reconstruction of swine industry, we set up New Pig Project Council and aimed keeping "Aujeszky's Disease Free, PRRS Free" region. So we had our rules for pig introduction and periodical monitoring survey.

Farm A is farrow-to-finish farm with 110 sows. Gilts are selected from growing pigs and all mating is AI. Pig flow is one-site continuous production.

Unfortunately, PRRS was confirmed at the neighbor farm in September 2014, 500M west of Farm A. The neighbor farm uses the same feed company and slaughterhouse.

Materials and methods

To monitor PRRS situation, PCR for PRRS virus and ELISA antibody against PRRS were measured by using Sows sera. After PRRS invasion, Aivlosin® and Marine mineral formulation 2 (MCM solution) were added to sow and weaning pigs' feed. (Table 1)

*Table.1 Counter measures against PRRS*

| Measuers | Action | |
|---|---|---|
| First total plan | Sows | Weaning |
| | Aivlosin 100ppm in feed | Aivlosin 100ppm in feed |
| | MCM liquid in feed | MCM liquid in drinking water |
| Biosecurity | complete dissemination of Ca(OH)2 | |
| | change boots and wear | |
| For fatteninng pigs | Early detection, isolation, culling | |
| For gilts | Internal breeding (GP introduction stop) | |
| Fo weaning unit | AIAO | |

We didn't ELISA test again about ELISA(+)PCR(-) samples. PRRS vaccine was not used. In addition, temporary skip weaning unit to make vacant unit period and to make the partition in the center of unit to do all-in-all-out (AIAO) in each line.

## Result

Fig 1 shows productive performance. Live litter size was very low in September, PRRS invasion period. It recovered to the same level as before, 10.9 in litter size, 5.2% in stillbirth ratio, mortality in 2.1% in March 2015.

Fig 2 shows Serum examination results (ELISA, PCR). At the beginning, there were many PCR(+) sows. But two months after counter measures, PRRSV couldn't be detected by PCR. Even two sows (1.9%) was still PCR(+), others were 65% in PCR(-)ELISA(+) or 31.1% in PCR(-)/ELISA(-) in January 2015.

***Figure 1 Productivity***

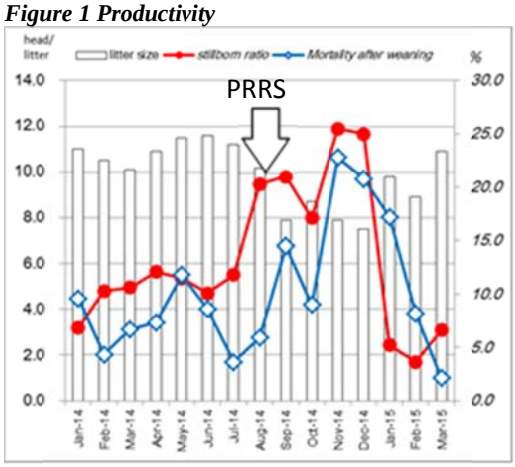
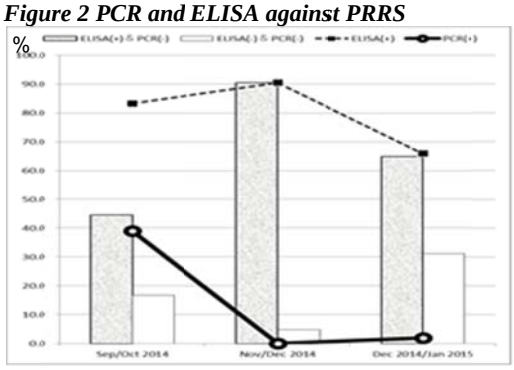



***Figure 2 PCR and ELISA against PRRS***




## Discussion

Marine mineral formulation 2 (MCM solution) which has been observed immuno-stimulatory effect [1]. It is reported that Aivlosin® has an effect against PRRS virus [2]. PRRS invaded Farm A around September 2014. There must be several factors to eradicate PRRSV infection. So we have to act as soon as we find out PRRS invasion in the farm. Not only decrease of PCR(+) but also decrease of ELISA(+) means no further infection in Farm A. We confirm the effect to minimalize and recover from PRRS damage in mortality, litter size and productivity by the early counter plan of Aivlosin®, MCM solution and AIAO.

Reference

[1] Shiga, Pig Journal Japan, 2015, 18-1, 58-61
[2] STUART et al. The Pig Journal, 2008, 61, 42-44

# EFFICACY OF AIVLOSIN® PREMIX FOR PRRSV INFECTED FARM

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## Introduction

PRRSV infection is major problem in commercial pig production and is difficult to control. Whilst aiming for a high level of biosecurity PRRS-negative farms are at risk from PRRS-positive neighboring farms in high density pig areas. Following reports of use of Aivlosin® in the field (1,2,3,4) and in vitro (5,6) against PRRS we tried to use it to control PRRS in our area. We report a trial with Aivlosin® Plus 50 (active ingredient tylvalosin) following PRRS virus entry into a pig farm. The farm is commercial unit with 250 sows, farrow-to-finish. In August 2014, there was an increase in reproductive disorders and mortality.

## Materials and methods

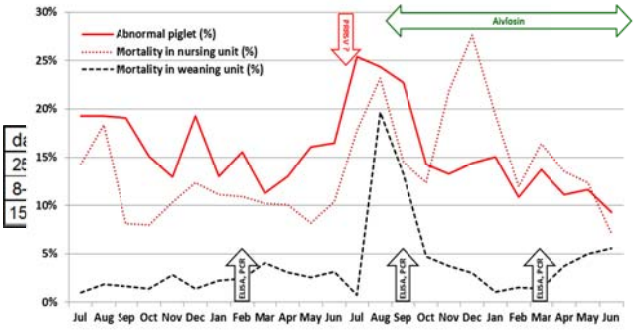
Reproductive disorders (immature, abnormal, still born, mummified fetus), mortality in nursing period, mortality after weaning and mortality in fattening period were recorded. Serological examination used PRRS ELISA (IDEXX) and PCR on pooled serum samples for detecting PRRS virus and antibodies.
After confirming PRRS virus infection, Aivlosin® Plus 50 administration started in feed (Table 1). In addition more severe hygiene control also started.

| Stage | Sow in stall | Farrowing to wean | Artificial Milk | Growing |
|---|---|---|---|---|
| Dose | 50ppm, one week interval | 100ppm | 100ppm | 100ppm |

***Table1. Dose in Feed PCR test***

## Result

Fig 1 shows mortality in each stage. In February 2014, the farm condition was stable. In July, mortality increased, especially in the nursing period




and after weaning. All mortalities peaked in August. All mortalities and reproductive disorders decreased after using Aivlosin®.

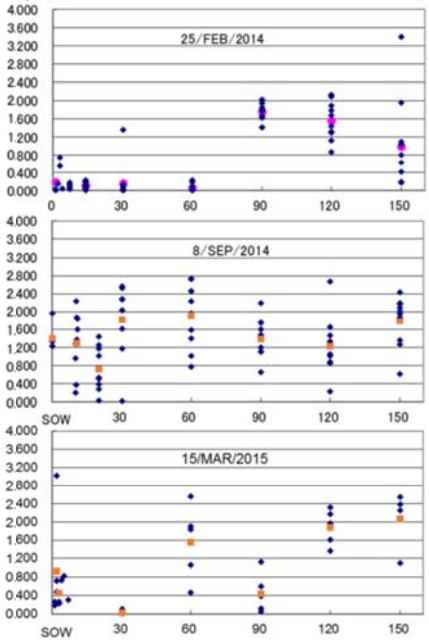
***Figure 1.*** *Mortality after PRRS virus infection*

***Table2. Dose in Feed PCR test***

| days-old | Sow | 30 | 60 | 90 | 120 | 150 |
|---|---|---|---|---|---|---|
| 25-Feb-14 | — | — | — | + | — | — |
| 8-Sep-14 | + | + | + | + | + | + |
| 15-Mar-15 | +/- | — | + | + | + | + |

Table 2 shows PCR and Fig 2 PRRS ELISA results. In February 2014, only 90 days-old pig showed PRRS positive in PCR test. In September, all stage of pigs resulted in PCR positives. In November, Sow, 120 and 150 days-old samples were negative. And ELISA S/P ratios of sow samples were lower in September.

***Figure 2. ELISA test***




## Discussion

PRRS virus invaded before September, maybe in July associated with a marked increase in mortality, and an increase in abnormal piglets born. PRRSV infection could be the major cause, as other pathogens were not isolated. Although PRRSV is still detected in November, mortality decreased after using Aivlosin®. The farmer reports that the condition of weaned piglets has gradually returned stable, and the condition in growing unit is recovering. Our results are similar to those previously reported (1,2,3,4). The mechanism of tylvalosin against PRRSV is not clear, but its effect was confirmed in vitro studies (5,6). There is evidence for a direct effect on PRRS virus, and indirect immuno enhancing effects of macrolide antibiotics. Aivlosin made the difference in production in PRRS problem farm.

## Reference

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[6] Takagi et al. (2014). IPVS 2014, 562

# FIELD TRIAL TO EVALUATE THE SAFETY AND EFFICACY OF PROVAC-TP VACCINE AGAINST TGE AND PED IN PIG IN VIETNAM

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**Introduction**

TGE (Transmissible Gastro-Enteritis) and PED (Porcine Epidemic Diarrhea) have caused a big loss in pig production in Vietnam. In some cases, mortality of piglet was over 80%. The disease spread from farm to farm depended on the distance, farm scales and the management measure. Many prevention methods have been applied including auto-vaccine with variant results. The good vaccine really need to prevent or lessen the symptom the infection of this disease. In order to evaluate the safety and efficacy of PRO-VAC TP vaccine under field conditions, the field trial was carried out. These results are the important criterion, basis for permission to provide this vaccine for the market to prevent or lessen the infectious diarrhea diseases caused by TGEV and PEDV in pigs.

**Materials and methods**

- Product tested: PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea
- Animal: 50 pregnant sows for trial have never immunized any TGE and PED vaccines devided by three groups, group one for control with 10 sows non vaccination, group two for safety evaluation with 10 sows applied double recommendation dose and group three with 30 sows vaccinate as recommenadion dose.

**Result**

*Safety criteria*: There was no death pigs, no abnormally clinical signs caused by vaccine during experimental period. There was no adverse reactions in the first two hours and during 28 days after vaccination. There was no local reactions at injection position during the experimental period. Weight gain ability of vaccinated pigs was not influenced by vaccine. These results demonstrated that PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea, which was used to prevent the infectious diarrhea diseases caused by TGEV and PEDV, was safe when vaccination for experimental sows. After administration of a double recommendation dose, all of the vaccinated pigs had normal feeding and no any adverse reactions.

*Efficacy criteria*: After injection of the trial vaccine, all of sows were health and normal feeding. Immune response were measured on sows (colostrum and serum samples after delivery) and piglets (serum samples).

- *The result of colostrum antibody in sows*: Antibody against to TGE in colostrum: 95% (19 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. Antibody against to PED antibody in colostrum: 80% (16 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.
- *The result of serum antibody level in sows after delivery*: The antibody against to TGE in serum: 90% (18 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. The antibody against to PED in serum: 90% (18 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.
- *The result of serum antibody in piglets after to be delivered*: TGE antibody in serum: 80% (16 out of 20 samples) samples of vaccination group had TGE antibody. Control group had 100% negative samples. PED antibody in serum: 85% (17 out of 20 samples) samples of vaccination group had PED antibody. Control group had 100% negative samples.

**Conclusion**

- PRO-VAC TP vaccine, produced by Komipharm International Co., Ltd. Korea, which was used to prevent the infectious diarrhea diseases caused by TGEV and PEDV in pigs, was safe when vaccination for experimental pigs.
- When vaccinate PRO-VAC TP vaccine for experimental pigs, pigs had the immunization to against the infectious diarrhea diseases caused by TGEV and PEDV.

**Acknowledgement**

- This experiment was carried out at Phon Thinh Breeding farm, Thanh Long Co. Ltd located at Cu Yen ward, Luong Son district, Hoa Binh province, Vietnam.
- This experiment was supervised by National Center for Quality Control of Veterinary Medical Products, located at 30/78 Giai Phong street, Dong Da district, Hanoi city, Vietnam.
- All samples were tested at National Center for Quality Control of Veterinary Medical Products.

# Antibiotics and Human Health

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# Antibacterial efficiency of Dicol and reduction of antibiotics use in boar semen doses.

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## Introduction

Dicol® is an extender containing a combination of bactericides acting against a large spectrum of bacteria. The aim of this study was to demonstrate the effect of Dicol® on control of semen contamination *in vitro* and the use of antibiotics-free extender for final dilution.

## Materials and methods

Five ejaculates from five different boars were collected and divided into 3 groups which were initially diluted (1:1) with Dicol, Vitasem or Duragen extender. Subsequently, all groups were divided into 10 aliquots, and infected with $10^6$ cfu/ml of final concentration from pure isolated multi-resistant strains: *Klebsiella oxytoca, Burkholderia cepacia, Proteus mirabili, Serratia marcescens, Myroides spp, Morganella morganii, Providencia rettgeri, Achromobacter xylosoxidans, E. coli, Pantoea spp.* After 25 or 50 min, final dilution was carried out with Duragen, Vitasem or antibiotics free extender to reach a $3x10^7$ spermatozoa/ml. All samples were stored at 16ºC and bacterial load determined at 24 hours.

## Results

The results showed almost no growth (<1cfu/ml) of bacteria in samples of Dicol group even in the case of final dilution with antibiotics-free extender. In parallel, samples of Duragen group showed small growth of *Serratia marcescens* up to 100 ufc/ml. However, Vitasem group was more sensitive to bacterial growth such *Serratia marscence* and *Achromobacter xylosoxidans*.

## Conclusion

We concluded that Dicol is suitable tool for contamination control in ejaculates, and final dilution is also effective with free antibiotics extender.

**Simultaneous administration of ceftiofur hydrochloride and vaccines in pigs: effects on the postvaccinal immunity***

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**Introduction**

Ceftiofur (CEF) is a popular in veterinary medicine 3rd-generation cephalosporin with a broad spectrum of activity. It has been shown that beyond its antibacterial activity, it can inhibit cytokines secretion and potentially affect the host immune response [2,3]. The influence of CEF on the immune response has not yet been studied in pigs. In the present study we evaluated the influence of therapeutic doses of CEF hydrochloride on the postvaccinal immune response after vaccination with two model vaccines (live and inactivated).

**Materials and methods**

Seventy pigs were divided into 5 groups: control, unvaccinated (C), control vaccinated against swine influenza (SI-V), control vaccinated against pseudorabies (PR-V), vaccinated against swine influenza during ceftiofur administration (SI-CEF) and vaccinated against pseudorabies during ceftiofur administration PR-CEF.

The commercial product containing CEF hydrochloride (Ceftiocyl, Vetoquinol Biowet Sp. z o.o.) and 2 model vaccines (Akipor 6.3, Merial, France; GRIPOVAC, Merial, France) were used.

Pigs from SI-CEF and PR-CEF groups received recommended dose of CEF for 5 days (-1 to day 3). Pigs from SI-CEF, PR-CEF, SI-V and PR-V groups were vaccinated twice at day 0 and 14 of study. Antibodies to the glycoprotein B (gB) and gE antigen were determined with the use of ELISA tests (HerdChek*Anti-PRVgB and HerdChek*Anti-PRVgp1, IDEXX Laboratories, USA). Humoral responses to SIV were assessed based on haemagglutination inhibition (HI) assay. The proliferation assay was done at day -1, 6, 9, 14, 28, 42, 56 and 70 of study, as described previously [1].

The concentration of Th1 or Th2-type cytokines (IFN- γ and IL-4) in culture supernatant after ex vivo re-stimulation of PBMC with PRV and H3N2 SIV were determined with the use of ELISA kits specific for porcine IFN-γ and IL-4 (Invitrogen Corporation, USA). Unstimulated cells served as control (mock control).

**Result**

The significant delay in the development of humoral response against PRV and a significant suppression of production of anti-SIV antibodies was found in pigs receiving CEF hydrochloride at the time of vaccination. The cellular immune response against PRV was also significantly affected by CEF. In contrast, there were no significant differences between vaccinated groups with regard to the T-cell response against SIV. The concentration of INF-γ in culture supernatants were significantly lower in group treated with CEF after restimulation with PRV. While, no significant differences were observed after restimulation of PBMC with SIV.

**Discussion**

The results of the present study indicate that both, humoral and cell-mediated postvaccinal immune responses can be modulated by CEF treatment. The significant reduction in ELISA S/N ratio against gB PRV antigen was observed before the second dose of vaccine. It may suggest that CEF hydrochloride exert its effect mainly on the IgM isotype. The marked reduction in the anti-HA antibody titer may be of great importance since anti-HA antibody has an important role in the protection against swine influenza. In pigs vaccinated during CEF therapy the mean HI titre were significantly lower than in control pigs. The results of our study point out that caution should be taken when CEF is given during vaccination of pigs.

**Reference**

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# Susceptibility levels of porcine respiratory bacteria to tiamulin and chlortetracycline and their combination efficacies

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## Introduction

Porcine respiratory disease complex (PRDC) has multifactorial etiology involving a variety of bacterial and viral infections and environmental stressors. The disease is a major cause of economic loss in pig industry. The major respiratory bacterial pathogens of nursery and fattening periods are included *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis, Streptococcus suis* type II, and *Pasteurella multocida.*

The routine antimicrobial groups used for controlling bacterial respiratory infection are pleuromutilin, macrolide, beta-lactams, lincomycins and tetracyclines. However, the situation of antimicrobial resistance has been thought as a threat of production, which becomes an impact to pig health and net cost of unit assessment. Up to date, there has not been a launch of novel antimicrobial in animal used for 40 years and use of alternative agents or formulation against the microbes are taken into practical consideration. Thus, update data of susceptibility level against the field pathogens are always useful for making antibiotic of choice and selection of antibiotic combination is needed to validate before practical use.

The aims of this study was to determine the susceptibility level of tiamulin and chlortetracycline to 4 respiratory pathgens comprising *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis, Streptococcus suis* type II, and *Pasteurella multocida.* The usable of tiamulin and chlortetracylcine combination was confirmed.

## Materials and Methods

*Susceptibility test by broth microdilution.*

Five strains of *Pasteurella (P.) multocida, Actinobacillus (A.) pleuropneumoniae, Haemophilus (H.) parasuis* and *Streptococcus (S.) suis* type II isolated from respiratory clinical sign pigs during 2013-2014 and species identification by biochemical test were used in susceptibility test. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27853 were used as a control group. Broth microdilution susceptibility test against tiamulin (Sandoz, Austria) and chlortetracycline (Zhumadian Huazhong Chia Tai, China) was determined following CLSI standard (3). For APP and *H. parasuis* broth medium were added 0.5% yeast extract and 1% hemophilus test medium supplement SR0158E incubated at 37$^0$C and interpreted the results at 48 hour of incubation.

*Synergistic effect of tiamulin combined with doxycycline finding by microdilution checkerboard susceptibility test*

The highest concentration of antimicrobial used in experiments was higher 4 times of MIC and 2-fold dilution until lower 4 times of MIC. The concentrations of each antimicrobial was 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 (4).

## Results and Discussion

The MICs of *H. parasuis* to tiamulin and chlortetracycline were in susceptible level whereas the rest bacteria were resistant (Table 1). Use of tiamulin and chlortetracycline combination could reduce the individual MICs of *P. multocida* and *H. parasuis*, *in vitro*. Controversially, the combination MICs of *Streptoccus suis* type II and *A. pleuropneumonia* were not reducible and still ranged in resistant level. The results showed that tiamulin mixed chlortetracycline had synergistic effects against *H. parasuis* and *P. multocida* but showed indifferent effects with *Streptococcus suis* type II and *A. pleuropneumoniae.*

**Table1** Single MICs and tiamulin combined chlortetracycline MICs of the respiratory pathogenic bacteria

| Organisms | Tiamulin | | Chlortetracyclines | |
|---|---|---|---|---|
| | MIC | Combined MIC | MIC | Combined MIC |
| *S. suis* type II<br>Resistant<br>Breakpoint | 64<br>≥*32* | 64 | >1024<br>≥*2* | >1024 |
| *A. pleuropneumoniae*<br>Resistant<br>Breakpoint | 8<br>≥*32* | 8 | 64<br>≥*2* | 64 |
| *H. parasuis*<br>Resistant<br>Breakpoint | 1<br>≥*32* | 0.5-1 | 2-8<br>≥*2* | 0.5-4 |
| *P. multocida*<br>Resistant<br>Breakpoint | 8-16<br>≥*32* | 2-4 | 16-32<br>≥*8* | 1-4 |

**Table 2.** Interpretation of the combination efficacy between tiamulin and chlortetracycline.

| Bacteria | synergistic | indifferent | antagonistic |
|---|---|---|---|
| *S. suis* type II | 0 | 5 | 0 |
| *A. pleuropneumoniae* | 0 | 5 | 0 |
| *H. parasuis* | 0 | 5 | 0 |
| *P. multocida* | 5 | 0 | 0 |

The bacterial used were recently isolated from diseased pigs in central area of Thailand, where routinely used of intensive antibiotics. Increase of resistant level among tested isolates were revealed in this study (2). There was no antagonist effects between the couple, *in vitro*. The ability of antibiotic combination to reduce the individual MICs was found on *P. multocida* and *H. parasuis,* and FIC index could confirm the synergistic effect for *P. multocida.*

## References

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# The use of a high concentration enrofloxacin (Baytril100®) to control post-parturient disorders in primiparous sows

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## Introduction

Since most of the postparturient disorders in sows involve bacterial infection and endotoxins, the quality of antibiotic used in sows therefore needs to be addressed. Enrofloxacin is a fluoroquinolone antibiotic developed for use in veterinary medicine. In general, this drug has a broad distribution in the organism, excellent tissue penetration and long half-life in the blood [1]. In practice, many formulations of enrofloxacin are used in veterinary medicine in numerous species. However, the efficacy of different enrofloxacin formulations based on clinical observation has never been comprehensively evaluated. The objective of the present study was to determine the use of a high concentration enrofloxacin (Baytril100®) to control postparturient disorders in primiparous sows.

## Materials and Methods

The study was conducted in a commercial swine herd in Thailand and included 42 primiparous sows. The sows were housed in an open-housing system. The gilts entered the farrowing house one week before farrowing. Duration of parturition was carefully determined. The sows were randomly divided into two groups, i.e., group I (n=16) and group II (n=26). Group I sows received enrofloxacin type one 7.5 mg/kg IM (enrofloxacin 100 mg/ml, Syvaquinol®100), an anti-inflammatory drug (Tolfédine®) and vitamins (Fercobseang®) for three days postpartum. Group II sows received the same supportive treatment as group I and enrofloxacin type two 7.5 mg/kg IM (enrofloxacin 100 mg/ml, Baytril100®). The rectal temperature and the presence of abnormal vaginal discharge were determined at Days 0, 1, 2, and 3 of parturition. Sows with a rectal temperature of ≥39.0°C were regarded as having a fever. Abnormal vaginal discharge was defined as '1' when a certain amount of abnormal discharge (≥5 ml) was observed and '0' if abnormal discharge was absent. The data were analysed by Chi-square or Fisher's exact tests. $P<0.05$ was considered to be statistically significant.

## Results

The effects of type of antibiotics on postparturient disorders are presented in Table 1. On Day 2 postpartum, incidence of abnormal vaginal discharge in group II was less than in group I ($P=0.002$) (Table 1).

**Table 1** Postparturient disorders in sows treated with enrofloxacin type I compared with enrofloxacin type II

| Variables | Group I | Group II |
|---|---|---|
| Total born/ litter | 11.4[a] | 11.7[a] |
| Live born/ litter | 10.0[a] | 9.8[a] |
| Stillborn (%) | 10.5[a] | 7.9[a] |
| Mummy (%) | 3.4[a] | 9.4[a] |
| Farrowing duration (h) | 93.8[a] | 88.9[a] |
| Fever D0 (%) | 87.5[a] | 84.6[a] |
| Fever D1 (%) | 68.8[a] | 53.8[a] |
| Fever D2 (%) | 50.0[a] | 50.0[a] |
| Vaginal discharge D0 (%) | 18.8[a] | 19.2[a] |
| Vaginal discharge D1 (%) | 68.8[a] | 50.0[a] |
| Vaginal discharge D2 (%) | 87.5[a] | 38.5[b] |

[a,b] different superscript differ significantly ($P<0.05$)

## Conclusions and Discussion

Different formulations and/or manufacturing of different antibiotics may lead to different pharmacokinetics and different drug penetration in different tissues [1,2]. Thus, the response of each individual sow after the antibiotic treatment should be carefully determined. The present study revealed that the use of highly efficient antibiotic in sows postpartum may help to eliminate some of the infected bacteria and hence reduce the incidence of abnormal vaginal discharge. Based on the present findings, intensive care postpartum should be increased in primiparous sows.

## References

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# Antimicrobial Resistance in Enterohemorrhagic *Escherichia coli* of Edema Disease Isolated from Swine in Taiwan

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## Introduction

Edema disease is one of the most prevalent porcine diseases worldwide, which is caused by enterohemorrhagic *Escherichia coli* (EHEC). Shiga-like toxin 2e (Stx2e) is the most important virulence factor of EHEC. This study was conducted to determine the multiple drug resistance in EHEC. First, polymerase chain reaction (PCR) technique was used to detect the gene of Stx2e toxin to identify EHEC. Then, broth micro-dilution technique was used for antimicrobial susceptibility testing to analyze minimal inhibitor concentration (MIC) values of antibiotics in EHEC. This study was performed in order to understand the current situation of EHEC in Taiwan.

## Materials and methods

In this study, *E. coli* were isolated from sick pigs which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan from 2011 to 2015. The isolates were processed for DNA extraction and amplification of *stx2e* gene by PCR. *E. coli*, which were positive for *stx2e* gene, were tested using a broth micro-dilution technique to antimicrobial susceptibility testing as described in the Clinical and Laboratory Standards Institute (CLSI) [1]. The following antibiotics were selected for antimicrobial susceptibility testing: amikacin (AK), amoxicillin (AMO), cefazolin (CZ), ceftiofur (EFT), ciprofloxacin (CIP), colistin (CO), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFC), gentamicin (GN), oxytetracycline (OTC) and sulfamethoxazole-trimethoprim (TS).

## Results

The results showed that 65 isolates of *E. coli* were positive with *stx2e* gene. And, all isolates were isolated from weaning pigs (5-8 week-old pigs). The percentage of antibiotics resistance of these isolates for AK, AMO, CZ, EFT, CIP, CO, DO, ENR, FFC, GN, OTC and TS were 8 %, 97 %, 92 %, 89 %, 94 %, 22 %, 58 %, 89 %, 100 %, 72 %, 92 % and 92 %, respectively (*Figure 1*). Fifty-six isolates (86 %) had multiple drug resistance for more than eight drugs. Moreover, a significant proportion (38 %) of EHEC had resistance to AMO, CZ, EFT, CIP, DO, ENR, FFC, GN, OTC and TS.

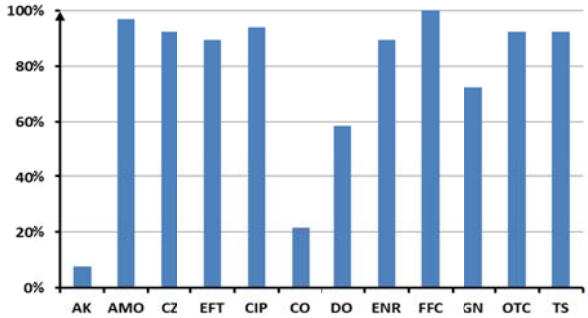


***Figure 1.*** The percentage of antibiotics resistance in EHEC. AK: amikacin, AMO: amoxicillin, CZ: cefazolin, EFT: ceftiofur, CIP: ciprofloxacin, CO: colistin, DO: doxycycline, ENR: enrofloxacin, FFC: florfenicol, GN: gentamicin, OTC: oxytetracycline, TS: sulfamethoxazole-trimethoprim.

## Discussion

In Taiwan, edema disease occurs frequently and causes huge economic losses to farmers. However, our study indicated that drugs resistance of EHEC was serious and widespread. Only amikacin and colistin are more sensitive to EHEC. Therefore, it's difficult to control edema disease by treatment with antibiotics. These results gave important hints that we should search for other methods to prevent and control the swine edema disease in Taiwan.

## References

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# Prevalence of Plasmid-mediated Quinolone Resistance Genes in *Escherichia coli* Isolated from Diseased Pigs in Taiwan

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## Introduction

The mechanisms of quinolone resistance were initially identified to be mediated by mutations located in the quinolone resistance-determining region (QRDR) of the *gyrA* and *parC* genes. To date, three types of plasmid-mediated quinolone resistance (PMQR) determinants, including a Qnr-mediated inhibition of quinolone binding to DNA, a QepA encoded efflux pump, and the *aac(6')-ib-cr* mediated fluoroquinolones (FQ) acetylation, have been identified in clinical isolates[1]. The aims of this study were to investigate the prevalence of PMQR gene in *E. coli* from diseased pigs in Taiwan, evaluating the occurrence of QRDR mutations among the PMQR-positive isolates and the correlation of genotypes with the FQ resistance phenotypes.

## Materials and methods

In this study, *E. coli* were isolated from diseased pigs, which were sent to the Animal Disease Diagnostic Center of National Chiayi University in Taiwan from 2011 to 2014. *E. coli* were screened and selected according to Clinical and Laboratory Standards Institute guidelines, minimal inhibitory concentration test (MIC) was used for antimicrobial susceptibility testing. The selects of drugs were concluded to be nalidixic acid (NAL), flumequine (FLU), enrofloxacin (ENR), ciprofloxacin (CIP) and moxifloxacin (MOX). *E. coli* isolates were processed for plasmid extraction and screening for the *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA* and *aac(6')-ib-cr* genes was carried out by PCR amplification. PCR products were sequenced and searched using BLAST for identifying positive isolates. PCR amplifications in QRDR of the *gyrA* and *parC* genes among PMQR-positive isolates were carried out using specific primers, and all PCR products were sequenced. The sequences were analysed with DNASTAR and compared with wild-type GyrA (No. NP_416734) and ParC (No. NP_417491) of *E. coli* K-12.

## Results

The analysed results of the 595 isolates of *E. coli* from diseased pigs in Taiwan from 2011to 2014 whoed thatthe percentages of resistance of these isolates for NAL, FLU, ENR, CIP and MOX were 72.61 %, 71.76 %, 48.07 %, 39.83 % and 47.06 %, respectively. Of the 595 *E. coli* isolates, 22.52 % (134/595) harboured at least one PMQR gene. The most common PMQR gene was *qnrS* (14.29 %), followed by *aac(6')-ib-cr* (8.40 %). Alterations in the GyrA were detected in 73 (54.48 %) of the 134 PMQR-positive strains tested. Of the isolates harbouring additional mutations in ParC, 54 out of the 73 strains (74.00 %) were with GyrA mutations. The correlation between genotype and phenotype of 134 PMQR-positive *E. coli* isolates are shown in Table 1.

**Table 1.** Results of sequence analysis of the QRDR and MICs of the quinolones for PMQR-positive *E. coli* strains

| PMQR | QRDR [b] | | | | $MIC_{50}$ (mg/L) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gyr A | | Par C | | NAL | FLU | ENR | CIP | MOX |
| | 83S | 87D | 80S | 84E | | | | | |
| *qnr S* | - | - | - | - | 16 | 8 | 1 | 0.5 | 1 |
| | A | - | - | - | 64 | 16 | 2 | 1 | 4 |
| | L | - | - | - | 512 | 32 | 8 | 4 | 8 |
| | L | - | R | - | >1024 | 512 | 4 | 4 | 4 |
| | L | - | I | - | >1024 | 512 | 32 | 32 | 64 |
| | L | N | I | - | >1024 | 1024 | 32 | 32 | 64 |
| *qnr S*, *aac* [a] | L | N | I | - | >1024 | 1024 | 128 | 128 | 128 |
| *aac* [a] | - | - | - | - | 8 | 1 | ≦0.125 | ≦0.125 | ≦0.125 |
| | - | G | - | - | 128 | 4 | ≦0.125 | 0.25 | ≦0.125 |
| | A | - | - | - | 128 | 16 | 0.5 | 1 | 0.5 |
| | L | - | I | - | >1024 | 1024 | 2 | 4 | 1 |
| | L | - | R | - | >1024 | 512 | 4 | 16 | 2 |
| | L | N | I | - | >1024 | 1024 | 128 | 128 | 64 |
| | L | Y | I | - | >1024 | 1024 | 64 | 64 | 32 |
| | L | N | I | A | >1024 | 1024 | 128 | 64 | 32 |
| | L | N | I | G | >1024 | 1024 | 256 | 128 | 64 |

[a]: *aac(6')-ib-cr*
[b]: S: Serine, D: Aspartic acid, E: Glutamate, A: Alanine, L: Leucine, R: Arginine, I: Isoleucine, N: Asparagine, G: Glycine, Y: Tyrosine.

## Discussion

These results indicated that FQ resistance of swine *E. coli* isolates was serious in Taiwan. The number of mutations in *gyrA* and *parC* correlated significantly with the quinolone MICs. Overuse of various antimicrobials in animal farms may have served as a major selection pressure for horizontal transfer of these resistance elements, and these PMQR determinants can facilitate the selection of resistant mutants. Based on our study results, conservative use of antimicrobial agents in animal farms in Taiwan is strongly recommended.

## Reference

1. Hopkins K. L. *et al*. 2005. Int J Antimicrob Agents 25: 358-373.

# Tylan Efficacy to Reduce Fecal Shedding of *Lawsonia intracellularis* in Pigs: Case Report

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## Introduction

*Lawsonia intracellularis* (LI) is the causative bacterial agent of ileitis or porcine proliferative enteropathy (PPE). Pigs with LI infection can show clinical and also subclinical signs[1]. The disease severity depends on the infectious dose of LI[1,2]. With higher infectious doses of LI, the clinical disease symptoms are more severe. Even low infectious doses in subclinically affected pigs not showing clinical signs, cause significant economic losses when compared with non-infected pigs[1,2]. Nowadays, control of LI infection is based on antimicrobial application rather than vaccination due to limitations in case of vaccination[3]. The advantage to use effective antimicrobials is due to the reduction of clinical signs, disease prevalence and performance losses but also based on reduction of problems from other bacterial endemic disease in pig herd[3]. The aim of this study was to quantify the amount of LI shedding via feces after using Tylan in growing pigs on a farm affected by ileitis endemic disease.

## Materials and Methods

This study was conducted in the finishing unit of one commercial farm in Thailand. This farm was a one-site production and had 2,500 sows with a history of enteric disease associated with LI infection during the finishing period (age 11-12 and 16-17 weeks). Ileitis prevalence was 15.56% in grower-finisher period. Eight week old-pigs (n=562), moved from nursery to finishing unit, were medicated in feed with Tylan (Tylosin, Elanco Animal Health) at 110 ppm (3-6 mg/kg BW) for 2 time periods, at 11-13 week and 16-18 weeks of age, based on history of the infection time periods

Fifteen pigs were randomly selected and tattooed for individual collection of fecal samples. Fecal samples from the same pig were collected 3 times before (day 0) and during the treatment at 2 weeks (day 15) and 3 weeks (day 22) of 11-13 week old pigs. All fecal samples were submitted to Farm Animal Hospital of the Faculty of Chulalongkorn University, Nakornpathom province. Real-time PCR was used to count the amount of LI in feces. ANOVA was used for statistical analysis - result significant at $P \leq 0.05$.

## Results

The percentage of positive pigs after 2 and 3 weeks of medication reduced significantly compared before onset of medication (Figure 1). The amount of LI in one gram of feces was also reduced after 2 and 3 weeks of medication with Tylan (Figure 2).

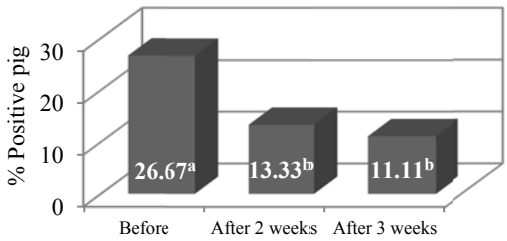


**Figure 1** Percentage of positive pigs at different time periods of medication

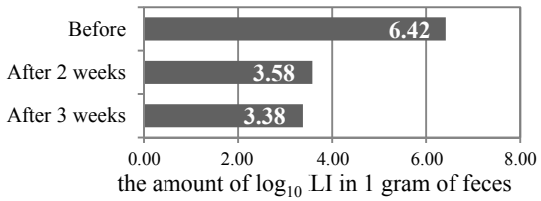


**Figure 2** Amount of LI shedding in feces pre and during medication

## Discussion

Results of this study indicate that Tylan fed at 110 ppm for 3 weeks in a farm with history of ileitis problems even at low disease prevalence can reduce significantly the percentage of LI positive pigs. Moreover, LI fecal shedding is reduced. In conclusion, Tylan medication of LI infected pigs at treatment dosage will reduce disease severity and LI spreading which will help to prevent pig performance losses. Therefore, Tylan is considered as an effective medication of LI infections.

## References

1. Paradis et al., 2012. Journal of Swine Health and Production. 20(3):137-141
2. Pedersen et al., 2012. http://www.actavetscand.com/content/54/1/58
3. Holyoake et al., 2009. Australian Veterinary Journal. 87(1-2): 33-34

# Update of Ileitis Seroprevalence status in Grower-Finisher Pigs in Thailand

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## Introduction

Ileitis is an important enteric disease in grower-finisher pigs caused by Lawsonia intracellularis (LI). Ileitis prevalence had been reported in many countries and tested by using fecal PCR or serological diagnosis. Advantages of serological tests or called seroprevalence versus fecal PCR: low costs, time effectiveness, simple to perform, high throughput1. Moreover, seroprevalence tests provide useful informations on time of seroconversion to determine the onset of infection in swine herds and also to detect clinical and subclinical forms of ileitis2,3. Knowledge of time of infection helps to establish proper ileitis treatment strategies to minimize disease impact and to prevent production losses2. Ileitis seroprevalence in Thailand had been reported in 20104. Chanunda et al. found that 90% of grower-finisher herds were seropositive, during 8-12 weeks of age the animals seroconverted and seropositive status decreased until 20 weeks of age. The objective of this study was to update the seroprevalence situation in grower-finisher pigs in Thailand during March 2014 to March 2015.

## Materials and Methods

Blood samples were collected from 7 commercial pig farms located in Western, Eastern, Northern and Southern part of Thailand. All farms had a history of diarrhoea, low mortality and weight variation in grower-finisher period. No farm in this study controlled ileitis by vaccination. Five hundreds and fifteen blood samples were randomly collected from each age of pigs at 3, 6, 9, 12, 15, 18, 21 and 24 weeks of age as cross-section. All samples were sent to the Farm Animal Hospital of the Faculty of Chulalongkorn University at Nakornpathom province and were tested by IgG antibody determination against LI infection by using immunoperoxidase monolayer assay (IPMA) technique.

## Results and Discussion

The study results show that 100% of the grower-finisher farms (7 farms) were seropositive on ileitis. The percentage of positive farms in this study was slightly higher than in a previous study in Thailand (4). Average ileitis seroprevalence in each farm was around 34% (173 out of 515). Percentage of positive samples in 3, 6, 9, 12, 15, 18, 21 and 24 week old pigs was 43.33%, 12.73%, 17.14%, 30.88%, 40.63%, 31.63%, 50.00% and 53.33%, respectively (Figure 1). High percentage of positive samples at 3 weeks of age is based on acquired maternal immunity and then percentage of seropositive reaction decreased until 6-9 weeks of age. The time of seroconversion was found during 9-12 and 18-21 weeks of age. Obviously pigs were LI-infected during two different time points during their live. It can be estimated from our results that natural infection with LI takes place at around 10 and 19 weeks of age. These results are in contrast with a previous study (4) that showed only one-time point of pig production was exposed with LI. Possibly the differences are based on different age of sampling or the patterns of infection have changed.

Figure 1 Percentage of sero-positive samples at different ages

In summary, pattern of LI infection was dynamic. Many factors can impact the percentage of positive sampling and disease prevalence. Implementation of routine seroprevalence monitoring is considered important to determine proper timing for antimicrobial administration and effective ileitis control.

## References

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## Acknowledgement

The results was reviewed and commented by Dr.Suphot Wattanaphansak from Chulalongkorn University.

# Field Study: The Efficacy of Denagard 20% Injection for Control of Lameness and Arthritis in Nursery

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## Introduction

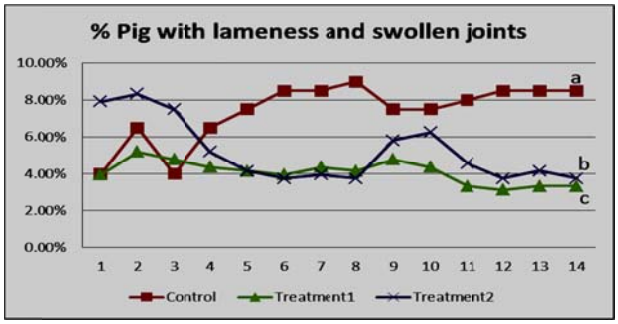
Lameness and arthritis in nursery pigs can be caused by several bacteria species including *Streptococcus suis* (SS), *Haemophilus parasuis* (HP) and *Mycoplasma hyorhinis* (MHR). Similar clinical signs and gross lesions based on those infections are found: polyarthritis with swollen joints, respiratory signs, pleuritis and peritonitis. Large amounts of synovial fluid in affected joints are a characteristic of MHR-based arthritis.[1,3]. Tiamulin is one of the antimicrobials being most susceptible for MHR strains[2]. In case of arthritis caused by MHR, the injection of Denagard® 20% (Tiamulin) injection has been proven to reduce swollen joints in nursery pigs[4]. This study aims to investigate the efficacy of Denagard® 20% injection to control lameness and arthritis in a herd infected by MHR.

## Materials and methods

In a 7,000 sows farrow to finish farm MHR infection was confirmed in weaning and nursery by bacteria isolation and direct PCR. Lameness and polyarthritis with swollen joints and huge amounts of synovial fluid was found. 1,160 weaning piglets were divided into three groups. Control group, 200 pigs, received no parenteral injection. Treatment group 1&2, 480 pigs each, received Denagard® 20% injection at 0.5 ml/pig for 1&2 days, respectively. Number of pigs with swollen joints and lameness were determined every day for 14 consecutive days (D1 is the 1st injection day). All groups were located in the same building and received medicated feed contained Amoxicillin500ppm + Colistin200ppm+Tylvalocin150 ppm during the study. Data was converted to percentage and analyzed by T-Test at $P<0.05$.

## Results

The results of the study are summarized in Figure1.




**a,b,c: *P*<0.05**

Figure 1 Percentage comparison of pigs showing lameness and swollen joints from control, treatment 1 and treatment 2 groups in 14 days during and after treatment.

## Discussion

The results indicate that the percentage of pigs with lameness and swollen joints before treatment onset were 4.00%, 3.96%, and 7.92% in control, treatment1, and treatment2, respectively. After injection of Denagard® the percentage of pig with lameness and joint swelling were decreased especially in treatment 2. After 14 days the percentage of pigs with lameness and swollen joints were 8.50%, 3.33%, and 3.75%, in control, treatment1&2 groups, respectively. In conclusion, once or twice parenteral injections of Denagard® 20% at treatment dosage can significantly control the incidence of lameness and joint swelling in weaning to nursery pigs in cases of mycoplasmal arthritis.

## Reference

1. Makhanon et al. 2012. Trop. Anim. Health Prod. 44 p.313-318.
2. Makhanon et al. 2014. Proc. 23rd IPVS, C-003, p. 302.
3. Reddy et al. 2000. Infection and Immunity. 68(3), p.1150-1155.
4. Talummuk et.al. 2010. Proc. 21st IPVS, P-706, p.1012.

# Field Study: The Efficacy of Denagard 20% Injection for Treatment of Lameness and Arthritis in Nursery

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## Introduction

Lameness and arthritis in nursery pigs can be caused by several bacteria including *Streptococcus suis* (SS), *Haemophilus parasuis* (HP), and *Mycoplasma hyorhinis* (MHR). Similar clinical signs and gross lesions due to those infections are identified: polyarthritis with swollen joints, respiratory signs, pleuritis, peritonitis. Large amount of synovial fluid in affected joints are characteristic for MHR-infections[1,3]. Tiamulin is one of the antimicrobials most susceptible for MHR strains[2]. In case of arthritis caused by MHR, the injection of Denagard® 20% (Tiamulin) injection has been proven to reduce the joint swelling in nursery pigs[4]. This study aims to confirm the treatment effect of Denagard Injection against lameness and arthritis in a herd infected by MHR.

## Materials and methods

In a 7,000 sows farrow to finish farm MHR infection was proven in weaning and nursery by bacteria isolation and direct PCR. Lameness and polyarthritis with swollen joints and huge amounts of synovial fluid occurred in every batch of animals. Forty weaning piglets with joint swellings at 4-5 weeks of age were divided into two equal groups. Control group received no injection and treatment group received Denagard® 20% injection at 1ml/20kg bw for 3 consecutive days. The perimeter of four legs were measured before injection (D0), 24hrs after the 1st and the 2nd injection (D1 and D2), and 8 days after the 3rd injection (D11). Both groups received medicated feed containing Amoxicillin500ppm+Colistin 200 ppm +Tylvalocin150ppm during the study. Data was analyzed by T-Test at $P<0.05$.

## Results

Results of the study are summarized in Table1.

**Table.1 Comparison of mean joint perimeter (in cm)**

| Legs | Observed days | Mean Joint Perimeter (cm) | |
|---|---|---|---|
| | | Control (N=20) | Treatment (N=20) |
| Left Fore Leg | D0 | 9.21±0.41 | 9.56±0.72 |
| | D1 | 9.36±0.47 | 9.36±0.64 |
| | D2 | 9.40±0.53 | 9.11±0.48 |
| | D11 | 9.68±0.50 [a] | 8.96±0.54 [b] |
| Left Hind Leg | D0 | 11.05±0.59 [a] | 12.35±0.84 [b] |
| | D1 | 11.52±0.69 [a] | 12.12±0.75 [b] |
| | D2 | 11.82±0.64 | 11.69±0.54 |
| | D11 | 12.00±0.72 [a] | 11.41±0.53 [b] |
| Right Fore Leg | D0 | 9.03±0.51 [a] | 9.47±0.72 [b] |
| | D1 | 9.33±0.54 | 9.35±0.55 |
| | D2 | 9.39±0.55 | 9.18±0.38 |
| | D11 | 9.64±0.69 [a] | 9.11±0.39 [b] |
| Right Hind Leg | D0 | 11.33±0.47 [a] | 12.23±0.66 [b] |
| | D1 | 11.76±0.58 [a] | 11.80±0.49 [b] |
| | D2 | 11.93±0.54 [a] | 11.57±0.50 [b] |
| | D11 | 12.32±0.62 [a] | 11.32±0.58 [b] |

**a,b: $P<0.05$**

## Discussion

The results confirm that the mean perimeter of pigs in the treatment group was larger than control group before injection of Denagard®. The perimeter of pigs in the treatment group changed to significantly smaller than in the control group at 8 days after the 3rd injection for every leg. Data confirm previous study results that injection of Denagard® can reduce amounts and incidence of swollen joints in case of mycoplasmal arthritis[4].

## Reference

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7. Reddy et al. 2000. Infection and Immunity. 68(3), p.1150-1155.
8. Talummuk et.al. 2010. Proc. 21st IPVS, P-706, p.1012.

# EFFICACY OF ALGAE-BASED COMPLEMENTARY FEED ECOPIGLET® IN REDUCING THE USE OF MEDICATION FOR PIGLETS IN MATERNITY

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## Introduction

At birth, the piglet's small intestine is not mature and it is challenged by dietary, pathogenic and environmental factors. As a consequence, health and growth performances of piglets during lactation are hampered. On the other hand, the heavy use of antibiotics in farms for the past decades in order to control animal performance has significantly contributed to increase bacterial antibiotic resistance and is a threat for both animal and human health. Therefore, research is being done to find solutions in protecting the development and integrity of the small intestine of farmed pigs, while aiming at reducing the use of antibiotics. In this context, Olmix has developed a complementary feed based on minerals and algae, whose active principles may stimulate the secretion of mucin in the intestine [1] and protect the gut mucosa. The objective of the present study was to evaluate the capacity of this complementary feed to improve growth and health performances of piglets as well as decrease the curative use of antibiotics during lactation.

## Materials and methods

The study took place in a commercial farm of 1,800 sows in Northern Vietnam. Litters from 80 sows (959 piglets) were involved in the study. Sows and their litter were randomly allotted to two groups of 40 litters: a control group and a test group. The number of first parity sows was the same (10) in each group. Litter size was balanced in both groups (12 piglets / litter) by piglets fostering. Ecopiglet® complementary feed was distributed to the test group every day from day 5 to weaning (23 days old). The daily dosage per day and litter was 70 g, distributed in liquid form (70 g of complementary feed with 20 ml of water) into piglet's feeders. Litters were weighed at birth and piglets were individually weighed at weaning. Diarrhoea incidence, mortality and veterinary treatments were recorded daily.

## Results

The average weight of control litters after fostering was slightly higher than in the test group (18.46kg vs 18kg respectively for control and test group). However, the average weight of litters at weaning was higher in the test group than in the control group (67.2 vs 65.4 respectively for test and control group). Control piglets weighed an average of 6.07 kg at 23 days while test piglets weighed an average of 6.23 kg at the same age. Mortality rate was 10.21% in the control group and 9.81% in the test group. Moreover, diarrhoea incidence was significantly decreased ($p=0.04$) in the test group in comparison with control. As a consequence, the use of enrofloxacin antibiotic was decreased (see Table 1).

***Table.1*** *Effect of Ecopiglet® complementary feed on piglets' diarrhoea incidence and use of enrofloxacin.*

| | Control group | Test group | P-value |
|---|---|---|---|
| Diarrhoea incidence | | | |
| Number of piglets* | 1019 | 760 | 0.04 |
| Percentage | 9.23 | 6.90 | 0.04 |
| Use of enrofloxacin | | | |
| Volume (ml) | 409 | 249.5 | |
| Days of use (n) | 22 | 14 | |

*Cumulated number of piglets which suffered diarrhoea every day.

## Discussion

Under the conditions of this study, Ecopiglet® improved growth performance of piglets and decreased diarrhoea incidence as well as the use of antibiotic (enrofloxacin) for curative purpose. Therefore, its use in lactation should be considered as a solution to decrease the use of antibiotics while improving piglets level of performance.

## Reference

[1] Barcelo A. et al. 2000. Gut 46:218-224.

# IMMUNOMODULATORY ACTIVITIES OF A SULFATED POLYSACCHARIDE-RICH EXTRACT OF GREEN ALGAE

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## Introduction

Antibiotics have been used for a long time in pig production to protect animals against pathogens. However, EU policy has been adopted to implement a sustainable production without adding antibiotics as growth promoters. Marine algae contain in their cell wall water soluble sulfated polysaccharides with potential biological activities such as anticoagulant, antiviral, antibacterial and immunomodulating activities that are being explored to be used as an effective alternative to antibiotics (1, 2). A crude extract containing sulfated polysaccharides was prepared from the green algae *Ulva armoricana*. The ability of this extract to stimulate the expression of the immune response mediators was evaluated using an *in vitro* system of porcine differentiated intestinal epithelial cells IPEC-1.

## Materials and methods

The tested algae extract is composed of 11.6% of neutral sugars, 7.3% of proteins, 12.2% of uronic acids and 26.4% of sulfated polysaccharides. The capacity of this extract to stimulate the expression of immune mediators has been evaluated by RT-qPCR, using an *in-vitro* culture system of differentiated porcine intestinal epithelial cells, IPEC-1. Three doses (1, 0.1 and 0.01 mg/ml) were tested in comparison with *E. coli* O111:B4 LPS as positive control and with cells incubated alone as negative control. The extract was also tested on a human embryonic renal cell line, HEK293 which expresses TLR4/MD2/CD14, TLR2, TLR5, TLR9, NOD1 and NOD2, in order to identify which TLR or NLR receptor was involved. Expression of IL-8 with ELISA was used as a marker of stimulation. Statistical analysis was done with Kruskal-Wallis and Bonferroni-Dunn tests.

## Results

Analysis by RT-qPCR showed that the algae sulfated polysaccharide extract, at 1 mg/ml, induced an increased expression of several cytokines in comparison with non-treated cells (Table 1). CCL20 chemokine showed the highest stimulation (38 times higher than the control), followed by IL-8 (10.8 times higher), TNF-α (8.3 times higher), IL-1 α (7.1 times higher) and IL-6 (4 times higher). It also induced, to a lower extent, a significant increase of IL-1β, IL-12p40, TGF- β and PPAR γ expression. The algae extract did not seem to influence the expression of IL-10, CCL25, CCL28, TLR2 and TLR4 (Table 1). LPS did not induce the expression of these mediators in comparison with the algae extract.

When tested with HEK293 cells on several membrane receptors, it seemed that the algae extract stimulated the expression of immune factors via the activation of TLR4 membrane receptor.

***Table.1*** *Influence of the algae sulfated polysaccharide extract (1mg/ml) on cytokines expression by IPEC-1 intestinal cells (**: P<0.01)*

| Tested immune mediators genes | Rate of stimulation of the expression with regard to control |
|---|---|
| TNFα | 8,3** |
| IL-1α | 7,1** |
| IL-8 | 10,8** |
| CCL20 | 38,4** |
| IL-6 | 4** |
| IL-1β | 2,1** |
| IL-12 p40 | 2,0** |
| TGFβ | 1,7** |
| PPAR γ | 2,4** |
| TLR2 | 2,4 |
| IL-12 p35 | 1,8 |
| IL-10 | 1,1 |
| CCL25 | 2,2 |
| CCL28 | 1,5 |
| TLR4 | 1,6 |

## Discussion

These results showed that the sulfated polysaccharide-rich extract of green algae has the capacity to stimulate, *in vitro*, the expression of cytokines involved in the immune response. This suggests that this extract could be used as a new prophylactic strategy to stimulate the immune response of animals and reduce the use of antibiotics in farms.

## Reference

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# The influence of Lianol® Ferti supplementation during lactating-weaned sows on the weaning to oestrus interval (WOI)

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## Introduction

The number of litters produce per sow per year is influenced by litter size, farrowing rate and weaning to oestrus interval (WOI). It has been shown that sows that return to oestrus within 7 days after weaning have a high farrowing rate and litter size. However, oestrus is delayed in a variable proportion of sows. Nutritional deficit or inadequate level of FSH/LH during lactation is well known to increase the WOI, especially in primiparous sows. Lianol® Ferti is a highly digestible fermented potato protein of which the use results in prometabolic peptides. The fertility effect of the Negative Energy Balance (NEB) can be countered by feeding Lianol® Ferti to sows, increasing peptide levels and enhancing milk production. These effects are mediated by an elevated IGF-1 level in serum, known to trigger ovarian sensitivity response to FSH and LH for further follicular development. Lianol® Ferti has the proven ability to enhance milk and colostrum production and also sow fertility (Farrowing rate and litter size). However, there is no evident to show that Lianol® Ferti is able to shorten the weaning to oestrus interval in sows. It is, therefore, this study aimed to study the effect of Lianol® Ferti feeding to sows around weaning on WOI.

## Materials and methods

Sixty lactating sows (Landrace x Yorkshire) on a commercial breeding farm were equally divided into 2 groups: an untreated control group (average parity number of 3.9) and a Lianol® Ferti group (average parity number of 3.6). They were then received 10 g (1 tablet) of Lianol® Ferti per sow per day by top dressing, on 2 days before and after weaning. The oestrus detection was performed twice daily in the morning and afternoon at the presence of a boar, starting on the day of weaning and the first day of standing oestrus was recorded as weaning to oestrus interval (WOI).

## Results

The reproductive performances of treated sows are as follows: total number piglet born = 13.83; piglet born alive = 12.83; stillborn piglet = 0.67 and mummified fetus = 0.33. The weaning to oestrus interval in Lianol® Ferti group (WOI = 4.0 ± 0.64 days) is shorter than that of untreated control group (WOI = 5.42 ± 0.59 days).

## Discussion and conclusion

It is well documented that after feeding Lianol® Ferti an increase plasma level of IGF-1 can be found. This IGF-1 influence the ovarian activity by increasing the sensitivity and response of the follicles to FSH and LH, resulted in promotes oestrogen production. This mechanism may at least explain the shorter WOI found in a Lianol® Ferti group. In conclusion, feeding of 10 g (1 tablet) Lianol® Ferti per sow/day for four consecutive days at pre- and post-weaning improved WOI in sows by 1.42 days.

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[2]Lee, C.Y. et al. (1993), Expression of components of the insulin-like growth factor system in pig mammary glands and serum during pregnancy and pseudopregnancy: Effects of oestrogen, J. Endocrinol., 137, 473–483.

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# The effect of *Lactobacillus acidophilus* fermentation product as an antibiotic alternative on fecal bacteria and performance of weaned pigs

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## Introduction

To reduce the antibiotic resistance among bacteria, antibiotic alternative should be considered in animal husbandry. *Lactobacillus acidophilus* fermentation product (SynGenX) can be a good candidate since it is derived by culturing *L. acidophilus* on an appropriate media for the production of enzymes and microbial metabolites. The analysis proved that the product contains about 17% of crude protein, 22% of crude fiber and minerals. The aim of this study was to determine the effect of SynGenX on weaned pig with respect to fecal bacteria and performance.

## Materials and methods

At weaning, (25 d of age, 6.5 kgBW) pigs of mixed gender (n=500) were allotted to either 1) control diet (CON), or 2) SGX1 (1kg/ton), 3) SGX2 (2kg/ton), 4) OTC (oxytetracycline 100ppm) or 5) SGO (SGX 1kg/ton and OTC 100ppm). Pigs were provided free access to their respective dietary treatment and water during 4-week-study. Fresh fecal samples were collected on d 1 and 15 for *E.coli* and lactic-producing bacteria count. Feed consumption and diarrhea record were measured daily. Pig weights were measured on d 0, 14 and 28.

## Result

Feeding SGX2 resulted in the greatest bodyweight of the pig at the end of the study (Table 1). Weight gain of piglets in SGX2 was significantly higher than that in CON and SGX1. This figure was a bit higher than that in OTC or SGO. Piglets in SGX2 increased feed intake. Pig fed SGX2 or SGO had improved FCR compared with CON, SGX1 or OTC. Diarrhea rate was significantly lower in treatment SGX1, SGX2, OTC and SGO compared to the CON. No dead pig was found. The condemn rates were not significantly different between groups. Supplementation of antibiotic and/or SGX had improved from 20% to 39% of the treatment cost compared to the control. Fecal bacterial count was shown in Table 2.

***Table.1*** *Growth performance and economic value*

| TRT | CON | SGX1 | SGX2 | OTC | SGO | P |
|---|---|---|---|---|---|---|
| d 0 BW, kg | 6.66 | 6.65 | 6.65 | 6.64 | 6.62 | 0.976 |
| d 14 BW, kg | 8.94 | 9.07 | 9.23 | 9.03 | 9.21 | 0.187 |
| d 28 BW, KG | $15.1^{b}$ | $15.29^{b}$ | $16.11^{a}$ | $15.5^{ab}$ | $15.72^{ab}$ | 0.000 |
| AWG, kg | $8.43^{c}$ | $8.64^{bc}$ | $9.46^{a}$ | $8.85^{bc}$ | $9.11^{ab}$ | 0.000 |
| ADG, kg/pig/day | $0.3^{c}$ | $0.31^{bc}$ | $0.34^{a}$ | $0.3^{bc}$ | $0.33^{ab}$ | 0.000 |
| ADFI, kg/pig/day | $0.41^{b}$ | $0.43^{ab}$ | $0.45^{a}$ | $0.44^{ab}$ | $0.44^{ab}$ | 0.013 |
| FCR | $1.43^{a}$ | $1.43^{a}$ | $1.35^{b}$ | $1.42^{a}$ | $1.37^{b}$ | 0.000 |
| Diarrhea rate | $0.77^{a}$ | $0.71^{a}$ | $0.58^{b}$ | $0.59^{b}$ | $0.55^{b}$ | 0.000 |
| Death and condemn rate | 7.34 | 5.91 | 2.68 | 4.83 | 3.84 | 0.535 |
| Treatment cost (USD/pig) | $0.102^{a}$ | $0.089^{a}$ | $0.069^{b}$ | $0.069^{b}$ | $0.064^{b}$ | 0.000 |

***Table 2.*** *Fecal bacterial concentration.*

| TRT | | CON | SGX1 | SGX2 | OTC | SGO | P |
|---|---|---|---|---|---|---|---|
| ***E.coli*** | D1 | 8.83±1.18 | 8.90±0.93 | 9.08±0.70 | 9.18±1.05 | 8.51±0.67 | 0.404 |
| | SE | 0.376 | 0.292 | 0.22 | 0.333 | 0.212 | |
| (Log10 CFU) | D15 | $9.19±1.27^{a}$ | $7.82±1.15^{bc}$ | $6.95±0.96^{cd}$ | $7.91±0.81^{b}$ | $6.64±0.83^{d}$ | 0.000 |
| | SE | 0.4 | 0.36 | 0.3 | 0.26 | 0.26 | |
| **LPB** | D1 | 8.70±0.83 | 8.65±0.53 | 8.91±0.74 | 9.07±0.81 | 8.85±0.86 | 0.515 |
| | SE | 0.264 | 0.166 | 0.233 | 0.255 | 0.273 | |
| (Log10 CFU) | D15 | $8.85±0.63^{b}$ | $9.63±0.95^{ab}$ | $9.98±0.47^{a}$ | $9.19±0.44^{ab}$ | $9.78±0.47^{a}$ | 0.002 |
| | SE | 0.2 | 0.3 | 0.15 | 0.14 | 0.15 | |

LPB, Lactic producing bacteria

## Discussion

Pigs fed SGX2 gained 1.01kg more weight than CON pigs after 28 days, while pigs fed the other dietary treatments were almost the same CON pigs. Using SGX for pigs at 2kg/ton improved FCR best. Diarrhea rate of pigs in SGX2, OTC and SGO was significantly lower than that in the CON treatment. This may be explained by the fact that *E.coli* number in feces of piglets after 14 days of using SGX2 or SGO was lowest compared to other treatments. In these two treatments, the levels of beneficial bacteria in fecal samples were highest. Cost in the treatments SGX2, OTC and SGO significantly decreased 32-37% compared to the control treatment.

In conclusion, we can use either SGX for piglets at 2kg/ton to obtain a better performance in weaning stage. SGX can be a solution for antibiotic alternative.

## Reference

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# Characterization of the quinolone resistance mechanism in *Salmonella* Choleraesuis and *Salmonella* Typhimurium isolated from sick pigs in Taiwan.

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## Introduction

*Salmonella enterica* serotype Choleraesuis and Typhimurium isolated from pigs in Taiwan has been reported with multidrug resistance (MDR). Quinolone resistance can be achieved in three ways: spontaneous mutations in the DNA gyrase (GyrA and GyrB) or topoisomerase IV (ParC and ParE) encoding genes, decreased accumulation of quinolones by overexpression of efflux pump and protection of DNA gyrase by the plasmid mediated quinolone-resistant (PMQR) proteins produced from plasmid or transposon. This study was aimed to identify the relationship between the mechanisms of quinolone resistance and quinolone-resistant *Salmonella* Choleraesuis and Typhimurium isolated from sick pigs in Taiwan.

## Materials and methods

From 2010 to 2014, we collected 64 *S.* Choleraesuis and 112 *S.* Typhimurum from clinical cases in Animal Disease Diagnostic Center (ADDC) in National Chiayi University (R.O.C.). Antimicrobial susceptibility tests were conducted by minimal inhibitory concentration (MIC) method according to Clinical and Laboratory Standards Institute. The tested quinolones include nalidixic acid, flumequine, enrofloxacin and ciprofloxacin. To know the contribution of efflux pump activity to antimicrobial resistance, MICs of nalidixic acid were performed in the presence or absence of the efflux pump inhibitor Phe-Arg-β-naphthylamide (PAβN [20 μg/ml]). To detect mutations in the DNA gyrase or topoisomerase IV of quinolone target genes, PCR amplification and sequencing were used. The presence of PMQR genes was determined by PCR.

## Result

All of 64 *S.* Choleraesuis were resistant to the four quinolones. The resistant percentage of 112 *S.* Typhimurium to nalidixic acid, flumequine, enrofloxacin and ciprofloxacin were 50 %, 52.68 %, 27.68 % and 12.5 %, respectively. In addition, the MIC of *Salmonella* isolates showed 2-64 folds decrease in the presence of PAβN (Table 1).

The most common GyrA mutation was S83F ($n$ = 66, 54.1 %) followed by D87N ($n$ = 65, 53.28 %) and S83Y ($n$ = 3, 2.46 %) in the 122 nalidixic-acid-resistant isolates. Amount all the S83F isolates, 65 both carried D87 to N substitution (Table 2). Mutations in ParC were detected in 64 *S.* Choleraesuis with S80I, while there was no substitution in ParC in *S.* Typhimurium (Table 2). In all 176 isolates, 14 of *S.* Choleraesuis and 77 of *S.* Typhimurium carried PMQR genes (*qnrS*) (Table 2).

***Table 1*** Decreased MIC range of 176 *Salmonella* isolates by nalidixic acid resistance.

| Serovar | Resistant pattern | NA | NA$^{P}$ | Fold change |
|---|---|---|---|---|
| SC | R | >1024 | 512 | >2 |
| | S | | | |
| ST | R | >1024 - 32 | 64-32 | 64 - 2 |
| | S | 16 - ≦0.5 | 2 - ≦0.5 | |

SC: *S.* Choleraesuis. ST: *S.* Typhimurium. NA: nalidixic acid. P: with PAβN.

***Table 2*** Comparison of mutations in GyrA and ParC, PMQR profiles and MICs in *Salmonella* isolates.

| Serovar | Number | Mutation | | | *qnrS* | NA | CIP |
|---|---|---|---|---|---|---|---|
| | | GyrA | | ParC | | | |
| SC | 50 | S83F | D87N | S80I | - | >1024 | 32 - 8 |
| | 14 | S83F | D87N | S80I | + | >1024 | 16 - 8 |
| ST | 2 | S83F | | | - | >1024 - 128 | ≦0.025 |
| | 1 | S83Y | | | - | 1024 | 0.25 |
| | 2 | S83Y | | | + | >1024 | 0.25 |
| | 74 | | | | + | 128 - ≦0.5 | 0.5 - ≦0.025 |
| | 33 | | | | - | 64 - ≦0.5 | 0.5 - ≦0.025 |

SC: *S.* Choleraesuis. ST: *S.* Typhimurium. NA: nalidixic acid. CIP: ciprofloxacin.

## Discussion

In Taiwan, quinolone-resistant *Salmonella* isolated from economic animals and human has been reported for years [1]. In the present study, we find the resistance to quinolones is mediated by mutations in quinolone target genes and by the overexpression of efflux pump. Although the presence of PMQR does not show its importance in quinolone-resistance, it is still a public health concern since it can be transported horizontally by plasmid or transposon [2]. In conclusion, prudent use of antimicrobials is needed to reduce *Salmonella* contamination and to inhibit the prevalence of drug resistance.

## Reference

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[2] Robicsek A, Jacoby GA, Hooper DC, Lancet Infect Dis 6: 629-640.

**Antimicrobial Agents Resistance and Pulse Field Gel Electrophoresis of *Pasteurella multocida* Causing Pig Pneumonia in Taiwan**

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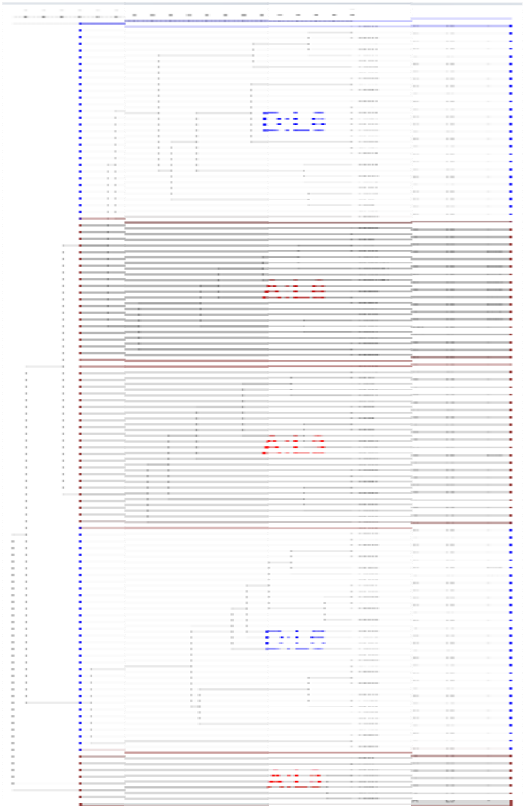
**Introduction**

*Pasteurella multocida* (PM) is a pathogen to wild range of animals. In pigs, PM can cause atrophic rhinitis, pneumonia, and hemorrhagic septicemia. PM has been differentiated by serological methods. Strains are classified into five capsular serogroups (A, B, D, E and F), and 16 lipopolysaccharide (LPS) serotypes. The capsule type A and D are the most two type in pig. PM causing atrophic rhinitis is owing to dermonecrotic toxin, which expressed from *toxA* gene. In Taiwan, there was without any study about the anti-microbial agent susceptibility of PM in pigs. Therefore, this study would test the minimal inhibitory concentration (MIC) and differentiated the serotype and genotype by pulse field gel electro-phoresis (PFGE).

**Materials and methods**

PM was isolated from the sick pig with pneumonia in the animal disease diagnostic center (ADDC) in National Chiayi University (R.O.C.), since 2012 to 2015. PM serotype and *toxA* gene were identified by polymerase chain reaction. The MIC test was according to Clinical and Laboratory Standards Institute operating rules. Amoxicillin, cefa-zolin, doxycycline, flumequine, enrofloxacin, flor-fenicol, kanamycin, lincomycin, lincospectin, tylosin, tilmicosin, erythromycin, and tiamulin were tested. Restriction enzyme, *Apa*I, was used in PFGE.




**Results**

In this study, 62 PM isolates were examined. The most serotype was capsule type D and LPS serotype L6 (D:L6) (35/62), the second was A:L3 (17/62), and the third was A:L6 (8/62). Besides, there were 8 isolates *toxA* positive, which 6 were serotype A:L6 and 2 were D:L6. The result of MIC displayed PM was highly resistance against tested antimicrobial agents, except for cefazolin. The resistant percentage of each drugs were amoxicillin (53 %), cefazolin (24.2 %), doxycycline (86.4 %), flumequine (69.7 %), enrofloxacin (62.1 %), florfenicol (90.9 %), kana-mycin (42.4 %), lincomycin (100 %), lincospectin (97.0 %), tylosin (100 %), tilmicosin (98.5 %), erythromycin (100 %), and tiamulin (98.5 %.) Interestedly, the serotype D:L6 was higher resistance than serotype A:L3. Besides, PFGE seems could distinguish different serotype and toxin isolates (Fig. 1.)

Fig. 1 The distribution of serotype in phylogenetic dendrogram of PFGE.

**Discussion**

The strains causing atrophic rhinitis, most belong to A:L6, might descending infection causing pig pneumonia [1]. However, the high resistance were major caused by D:L6. According to Michael *et al*., the multiple resistance genes can transfer via plasmid [2]. But it needs more deeply research about the resistant mechanisms.

**Reference**

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[2] Michael et al. J Antimicrob Chemother 67: 84-90.

# The influence of feeding Lianol® Ferti in sows during pre- and post-partum period on the weaning weight

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## Introduction

In pig industry, it has been well documented that heavier weaning piglets reach market weight earlier than lighter weaning piglets[1,2]. This has inspired scientist worldwide in discovering strategies to increase the body weight gain during lactation period. Basically, besides genetics background, milk consumption is a primarily factor for piglet survival, growth during suckling period and also influenced the body weight at weaning. In addition, sufficient sow colostrum production and colostrum intake by piglets are vital for the piglet immune status. It has been reported that Lianol® Ferti, a fermented potato protein added to the feed of sows, increases potency and colostrum intake of piglets and sow colostrum production. These effects are mediated by an elevated IGF-1 level in serum, known to increase these parameters. Therefore, we aimed to improve whether Lianol® Ferti feeding in sow during pre- and post- partum influenced the body weight at weaning.

## Materials and methods

Forty six pregnant sows (Landrace x Yorkshire) on a commercial breeding farm were equally divided into 2 groups: an untreated control group (average parity number of 4.17) and a Lianol® Ferti group (average parity number of 4.91). They were then received 10 g (1 tablet) of Lianol® Ferti per sow per day by top dressing for 6 consecutive days, i.e. 5 days before and 1 day after farrowing. Sow reproductive performances such as total number piglet born (TB), piglet born alive (BA), stillborn piglet (SB), mummified fetus (MM), number of weaned piglets (NWP) and piglet birth weight (BW). At weaning (on day 21), the body weight of piglets were recorded (BW-WP).

## Results

The results of sow reproductive performances and piglets body weight are presented in Table 1. A higher birth weight (27 g more) and body weight at weaning (403 gram more) was found in Lianol® Ferti group than control group.

Table 1 Sow reproductive performances and piglets body weight (gram)

| Groups | TB | BA | SB | MM | BW | NWP | BW-WP |
|---|---|---|---|---|---|---|---|
| Control | 13.83 | 12.48 | 0.91 | 0.39 | 1641 | 9.52 | 6018 |
| Lianol® Ferti | 12.87 | 11.57 | 1.00 | 0.30 | 1668 | 9.35 | 6421 |
| Differences | 0.96 | 0.91 | 0.09 | 0.09 | 27 | 0.17 | +403 |

## Discussion and conclusion

The results in the present study clearly showed that a higher weaning weight was found in Lianol® Ferti feeding sows than the control group. The reason might that the Lianol® Ferti feeding in sows resulted in an elevated IGF-1 level in their serum, which in turn, promote milk production for their piglet. As a result, the piglets have a chance to consume some more milk than those the piglet in control group. This heavier weaning piglet may certainly reach market weight earlier than lighter weaning piglets as has been shown in many other studies[1,2].

## Reference

[1] Mahan DC et al., (1991). Effect of pig weaning weight and associated nursery feeding programs on subsequent performance to 105 kg body weight. J. Anim. Sci. 69:1370-1378.

[2] Dunshea FR et al. 2003. Life time and post-weaning determinants of performance indices of pigs. Austral. J. Agri. Res. 54: 363-370.

**Appropriate metaphylactic treatment of *S. suis* infections of pigs in nursery with Vetrimoxin LA®**

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**Introduction**

*Streptococcus suis* belongs to the most frequent bacterial pathogens in pigs raised in industrial farms. Losses of pigs in the nurseries are associated mainly with *S. suis* serotype 2. Clinically ill animals should be treated with the anti-inflammatory product and with the antibiotic together with all penmates. Amoxicillin is the antibiotic of the first choice in the treatment and control of *S. suis* infections (Gottschalk 2002). Metaphylactic treatment at weaning is a common practice to reduce the risk of the development of the disease after stress and contamination of comingled piglets. The aim of the study was to compare the efficacy of Vetrimoxin LA® containing long acting injectable amoxicillin with Naxcel® - a long acting ceftiofur-based product.

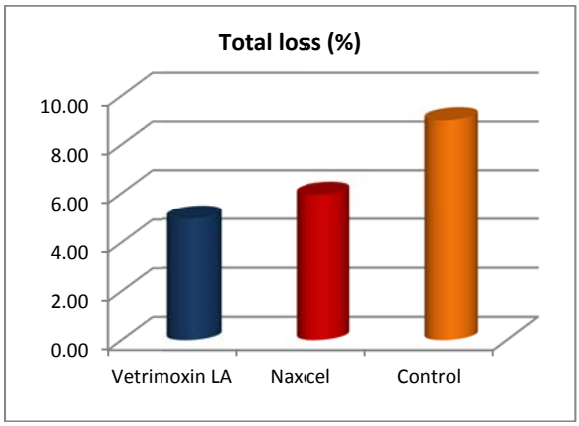
**Materials and methods**

A farrow-to-finish farm with the history of *S. suis* infections was selected for the trial. About 100 piglets were randomly assigned into three treatment groups after weaning. G1 and G2 group piglets received either Vetrimoxin LA® or Naxcel® in the recommended dosage. Piglets of G3 served as the non-treated control. Mortality and emergency treatments were recorded and evaluated per group in the whole post-weaning period. The economic impact was measured by using the Ceva Respinomics™ calculations.

**Results**

The total loss in different groups G1, G2 and G3 was 4,95%, 5,94% and 9% respectively. This loss included mortality and culling. The clinical observing revealed nervous symptoms and septicemia, in one case (G1) respiratory distress. *S. suis* strain isolated from dead pigs was susceptible to AMX and CFT.

*Figure 1. Total loss in nursery within different treatment groups*




The additional treatment with injectable antibiotics was required in sick pigs. The overall benefit-to-cost amounts to 1.97€ per pig into trial in the Vetrimoxin LA® group and 0.97€ in the Naxcel® group in comparison to the control.

**Conclusion**

The metaphylactic treatment of piglets at weaning with Vetrimoxin LA® demonstrated high efficacy in prevention of losses associated with the *S. suis* infections in post-weaning pigs. This efficacy was higher yet not significantly, than in case of the use of long acting ceftiofur. Vetrimoxin LA® appears an appropriate, cost-efficient product for such use.

**Reference**

Gottschalk M., Proc AASV, 2002

**Comparison of the efficacy of amoxicillin with other antimicrobials against *Streptococcus suis***

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**Introduction**

*Streptococcus suis* represents one of the major threats in swine as regards to bacterial infections in industrial farms. It is a very early colonizer, the first contaminations can occur already during parturition. In suckling piglets the clinical signs develop mostly at the age of 10-21 days, which requires an early control measures to be implemented. Antibiotics remain the major tool used in newborn pigs. Amoxycillin (AMX) is the antibiotic of the first choice in the treatment and control of S. suis infections[1]. Other molecules such as tulathromycine, cephalosporins, fluoroquinolons or tetracyclines are also sometimes used in young piglets for their broad spectrum of activity and convenience. The aim of this study was to compare the efficacy of AMX with those antimicrobials against *S. suis* field isolates.

**Materials and methods**

22 Isolates of *S. suis* were chosen from piglets with clinical disease and each isolate represented one piglet (age 14 to 90 days) and different farm per year in the Czech Republic in the period 2009-2015. Identification was performed based on biochemical activity (API20Strep, BioMerieux, France) and by using of MALDI TOF (mass spectrometry) (Bruker). Final serotypisation was done by specific PCR. Total 22 strains were analysed according this procedure (arthritis- 10, pneumonia 4, sepsis- 8). The MICs were determined using the agar dilution method with Mueller-Hinton agar (MHA), (Oxoid, UK), with the addition of 5 % ovine blood (CLSI, 2008).

**Result**

MIC50 and % of sensitive isolates were established in group of tested strains of *S.suis* (serotype 1 and 2), the lowest MIC50 and 100 % sensitivity were evaluated for amoxicillin. We have found widely spread resistance especially for tetracycline. There is no CLSI criteria available for tulathromycin, but according to published Cmax plasma parameter 0.62 µg/ml we could consider it as clinically not effective treatment option for selected isolates of *S. suis*[2]. Among the selected molecules amoxicillin was the most effective antimicrobial based on in-vitro sensitivity data without any trend towards creating the potential resistance pattern during the testing period.

***Table.1*** *MICs for the tested antimicrobials*

| Atb | $MIC_{50}$ | % of sensitive isolates | Range of MIC | MIC breakpoint for resistance (µg/ml) |
|---|---|---|---|---|
| amoxicillin | < 0,125 | 100 | ≤0.125-0,5 | ≥ 2.0 |
| tulatromycin | 8 | ND | < 4,0- >256 | unavailable |
| ceftiofur | < 0,25 | 100 | < 0,25-1,0 | ≥ 8.0 |
| enrofloxacin | 0,5 | 100 | < 0,125-1,0 | ≥ 2.0 |
| tetracyklin | > 8,0 | 36 | 0,5- >8,0 | ≥ 2.0 |

**Discussion**

The prudent use of antimicrobials in food animals considers the right choice of the molecule to expect maximum clinical response with the respect to the risk of resistance. Recent surveys confirmed high susceptibility of *S. suis* to AMX[3], however data regarding tulathromycine were missing. This study confirmed AMX as an optimal choice for the control of *S. suis*, for its highest efficacy and lowest resistance rate compared to other molecules in test.

**Reference**

1 Gotschalk, AASV, 2002
2 (Benchaoui, 2004
3 Butty, APVS, 2013
4 CLSI, VET01-S2, 2013

# In vitro study on antimicrobial susceptibility of *Haemophilus parasuis* in Taiwan

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## Introduction

*Haemophilus parasuis*, a small pleomorphic, nicotinamide adenine dinucleotide (NAD)-dependent Gram-negative rod of the family Pasteurellaceae (1). *H. parasuis* is a commensal of the upper respiratory tract of pigs but is also the causative agent of Glässer's disease, which is characterized by fibrinous polyserositis, polyarthritis and meningitis (2). Nowdays Glässer's disease has been described as an emergence disease which causes important economic losses in swine industry. While the antimicrobial resistance of *H. parasuis* has already been identified in other countries, the current knowledge is unavailable in Taiwan. Thus, the aim of the present study was to determine the Minimal inhibitory concentration (MIC), with a microdilution method of Taiwan swine clinical isolates of *H. parasuis*.

## Materials and methods

A total of 40 *H. parasuis* strains were isolated from pigs with fibrinous polyserositis and polyarthritis in Taiwan during 2013-2015. Identification of this isolates was carried out by biochemical tests and the species-specific polymerase chain reaction (3). MICs determinations were performed in microtiter wells according to NCCLSs guidelines. The antimicrobial agents used and their respective dilution ranges were as follows: enrofloxacin and florfenicol, 0.0075–16 μg/ml; amoxicillin, gentamicin, ceftiofur and doxycycline 0.0015–32 μg/ml; tylosin, 0.03–64 μg/ml; spectinomycin, 0.12–256 μg/ml; tiamulin, tilmicosin 0.06-128 μg/ml. The inocula were prepared from a 24 hours chocolate agar plate by adjusting to 0.5 McFarland standard and further diluted 1/100 in Veterinary Fastidious Medium. Test plates were incubated at 37°C for 24 to 48 hours in atmosphere containing 5% $CO_2$. Ranges of susceptibility were recorded along with the MIC that inhibited 50% ($MIC_{50}$) and 90% ($MIC_{90}$) of the isolates.

## Result

The results of the susceptibility testing of the clinical isolates of *H. parasuis* as distribution of the MICs values and percentage of resistant strains are shown in Table 1.

***Table.1*** *In vitro* susceptibility of *H. parasuis* isolates from swine.

| Antimicrobial agents | MIC (μg/mL) | | | Resistant % (N=40) |
|---|---|---|---|---|
| | Range | MIC 50 | MIC 90 | |
| Amoxicillin | 0.0015–32 | 0.03 | 8 | 0 |
| Enrofloxacin | 0.0075–16 | 0.5 | 2 | 0 |
| Ceftiofur | 0.0015–32 | ≤0.015 | 0.03 | 0 |
| Doxycycline | 0.0015–32 | 0.12 | 0.5 | 0 |
| Florfenicol | 0.0075–16 | 0.5 | 2 | 0 |
| Gentamicin | 0.0015–32 | 2 | >32 | 22.5 |
| Tylosin | 0.03–64 | 2 | 32 | 5 |
| Tilmicosin | 0.06-128 | 1 | 16 | 0 |
| Tiamulin | 0.06-128 | 1 | 4 | 2.5 |
| Spectinomycin | 0.12–256 | 128 | >256 | 20 |

## Discussion

Ceftiofur had the highest susceptibility rates against *H. parasuis* isolates *in vitro* in this study, followed by amoxicillin and doxycycline. A large proportion of *H. parasuis* strains were resistant to gentamicin. Since gentamicin and spectinomycin are commonly used antimicrobial agents for disease prevention and treatment in herds in Taiwan, this may partially account for the high percentage of resistant strains in this study. In conclusion, our results emphasize the importance of prudent use of antimicrobial agents in the treatment of Glässer's disease.

## Reference

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# Evaluation of the Bioavailability of Ivermectin at Feed Medication in Pigs

Piyapong Pienpinijtham[2], Kanjana Imsilp[1], Saranya Poapolathep[1], Amnart Poapolathep[1], Phanwimol Tanhan[1], Usuma Jermnak[1], Narumol Klangkaew[1], Nanthawan Khantee[2], Settasart Sonna[2], Metta Makhanon[2]
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## Introduction

Ivermectin is a combination of 22, 23-dihydroavermectin B1a (at least 80%) and 22, 23-dihydroavermectin-Bib (not more than 20%) with high lipophilic activity[1]. Both oral and parenteral application routes, are proven to be the first priority medications for Sarcoptic mange infection as well as the endoparasites[4]. It has high lipophilic activity and low recommended dose with a large safety margin. The concentration above 1 ng/g at target tissue level is accepted to be the effective concentration[1,4]. The pharmacokinetics of products are an important criterion for proving the efficacy of each commercial ivermectin product. This study aimed to evaluate the bioavailability of a single dose of VertinGard 0.6% Premix in breeder pigs.

## Materials and methods

Eight sows after weaning at 200-250 kg body weight were feed with VertinGard 0.6% in feed at a dosage of 100 µg/kg bw given for 7 consecutive days by top dressing. Before the study, selected sows have not been treated with ivermectin more than one month. Blood samples were collected at 10 ml from every animal at 0, 1, 3, 5, 7 days before the morning feed and 3, 6, 9, 12 hours and 1, 2, 3, 5, and 7 days after the last feeding. Each blood sample was centrifuged and plasma was collected at min. 3-5 ml in each pig and duration and stored at -80 °C until analysis. Plasma concentrations of ivermectin B1a were analyzed by LC-MS/MS using USDA-FSIS method CLG-AVR.04 (2011). Pharmacokinetic (PK) parameters: AUC ∞ (expo), $C_{max,}$ and $T_{max}$ were calculated using PK solutions 2.0x software. Statistical determination was performed using PK Solution2.0[2,3].

## Results

PK parameters are illustrated in Table 1 and Figure 1.

## Discussion

After oral administration of VertinGard 0.6 % a plasma concentration of Cmax at 14.88 ng/ml were found which is almost 15 times over the effective drug tissue concentration. Its estimated concentration in target tissues such as skin and intestinal mucosa are 1,976.5 ng/ml (>45% of plasma concentration) and 3,325.96 ng/ml (>163% of plasma concentration), respectively[5]. The results indicate a very high bioavailability of VertinGard 0.6% Premix in plasma of breeder pigs.

*Table1. Pharmacokinetic parameters of VertinGard 0.6% premix*

| PK Parameters | VertinGard 0.6% |
|---|---|
| Cmax (ng/ml) | 14.88 |
| AUC ∞ (expo) (ng-day/ml) | 1,363.10 |
| Tmax (hr) | 4.88 |

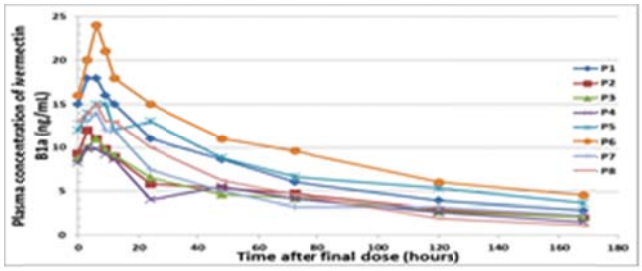


*Figure 1 Plasma concentration of Ivermectin B1a in 8 pigs after in feed administration of VertinGard 0.6% premix*

## Reference

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5. Pereira and Kohek, 1998. Rev. Bras. Parasitol. Vet. 7(1) p. 53-56.

# Evaluation of Antimicrobial Susceptibility of *Mycoplasma hyorhinis* Field Strains in 2015 from Thai Case Reports

Metta Makhanon [1],Rapeepat Kunalintip [1], Wipawee Rodpreecha[1], Kannika Aungtragoolsuk[1], Nalinee Chaiiesarakorn[1], Piyapong Pienpinijtham[1], Settasart Sonna[1], Chotika Pramarn[1], Pacharee Thongkamkoon[2]
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## Introduction

Mycoplasmal pneumonia, arthritis and synovitis caused by *Mycoplasma hyorhinis* (MHR) become high occurrence in nursery to grower pigs in Thailand[2,3,4]. Trends of reduced sensitivity are observed for several antimicrobials[5]. This study is part of a farm field surveillance program for integrated farms in different areas of Thailand. The objective of the study is to evaluate the MHR susceptibility (MIC) of strains isolated in four farms and the antimicrobial use on these farms..

## Materials and methods

MHR (24 isolates) was derived from pigs with severe respiratory signs and lameness in four integrated farms using the same medication and feed as well as the same source of breeders in March 2015. The MICs were conducted by using agar dilution method[1]. Six antimicrobials including Doxycycline (D), Lincomycin (LC), Tilmicosin (TM), Tylvalosin (TV), Tiamulin (TA), and Valnemulin (VN), were used in the MIC tests. MIC90s were determined for the different antimicrobials and for all isolates of each farm. The MIC90 results were evaluated in relation to the two year antimycoplasmal medication program of the farms.

## Results

Antimycoplasmal medication program for the last two years is summarized in Table 1. The MIC90 of individual farms and the summary for all farms are in Table 2.

## Discussion

The study results show that independent from the use of Doxycycline, Tiamulin, and Tilmicosin over a period of two years no trend of reduced sensitivity based on the proposed resistance breakpoints were found. Stop of Tylvalosin and Lincomycin application for one year did not affect the reduced sensitivity against both products[1]. The resistance development of MHR strains to antimicrobials seems not to be related to the drug rotation on the farms over a period of one year.

**Table.1 The antimycoplasmal program (ppm) for the last two years in four farms.**

| **Fattening feed** | **1-12 wk old** | **12-18 wk old** | **18-24 wk old** |
|---|---|---|---|
| 6mt** | D200 | none | C400* |
| 1yr** | TA200 | D250 | TA360 |
| 2yr** | TM200 D200 | TA150,C300* | none |
| **Breeder feed** | **Gilt** | **Gestation** | **Lactation** |
| 6mt** | TM400 | TM200+C500 * | D300 |
| 1yr** | TM220+C300 * | TM220 D300 | TA200 |
| 2yr** | TV100 | TA150 +C300* | TV100+C300 * |

*C=Chlortetracycline **before surveillance

**Table.2 MIC90 (µg/ml) of M. hyorhinis (MHR) derived from four farms to six antimicrobials**

| **MHR-MIC90(µg/ml)** | **D** | **LC** | **TM** | **TV** | **TA** | **VN** |
|---|---|---|---|---|---|---|
| Farm A, N=5 | 1 | >128 | 16 | 16 | 1 | 0.032 |
| Farm B, N=7 | 4 | >128 | 16 | 32 | 1 | 0.032 |
| Farm C, N=6 | 4 | >128 | 16 | 16 | 1 | 0.063 |
| Farm D, N=6 | 2 | >128 | 16 | 16 | 1 | 0.063 |
| All farms, N=24 | 2 | >128 | 16 | 16 | 1 | 0.063 |

## Reference

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4. Makhanon 2012. Doc. Thesis. CU Vet. Sci. BKK., Thailand
5. Makhanon et al. 2014. Proc. Of 23rd IPVS (C003) p.302

# Antimicrobial Susceptibility of *Mycoplasma hyorhinis* Field Strains

Metta Makhanon [1],Rapeepat Kunalintip [1], Wipawee Rodpreecha[1], Kannika Aungtragoolsuk[1], Nalinee Chaiiesarakorn[1], Piyapong Pienpinijtham[1], Settasart Sonna[1], Chotika Pramarn[1], Pacharee Thongkamkoon[2]
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## Introduction

Mycoplasmal pneumonia, arthritis and synovitis caused by *Mycoplasma hyorhinis* (MHR) become high occurrence in nursery to grower pigs in Thailand[3,4,5]. Trends of reduced sensitivity have been reported for several antimicrobials [6]. This study is part of a field surveillance for integrated farms including four farms in different areas. The objective of the study is to investigate the MHR susceptibility (MIC) of strains isolated in these farms.

## Materials and methods

MHR was derived from pigs with severe respiratory clinical signs and lameness using the same medication, feeding as well as the same source of breeders in March 2015. MHR isolates were kept in -80°C until use for MIC testing. Agar dilution method[2] .was used for MIC determination (MHR reference strain: BTS7). Serial dilutiobns of six antimicrobials including Doxycycline (D), lincomycin (LC), Tilmicosin (TM), Tylvalosin (TV), Tiamulin (TA), and Valnemulin (VN), were prepared. . MHR isolates were diluted to $10^8$ CFU/ml in Hayflick's broth. Five µl of each isolate was inoculated on the agar plate to be $10^5$ of the isolate per spot. The inoculated plates were incubated at 37° C in a humidified incubator with 5% $CO_2$ for 5 days. The lowest concentration of antimicrobial agent that inhibited the growth of mycoplasma was interpreted as the MIC value for each isolate. MIC ranges, MIC50, and MIC90 were were determined for the isolates. Proposed resistant breakpoints for each antimicrobial was compared with the MICs to evaluate the susceptibility of the isolates[1,2,7].

## Results

Antimicrobial susceptibility of MHR is illustrated in Table 1.

**Table 1. MIC of six antimicrobials against MHR (N=24)**

| Antimicrobials | D | LC | TM | TV | TA | VN |
|---|---|---|---|---|---|---|
| MIC range | 0.5-4 | 2->128 | 4-16 | 0.125-32 | 0.25-1 | ≤0.008-0.063 |
| MIC50 | 1 | 128 | 16 | 8 | 1 | 0.032 |
| MIC90 | 2 | >128 | 16 | 16 | 1 | 0.063 |
| Proposed resistance breakpoints | ≥16 | >8 | ≥32 | ≥4 | ≥16 | ≥16 |
| MIC of BTS7 | 0.25 | 2 | 1 | 0.125 | 0.25 | 0.016 |

## Discussion

The tested MHR field strains tend to show resistance against Tylvalosin and Lincomycin. However, no trends of resistance against Tilmicosin were found in this surveillance programme. Doxycycline and the pleuromutilins (tiamulin and valnemulin) show a higher antimicrobial effect in vitro against the tested MHR strains. In conclusion, establishment of a prudent use strategy for antimicrobials and routineous monitoring of the susceptibility of MHR organisms is recommended for future planning and selection of the antimicrobial medication programmes on the farms.

## Reference

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3. Kobayakhi et al. 2005. J.Vet.Med.Sci. 67(8) p.725-800
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# Evaluation of the Bioavailability of Ivermectin Post-Injection in Pigs

Piyapong Pienpinijtham[2], Kanjana Imsilp[1], Saranya Poapolathep[1], Amnart Poapolathep[1], Phanwimol Tanhan[1], Usuma Jermnak[1], Narumol Klangkaew[1], Nanthawan Khantee[2], Settasart Sonna[2], Metta Makhanon[2]
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## Introduction

Ivermectin is a combination of 22, 23-dihydroavermectin B1a at least 80% and 22, 23-dihydroavermectin-Bib not more than 20% with high lipophilic activity[1]. Both oral and parenteral administration routes, are proven to be the treatment options of choice for Sarcoptic mange infection as well as the endoparasites[4]. It has high lipophilic activity and low recommended dose with large safety margin. The concentration above 1 ng/g at target tissue level is accepted to be the effective concentration[1,4]. The pharmacokinetics of products are an important criterion for proving the efficacy of each commercial ivermectin products. This study aimed to evaluate bioavailability of a single dose of VertinGard 1% injections in breeder pigs.

## Materials and methods

Four sows after weaning at 200-250 kg body weight were injected single doses of VertinGard1% subcutaneously at 300 µg/kg bw (equi. to 1ml/33Kg bw). Before the onset study, selected sows have not been treated with ivermectin for more than one month. Blood samples were collected at 10 ml from every animal at the timespoints 0, 12 hours after SC and 1, 2, 3, 4, 5, 7, 9, 15, and 20 days after SC. Each blood sample was centrifuged and plasma was collected at min. 3-5 ml in each pig and duration and stored at -80 °C until analysis. Plasma concentrations of ivermectin B1a were analyzed by LC-MS/MS using USDA-FSIS method CLG-AVR.04 (2011). Pharmacokinetic (PK) parameters: AUC ∞ (expo), $C_{max,}$ and $T_{max}$ were calculated using PK solutions 2.0x software. Statistical determination was performed using PK Solution2.0[2,3].

## Results

PK parameters are illustrated in Table 1 and Figure 1.

## Discussion

After parenteral application of VertinGard 1% meaned plasma Cmax was determined at 16.5 ng/ml which is more than 16 times over the effective tissue concentration. Its estimated concentration in target tissues such as skin and intestinal mucosa are 245.59 ng/m (>45% of plasma concentration) and 276.09 ng/ml (>163% of plasma concentration), respectively[5]. The results after VertinGard 1% injection show a high Ivermectin bioavailability based on high plasma concentrations in breeder pigs.

| PK Parameters | VertinGard 1% |
|---|---|
| Cmax (ng/ml) | 16.5 |
| AUC ∞ (expo) (ng-day/ml) | 169.37 |
| Tmax (hr) | 1.75 |

**Table1. Pharmacokinetic parameters of VertinGard 1% Injection after single dose of SC.**

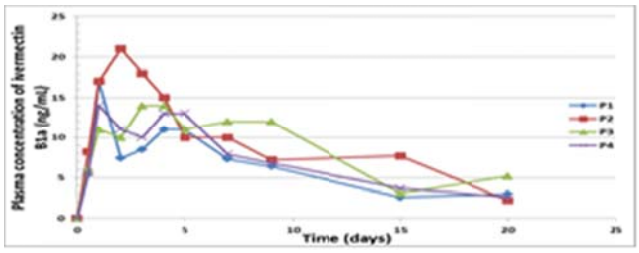


*Figure 1 Plasma concentration of Ivermectin B1a in 4 pigs*

## Reference

1. Canga et al. 2009 Vet. J. 179 p.25-37.
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# BIOAVAILABILITIES OF THREE AMOXICILLIN PRODUCTS IN PIGS FOLLOWING SINGLE ORAL ADMINISTRATION

Kanjana Imsilp[1], Saranya Poapolathep[1], Amnart Poapolathep[1], Phanwimol Tanhan[1], Usuma Jermnak[1], Narumol Klangkaew[1], Sujin Sukchai[2], Nanthawan Khantee[2], Settasart Sonna[2], Teerapol Jindawong[2], Prasit Ekapongpaisit[2], Metta Makhanon[2]

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## Introduction

Amoxicillin is a broad spectrum antimicrobial agent. It has extensively been used in pig industry. Currently, there are a large number of amoxicillin products available commercially. They are also marketed at different amoxicillin compositions. In very restricted countries, it is required by regulations that drug products should prove their effectiveness similar to the original drug reference product (s). However, there have been no original/ reference amoxicillin products for pigs specified. The objective of this study, thus, was to compare bioavailability of three amoxicillin products available in a country in Southeast Asia (SEA) market following single oral administration in pigs.

## Materials and methods

Twelve healthy Large White-Landrace-Duroc crossbred pigs (15.6–18.1 kg bodyweight) with no antibiotic administration for 14 days were used in this study. They were divided into 3 groups of 4 pigs. The animals were fasted for 12 h before receiving amoxicillin. Animals in each group were orally given only one amoxicillin product; i.e. Amoxicillin 1, Amoxicillin 2 (Aquacil), and Amoxicillin 3 commercially available in SEA at the dosage of 20 mg/kg body weight.

Blood samples were collected from every animal at timepoints 0, 30, 60, 90 minutes and 2, 3, 4, 5, 6, 8, 12, and 24 hours post-administration. Each blood sample was centrifuged and plasma was collected and stored at -80 °C until analysis. Plasma concentrations of amoxicillin were analyzed by UPLC-MS/MS using a modified method[1,2]. Pharmacokinetic (PK) parameters: AUC, $C_{max,}$ and $T_{max}$ were calculated using PK solutions 2.0x software. Statistical determination was performed using Systat 10 software.

| PK Parameters | Amoxicillin 1 | Amoxicillin 2 (Aquacil) | Amoxicillin 3 |
|---|---|---|---|
| Cmax (µg/ml)* | 6.12±1.49[a] | 7.01±1.96[a] | 5.74±1.54[b] |
| AUC 0-24hr (µg-hr/ml)* | 11.44±2.39[a] | 13.92±3.21[a] | 14.19±4.35[a] |
| Log AUC* | 1.05±0.09[a] | 1.13±0.11[a] | 1.13±0.15[a] |
| Tmax (hr) | 1.25±0.29[a] | 1.38±0.25[a] | 1.25±0.29[a] |

## Results

Determined PK parameters are summarized in Table 1.

**Table 1. Plasma pharmacokinetics of three amoxicillin products (Mean±SD) in 4 pigs/ group.**

[a,b] Treatments in the same row with the same superscript letter did not differ at 90% confidence interval.

## Discussion

No statistically significant differences for most PK parameters* important for bioequivalence determination at 90% confidence interval were found. Only the significantly higher Cmax concentration of Amoxicillin 2 (Aquacil) versus Amoxicillin 3 indicate differences in the pharmacokinetic profile of the products. This can be based on the higher absorption in Amoxicillin 2 medicated piglets after oral bolus administration.

## Reference

1. Dong et al. 2013. Biomed Chromatogr. 27 p.520-526.
2. Wen et al. 2008. J Pharm Biomed Anal. 48 p.829-834.

# Surveillance of Farm Medication to control *Mycoplasma suis* in Thailand

Metta Makhanon[1], Sasiwimon Talummuk [1], Piyanan Taweethavonsawat, Sudchit Chungpivat[2], Supot Wanttanapansak[2], Pornchalit Assavacheep[2], Ulrich Klein[3]
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## Introduction

*Mycoplasma suis* )*M. suis* (or formerly *Eperythrozoon suis* is an uncultivatable hemotrophic bacterium present at all ages of pigs. *M. suis* causes hemolytic or chronic anemia in pigs and results in economic losses for the pig industry. The transmission can be both vertical (in utero transmission) and horizontal (transmission by mosquito, biting flies, hog louse). Treatment and control by tetracyclines and organic arsenicals have been used. Caution in the case of organic arsenicals has to be considered[1,2]. This study aims to evaluate antimicrobials used in pig farms in Thailand considered to provide specific efficacy to control *M. suis*.

## Materials and methods

Three-hundred blood samples of suspected pigs in 18 farms with *M. suis* infection were collected (1 ml of blood/1 mg EDTA). 0.5 ml of the blood was kept at -20°C for DNA extraction and molecular assays. The remaining blood was preserved at 4°C within 24 hours for investigation by conventional techniques (10% Giemsa and acridine orange) and observation of blood parameters.
Records on clinical diseases observed including medication programmes were collected from the swine farms. . The positive/negative cases and the medication used were evaluated by descriptive analysis.

## Results

The overall prevalence of *M. suis* during the grower-finisher period was 70 out of 300 samples )avg.23.3%; 95% CI; 18.9-28.4%( based on nested PCR assay, while 24 samples )avg.8.0%; 95% CI; 5.4-11.6%( represented Giemsa-positive manifesting numerous basophilic discoid shapes located on the erythrozyte membrane.
The medication in these positive and negative farms is shown in Table 1.

***Table.1 Comparative M. suis positive results among different medication strategies.***

| Antimicrobials | | No.positive farms/no.farms (%) | No.positive samples/no.samples (%) |
|---|---|---|---|
| CTC / OTC | Tiamulin /Tylosin | | |
| yes | no | 2/2 (100%) | 12/60 (20%) |
| yes | yes | 1/5 (20%) | 13/126 (10.3%) |
| no | no | 11/11 (100%) | 37/72 (51.4%) |

## Discussion

The study results indicate a lower percentage of *M. suis* positive animals in farms with medication in comparison to farms without medication. Moreover, the percentage of *M. suis* positive animals in case of medication with tetracyclines (CTC/OTC) and tiamulin/tylosin combinations is lower in comparison to a single medication program only with tetracyclines.

## Reference

1. Groebel et al. 2009. Infection and Immunity. 77 (2) p.576-584.
2. Swine Disease manual. Iowa State University 2015.

# COMPARATIVE BIOAVAILABILITIES OF AMOXICILLIN PRODUCTS FOR APPLICATION IN PIGS IN THAILAND

Kanjana Imsilp[1], Saranya Poapolathep[1], Amnart Poapolathep[1], Phanwimol Tanhan[1], Usuma Jermnak[1], Aksorn Saengtienchai[1], Napasorn Phaochoosak[1], Narumol Klangkaew[1], Sujin Sukchai[2], Nanthawan Khantee[2], Settasart Sonna[2], Teerapol Jindawong[2], Kamolwan Na Lumphun[2], Wipawee Rodpreecha[2], Nakarin Okchim[2], Piyapawn Khamlue[2], Suchaya Kunanoppadol[2], Piyapol Tianladdawong[2], Thodsaporn Muangchamnan[2], Kannika Aungtragoolsuk[2], Atthariya Jiranaparat[2], Netchanok Malingam[2], Suthep Suksomsin[2], Chirattakorn Kerdaiem[2], Metta Makhanon[2]

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## Introduction

Antimicrobial agents have long been used in pig production. Among those, amoxicillin is popular based on its broad spectrum activity. A lot of amoxicillin products for pigs are available in Thailand. It is, thus, crucial to know more about the drug "performance effects" of those products in animals. This can be achieved by product tests in animal-based pharmacokinetic studies. The objective of the current study, hence, was to compare bioavailabilities of amoxicillin products used in Thailand for pigs following single oral bolus administration.

## Materials and methods

Fifteen healthy Large White-Landrace-Duroc crossbred pigs (average body weight of 21.0 kg for each group) were withdrawn from antibiotics for 14 days before the onset of this study. They were divided into 3 groups of 5 pigs. Pigs were fasted for 12 h before receiving medication. Animals in one of the groups were orally administered one of the amoxicillin products marketed in Thailand; i.e., Amoxicillin 1 (Amoxcyl), Amoxicillin 2, and Amoxicillin 3. Delivered amoxicillin dosage was 20 mg/kg body weight.

Blood samples were collected from each animal at timepoints 0, 30, 60, 90 minutes and 2, 3, 4, 5, 6, 9, 12, and 24 hours after amoxicillin administration. Each blood sample was centrifuged and plasma was collected and stored at -80 °C until analysis. Plasma concentrations of amoxicillin were analyzed by UPLC-MS/MS using a modified method[1,2]. Pharmacokinetic (PK) parameters: AUC, $C_{max,}$ and $T_{max,}$ were calculated using PK solutions 2.0x software. Statistical analysis was performed using Systat 10 software.

## Results

Results on different blood PK parameters of the tested amoxicillin products are shown in Table 1.

**Table 1. Blood pharmacokinetics of three amoxicillin products (Mean±SD) in 5 pigs/ group.**

| PK Parameters | Amoxicillin 1 (Amoxcyl) | Amoxicillin 2 | Amoxicillin 3 |
|---|---|---|---|
| Cmax (µg/ml)* | $3.83 \pm 2.38^{a}$ | $0.51 \pm 0.22^{c}$ | $1.10 \pm 0.84^{b,c}$ |
| AUC 0-24h (µg-hr/ml)* | $6.80 \pm 4.13^{a}$ | $0.75 \pm 0.32^{b}$ | $1.85 \pm 1.53^{a,b}$ |
| Log AUC* | $0.77 \pm 0.26^{a}$ | $-0.17 \pm 0.26^{c}$ | $0.08 \pm 0.51^{b,c}$ |
| Tmax (hr) | $0.90 \pm 0.22$ | $1.10 \pm 0.65$ | $1.60 \pm 0.42$ |

$^{a,b,c}$ Treatments in the same row with the same superscript letter did not differ at 90% confidence interval.

## Discussion

Statistically significant differences were determined for all *PK parameters used for drug bioavailability and bioequivalence measurement. Cmax of Amoxicillin 1 (Amoxcyl) was much higher and significantly different from Amoxicillin 2 and Amoxicillin 3. In addition, AUC of Amoxicillin 1 (Amoxcyl) was significantly different versus AUC of Amoxicillin 2. Obtained results indicate higher bioavailability behaviour of Amoxicillin 1 (Amoxcyl) in comparison to the other tested products.

## Reference

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# Environmental Health

# Effect of Probiotics (BACTOSAC-P) in Nursery and Fattening pigs Diets on Performance, Carcass Quality and Economic Benefits Return

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**Introduction**

Probiotics is a new class of feed additive which its advantages have been documented in animal performance and health. The action of probiotics is not only as growth promoters and feed savers, but also as nutritional bioregulator useful to animal production. Previous study reported that the use of yeast as a probiotics in pig diets results in improvement of appetite, palatability, digestibility of feedstuffs and feed efficiency in pig from wean to slaughter house [1-3]. In addition, administration of *Bacillus toyoi* in creep feed could prevent diarrhea and mortality in sucking piglet which indicated that the product could satisfactory replace antibiotics supplemented in feed [4]. Therefore, the objective of this study was to evaluate efficacy of probiotics (BACTOSAC-P; *L. acidophilus, L. plantarum, P. pentosaceus, S. faecium, B. subtilis, B. licheniformis and S. cerevisiae*) in nursery and fattening pig diets.

**Materials and Methods**

A total of 360 crossbred nursery piglets [Duroc x (Yorkshire x Landrace)] were divided into 3 treatments with 4 replications of 30 piglets each (2 replications for male and 2 replications for female). All weaned piglets were house in an evaporative regulated pens and fattening were house in conventional pen throughout the study (146 days period). All pigs were fed *ad libitum* access to feed and water. The three treatments consisted of control diet for treatment 1 and control diet supplemented with probiotics (BACTOSAC-P) two levels (0.5 and 1.00 kg/t) for treatment 2 and 3 for nursery, starter, grower and finisher diets. In treatment 2 and 3, antibiotics in nursery feed were deducted 20% compare to control diet of treatment 1. Body weight, feed intake, mortality were recorded. Carcass quality was evaluated at the termination of tested.

**Results**

For overall period of evaluation, supplementation of BACTOSAC-P 0.5 and 1 kg/ton in fattening diets showed remarkable increasing productive performance which resulting in increased economic benefits return (table1). The Carcass quality of pig administered of BACTOSAC-P showed improvement of carcass characteristic when compared to control group (table 2).

***Table.1*** *Effects of addition BACTOSAC-P to the diets on the performance in overall period (146 days)*

| Parameter | Control (Tx.1) | Supplemented with BACTOSAC-P | |
|---|---|---|---|
| | | 0.5 kg/t (Tx.2) | 1 kg/t (Tx.3) |
| Initial weight, kg | 6.93 | 6.76 | 6.81 |
| Final weight, kg | 93.67[b] | 96.09[ab] | 97.57[a] |
| Survival rate, % | 97.5 | 98.33 | 99.17 |
| ADG, g | 641[b] | 658[ab] | 668[a] |
| FCR | 2.336[a] | 2.228[b] | 2.187[b] |
| FCG, Baht/kg BWG | 34.39 | 33.56 | 33.56 |
| Economic benefits return per head, Baht | | 189.66 | 240.9 |

[a,b] Means within row with no common superscript differ significant(*P<0.05*)

***Table.2*** *Effects of BACTOSAC-P supplementation in fattening pigs diets on carcass characteristics.*

| Parameter | Control (Tx.1) | Supplemented with BACTOSAC-P | |
|---|---|---|---|
| | | 0.5 kg/t (Tx.2) | 1 kg/t (Tx.3) |
| Carcass evaluation at termination* | | | |
| Live weight , kg | 100 | 102.75 | 104.4 |
| Carcass weight, kg | 72.50[b] | 74.30[ab] | 75.90[a] |
| Dressing percentage , % | 73.36 | 75.79 | 75.63 |
| Carcass length , cm | 82.5 | 84.25 | 85 |
| 10th rib fat depth, mm | 23.25 | 22.25 | 20.25 |
| Loin eye area ($cm^2$) | 41.39 | 43.42 | 45.13 |
| Percentage of muscling ,% | 53.15 | 55.26 | 56.58 |
| Color score** | 1.588 | 1.603 | 1.599 |

[a,b] Means within row with no common superscript differ significant *P<0.05*)
*Direct carcass measurement on 4 pigs (2females and 2 males) per treatment
**Color score: 1.0=normal red +, 1.5=normal red ++, 2.0=normal red+++

**Conclusions and Discussion**

The results from this study clearly demonstrated that supplementation of BACTOSAC-P with two levels (0.5 kg/t and 1 kg/t) in fattening pig diets elicits favorable biological responses in both performance and carcass quality and promote higher economic benefits return with best response found when using 1 kg/t supplementation.

**Acknowledgements**

The author would like to thanks K.M.P.Biotech Co., Ltd.Thailand for support and operation. We also thank Reuang Siri Farm for providing animals and facilities in conduction the experiments.

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# Application and effect of pig farm OK movement

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## Introduction

OK Movement means the activity for improving management, agricultural environment and community environment by applying and practicing
arrangement · cleaning · hygiene · maintenance to pig farms.

As HACCP is applied to the pig farms, The Hazard Analysis Critical Control Point (HACCP) approach is a method that could transform the current system of safety and quality assurance of meat control system[1].

However, the environmental management of the basic GAP (Good Agriculture Practice) should be preceded to make sure the application of HACCP.[2]

With the purpose of upgrading pig farms and improving environment through smooth operation and the improvement of management efficiency of the pig farm system, this study actually applied arrangement · cleaning · hygiene · maintenance to pig farms and examined its effects.

## Materials and Methods

11 farms participated in OK Movement from March to December 2014. The total number of the sows of the participating farms were around 3,600 heads. This study compared the changes of appearances before and after OK Movement, and effectiveness evaluation on business operation was composed of 7 question items for the owners.

## Results

A total of 7 cases were completed from the survey conducted on the owners. Numbers of responses are listed parenthesis after question. It was possible to another duplicate check item. The most popular answers are reported in this abstract.

### Q1. What are positive effects after the progress of OK Movement?(11)

| | |
|---|---|
| 1. Increase of efficiency according to arrangement in the surroundings | 100% |
| 2. Although it does not seem to be perfect, the staff showed the behavior to do themselves. | 50% |
| 3. Recognition changes of all staff on arrangement | 40% |
| 4. Improvement of positive mind | 30% |
| 5. Improvement of negative image and recognition on the surrounding livestock industry | 20% |

### Q2. What Difficulties were in progressing OK Movement (9)

| | |
|---|---|
| 1. Difficulty at the initial stage in inducing all staff to actively participate | 70% |
| 2. Cost burden in progress | 40% |
| 3. Increase of additional troublesome unnecessary work | 30% |
| 4. More difficulty in arrangement than before participation | 0% |

### Q3. What is the most necessary consulting direction after application of OK Movement?(11)

| | |
|---|---|
| 1. Continuous maintenance inspection after completion of the certification | 100% |
| 2. Regular employee education and support | 30% |
| 3. Active public relations of OK Movement to other pig farms | 30% |
| 4. Benefits for OK certified farms | 0% |

### Q4. Do you have the intention to advertise and recommend OK Movement to other farms?(11)

| | |
|---|---|
| 1. Yes | 100% |
| 2. No | 0% |

## Discussion

It was confirmed that after applying OK Movement to pig farms, there were changes to the surroundings of farms such as site office, tool and chemical warehouses, etc. Among the farm owners who participated in the survey, all of them replied that the positive effect is the efficiency improvement of surrounding arrangement, and voluntary behavioral and recognition changes were 50% and 40% respectively. Therefore, it was found that OK Movement works effectively on farms. In addition, it is considered that the environmental improvement can upgrade pig farms to a higher stage through the improvement of management efficiency.

## References

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# Employee survey for the evaluation of the effectiveness of pig farm "OK Movement"

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## Introduction

Production of high quality pigs requires optimized agricultural environment and a system to support pig farms [1]. As the necessity of good agricultural environment to grow pigs and raise the efficiency of management is rising, in particular environmental approach for GAP (Good Agriculture Practice) demands partial revision of the existing farm system [2].

With the purpose of improving pig farm environment to upgrade and improve management efficiency, this study conducted a questionnaire survey on farm employees, who participated in application of OK Movement that is the activity of arrangement · cleaning · hygiene · maintenance to the whole farm and evaluation of its effectiveness.

## Materials and Methods

The questionnaire survey was conducted in 2014 on 7 OK certified farms affiliated to Dodram Pig Farmer's Cooperative. The total number of the sows of the participating farms were around 3,600 heads. A questionnaire survey on 12 items was performed to 35 employees of the 7 OK Movement certified farms to evaluate the effectiveness of OK Movement.

## Results

A total of 12 cases were completed from the questionnaire survey conducted on the farm employees.

Numbers of responses are listed parenthesis after question. It was possible to another duplicate check item. The most popular answers are reported in this abstract.

Q1. What was the most difficult thing in progressing OK Movement?(34)

| | |
|---|---|
| 1. The whole progress of arrangement | 23% |
| 2. Additional heavy work in addition to basic management | 19% |
| 3. Communication with employees | 23% |
| 4. General instructions of president and head of a team (downstream) | 0% |
| 5. Insufficient communication about foreign workers | 23% |
| 6. Progress by only persons participating | 23% |
| 7. Standardization of cases and boxes | 4% |
| 8. Disposal of waste resources | 19% |
| 9. Standardization | 19% |
| 10. Criteria to dump stuff | 23% |
| 11. Overcoming myself (inconvenient to change) | 19% |

Q2. What part makes a farm need OK Movement?(33)

| | |
|---|---|
| 1. Improvement and change of recognition | 54% |
| 2. Enhancement of self-belief in workplace and work | 23% |
| 3. Farm image improvement from surrounding communities | 38% |
| 4. Atmosphere improvement due to clean environment | 46% |
| 5. Business efficiency followed by surrounding arrangement | 50% |
| 6. Critical system for the future pig farm industry | 38% |

Q3. What should be progressed in the 2nd year pigsty?(35)

| | |
|---|---|
| 1. Throwing unnecessary stuff | 35% |
| 2. Making space for used stuff | 15% |
| 3. Management of chemicals stock on site | 12% |
| 4. Around the drinking foundation on site | 8% |
| 5. Facility arrangement on site | 31% |
| 6. Cleaning the feed weighing container in the pigsty | 31% |
| 7. Arrangement of the feed warehouse | 8% |
| 8. Improvement of isolated and dark parts in the pigsty | 42% |
| 9. Around corridor and passage in the pigsty | 4% |
| 10. Preparation for and improvement of the rest area on site | 35% |

## Discussion

54% out of the employees, who participated in the questionnaire replied that the effect of participation in OK Movement was recognition improvement and attitude changes about the general business of employees, and 50 % replied business efficiency followed by arrangement of tool and chemical warehouses, and it was found that even farm employees thought it works. Because there was an opinion that as for the pigsty where the second year OK Movement should be progressed, dark and isolated parts should be improved along with employees' welfare, it should be sufficiently considered with owners in case of progress of the second year.

## References

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# Effect of Dietary Probiotics (BACTOSAC-P) Supplementation on boar Semen Quality

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## Introduction

Probiotics are microorganisms that are believed to provide health benefits when consumed. [1]Commonly claimed benefits of probiotics include the decrease of potentially pathogenic gastrointestinal microorganisms; the reduction of gastrointestinal discomfort; the strengthening of the immune system; the improvement of the skin's function; the improvement of bowel regularity; the strengthening of the resistance to cedar pollen allergens; the decrease in body pathogens; the reduction of flatulence and bloating; the protection of DNA; the protection of proteins and lipids from oxidative damage; and the maintaining of individual intestinal microbiota in subjects receiving antibiotic treatment. If the boars have a good health they will give a good semen quality. Therefore, the objective of this study was to evaluate efficacy of probiotics (BACTOSAC-P; *L. acidophilus*, *L. plantarum*, *P. pentosaceus*, *S. faecium*, *B. subtilis*, *B. licheniformis* and *S. cerevisiae*) in diet on boar semen quality.

## Materials and Methods

Fourteen Duroc boars, average 2 years old of age, were divided into 2 dietary treatment groups. Each group consisted of 7 boars. All boars were raised in evaporative cooling system house. The boars were fed 2 kg per day of basal diet (2,934.08 kcal/kg feed and 14% of crude protein). The dietary treatments were (1) basal diet (Control group), (2) basal diet+ BACTOSAC-P 1 kilogram per 1 ton of feed. (Treatment group).Preliminary study period is 4 weeks before starting record the data of semen quality for analysis. During trail period 5 weeks, each boar was collected semen 1 time a week. CASA was used for semen quality analysis.

## Results

The results found that there were highly significant difference ($p<0.01$) on total sperm concentration and numbers of semen dose production in the treatment group which supplementation of BACTOSAC-P in basal diet. While, semen volume in treatment group was significant difference ($p<0.05$) improved higher than the control group (table1).

***Table1*** Effect of Dietary Probiotics (BACTOSAC-P) Supplementation on Boar Semen Quality

| Characteristics | Control | Treatment |
|---|---|---|
| Volume (ml) | 335.51±71.61[b] | 426.11±90.64[a] |
| Colour (0-3) | 2.97±0.16 | 3.00±0.00 |
| Motility rating (0 -5) | 3.98±0.33 | 3.97±0.34 |
| pH | 7.08±0.07 | 7.07±0.07 |
| Agglutination (1-3) | 1.48±0.50 | 1045±0.50 |
| Sperm concentration ($x10^6$/ml) | 417.08±51.60 | 428.14±59.67 |
| Total sperm concentration ($x10^9$) | 139.67±33.65 | 180.15±36.39 |
| Motile sperm (%) | 96.51±2.20 | 96.46±2.39 |
| VCL (µm/s) | 53.88±7.21 | 53.25±8.12 |
| VSL (µm/s) | 29.80±5.34 | 29.68±4.56 |
| VAP(µm/s) | 34.77±5.49 | 34.71±4.97 |
| Progressive movement (%) | 55.57±8.06 | 56.94±7.26 |
| Abnormal sperm (%) | 10.41±2.96 | 9.61±2.54 |
| Numbers of semen dose production(doses)* | 33.68±8.05[B] | 43.57±8.92[A] |

[a,b] Means within row with no common superscript differ significantly ($P<0.05$)
[A,B] Means within row with no common superscript differ significantly ($P<0.01$)
*numbers of motile sperm per dose is $4x10^9$ cells

## Conclusions and Discussion

The results from this study showed that supplementation of BACTOSAC-P could improve semen volume and total sperm concentration of the boars. Therefore, the farmers can produce numbers of semen dose per 1 ejaculation more than control group. The boar in this study will have higher economic benefits from supplementation of BACTOSAC-P in the boar diet.

## Acknowledgements

The author would like to thanks K.M.P.Biotech Co., Ltd.Thailand for support and operation. We also thank Kaonamsud Pig Farm for providing animals and facilities in conduction the experiments.

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**Single serving sows to reduce carbon emissions by enhancing the FCR of finishing pigs**

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**Introduction**

Pig production needs to accelerate its genetic improvement to produce pigs with improved food conversion, reduced carbon costs and a carcase quality that reflect local and international customer requirements. The use of the pig in biological products also demands the maximum output per boar.

Current breeding programmes utilise two or even three artificial insemination matings after weaning to achieve an 82+% farrowing rate and producing a total of 13+ pig born (alive and dead).

These production targets were taken as a base line to develop alternative methods of enhancing boar production.

Single serving sows with boars have been recognized as a successful method of breeding for over 30 years. But the technique has never become a routine breeding technique, partly because if the boar is infertile the results are disastrous.

**Methods**

Semen

Boars semen was pooled (minimum 2) to avoid the likelihood of any one boar being infertile (despite visual examination of the semen). Sperm concentration was a minimum of $2x10^9$ sperm per insemination in a semen dose of 75 ml.

Semen delivery system

Sows were served using a controlled boar exposure breeding stall in groups of 3. Seven minutes were allowed for each group of 3 matings. The sow was mated using a foam tipped catheter and a semen delivery system allowing for a hands free insemination. The sow was allowed to climax and move the semen into her uterus and oviduct under peristaltic contractions.

Mating programme for Single Served Group

*Weaned sows*

The sows were weaned on day 0

There was no boar exposure (boars not present in the building) until day 4

Sows were heat checked in the morning of day 4. Sows in oestrus were noted but not bred.

Sows were heat checked in the morning of day 5. All sows in oestrus were immediately mated and the quality of the mating recorded (good, average, poor).

Sows were heat checked in the morning of day 6. All sows which recorded a poor mating on day 5 and in oestrus on day 6 were remated (recorded as a double mating – there was less than 1% of matings in this group). All sows which recorded an average or good mating on day 5 were ignored. All sows, which were in oestrus for the first time on day 6, were mated and the mating recorded (good, average, poor).

Sows were heat checked in the morning of day 7. All sows which recorded a poor mating on day 6 and in oestrus on day 7 were remated (recorded as a double mating). All sows which recorded an average or good mating on day 6 were ignored. All sows that were in oestrus for the first time on day 7 were mated and the mating recorded (good, average, poor). They were remated on day 8 (recorded as double mated).

*Return sows and gilts Late Sows (in oestrus for the first time after day 7). (double mated).*

Sows or gilts that return to oestrus once were mated once in the morning of day 1 and by a boar on day 2.

*Gilts (double mated)*

Gilts were mated twice by AI in the morning of two consecutive days (AM,AM) and the quality of the mating recorded.

Mating programme for Double Served Group

All sows were mated AM/AM following heat detection on day 4 plus post-weaning. Returns and gilts were double mated AM/AM after their oestus was detected.

**Results**

| Breeding | # bred | # farrow | FR % | Total born/sow |
|---|---|---|---|---|
| Single | 3903 | 3455 | 89 | 13.3 |
| Double | 4066 | 3375 | 83 | 13.3 |

FR= Farrowing rate

There is a statistical difference between the results favouring single service, this is to be expected as the single serving group would be naturally more fertile.

*Cost implications*

There is an obvious benefit in reducing AI purchases to achieve pregnancy.

**Summary and conclusions**

Single serving appropriate weaned sows by AI can be easily achieved by today's pig industry. There are additional chemical assistants which can also be utilised to assist farm teams to embrace single serving.

The use of single serving will reduce the variability within the finishing performance by concentrating on only the very best boars with the lowest food conversions efficiency. This will have a significant impact on carbon emissions of pig farms.

# *Evaluation of the efficacy of ADV modified live vaccine with IDAL® injector*

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**Introduction**

Aujeszky's disease, or also known as Pseudorabies is endemic in some regions of Japan and it is known to cause large economic losses at the Aujeszky's disease virus (ADV) infected farm [1]. It has been proven that the modified-live vaccine is one of the options to control ADV. Vaccination of AD vaccine is usually performed by intramuscular injection using automatic syringes injector which has needles. However this vaccination has the risk that a needle is broken and the risk of the transmission of the microbe through needles. Moreover, it takes labor in changing needles. As a safer and convenient alternative method, IDAL®(Intra-Dermal Application of Liquid), needle-free intradermal injector, has been developed. IDAL® minimizes the risk and labors mentioned above. In this study, the efficacy of using IDAL® injector is compared to that of automatic syringe injector.

**Materials and methods**

The study was performed in a conventional farm in Gunma, Japan (2 site system, 300 sows). Forty ten weeks piglets were used in this study. Thirty piglets of them were vaccinated intradermally with 1 dose /0.2mL of Porcilis Begonia IDAL by IDAL® injector (IDAL® group), and 10 piglets of them were vaccinated intramuscularly with 1 dose / 2 ml of Porcilis Begonia DF by automatic syringes injector (control group). The vaccination was performed twice every 30 days. The clinical signs and the local observation were observed for 2 weeks after injection. Blood samples were obtained at each vaccination, and one month after the second vaccination from fifteen piglets of IDAL® group and ten piglets of control group. The presence of gI specific antibody was tested using ADV(gI) ELISA kit and the neutralization antibody titer determined.

Results

Vaccinated piglets did not show clinical signs or local lesions for 2 weeks after each vaccination.

All animals were negative for gI specific antibody during this study. It was confirmed that IDAL® group and control group did not receive the infection of the field ADV during this study.

VN antibody titer at each vaccination and one month after the second vaccination in IDAL® group was 1:12.1, 1:13.9 and 1:203.2 and VN antibody titer in control group was each 1:5.3, 1:32.0 and 1:298.6. The significant difference was not recognized between IDAL® group and control group. Antibody response after the first vaccination in IDAL® group was 40% and that in control group was 100%. Antibody response after the second vaccination in IDAL® group and control group were 100% together.

**Discussion**

It was considered that IDAL® injector was safe because of no clinical signs and local lesions after injection. The antibody response against ADV after the first injection was influenced by maternal antibodies but the antibody response after the second injection was equal to control group. Therefore the effectiveness of IDAL® injection was confirmed.

**References**

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# *In vitro* efficacy of Virusnip® against encapsulated yeasts contaminated in organic matter

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## Introduction

Disinfectants are routinely used for hygienic management in swine industry to promote sanitation. Virusnip® is a disinfectant used in pig and poultry farms that has virucidal, fungicidal and bactericidal effects. *Cryptococcus neoformans* is an encapsulated yeast that contaminates the environment, and pigeons are recognized as a major reservoir of this microorganism that can migrate worldwide (1). The yeast is spread by their droppings and contaminates the farm environment. The capsule of *C. neoformans* can prevent penetration and activity of disinfectants and is considered to be a good representative for evaluation of disinfectant efficacy against unicellular fungus. Furthermore, contamination of organic substance interferes with activity and decrease effectiveness of the disinfectant. The aim of this study was to determine the efficacy of Virusnip® against *C. neoformans* by duration and concentration with and without the presence of organic matter.

## Materials and Methods

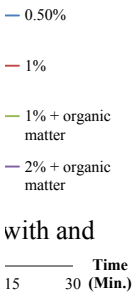
*Evaluation of fungicidal activity of Virusnip® against C. neoformans.*

Eight *C. neoformans* serotype A strains were identified by cell morphology, biochemical properties and multiplex PCR. The yeast inoculum was prepared and counted according to ISO 7218:1996 (2). Test method was conducted according to the European Standard EN1656:2000 (3). In brief, 1-ml inoculum containing $1x10^6$ yeast cells per ml were mixed with 9 ml of 1:50, 1:100, 1:200 and 1:300 Virusnip® to be 2%, 1% 0.5% and 0.33%, respectively and incubated at different times including 10 and 30 seconds, 1, 3, 5, 10, 15 and 30 minutes. One ml of mixture was neutralized by D/E neutralization, and surviving yeast cells were grown and counted by spreading on Sabouraud Dextrose Agar (SDA).

*Evaluation of fungicidal activity of Virusnip® against C. neoformans with the presence of organic matter.*

Organic matter was prepared by mixing 1 g of *C. neoformans*-free pigeon dropping with 9 ml of distilled water and then was autoclaved at 121°C for 15 minutes. One ml of sterile organic matter was mixed with 8 ml of 1:50, 1:100, 1:200 and 1:300 Virusnip®. Inoculum of *C. neoformans* were inoculated in each concentration and incubated at different time points as above. D/E neutralization was used to inactivate the reaction, and viable yeasts were grown and determined by fungal culture on SDA.

**Figure 1** Number of survival *C. neoformans* cells after exposure to each concentration of Virusnip® under conditions




with and without organic matter

## Results and Discussion

Virusnip® could completely inactivate *C. neoformans* as fast as 1 minute with 1% concentration and 2% concentration with organic matter (Figure 1). The presence of organic matter delayed the bactericidal effect. Even contaminated with organic matter, Virusnip® was able to destroy the encapsulated yeast cells within 10 minutes. One percent of Virusnip® effectively killed *C. neoformans* within 1 minute that might be applied for instrumental disinfection and killed the microorganism within 10 minutes with the presence of organic matter. This concentration is considered as a dose for bacterial and viral decontamination (4). Increase of concentration to 2% could rapidly destroy *C. neoformans* within only 1 minute. This study shows that 1% Virusnip®, which is recommended for disinfection of barriers in case of presence of organic matter, was effective for decontamination of *C. neoformans* within 10 minutes.

## References

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# Oral solutions based on toltrazuril: Are they all the same?

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## Introduction

Toltrazuril is a triazine-based antiprotozoal (TBA) agent with specific action against apicomplexan organisms. TBA agents are known for their lipophylic characteristics, and they are typically well absorbed following oral administration. If any compound is a solid and relatively insoluble in gastrointestinal (GI) fluids, it will have limited contact with the GI mucosa, and therefore, its rate of absorption will be low (Bates & Gibaldi, 1970). Based on the above considerations, it is necessary to maintain TBA agents in solution, thus allowing an increased rate of absorption following oral administration. Bioavailability is an important parameter in clinical trials because a major part of a drug's therapeutic effect is proportional to both dose and bioavailability. Moreover, when bioavailability is low, inter- and intra-subject variability in bioavailability are magnified and incomplete bioavailability becomes a major concern. Therefore, it is important to maximize oral bioavailability of TBA agents to maximize plasma drug concentrations and therefore its clinical efficacy. The goal of this study is to investigate the differences in pharmaceutics between four formulations of toltrazuril available in Vietnam.

## Materials and Methods

Three formulations of toltrazuril intended for oral treatment of coccidiosis in piglets were obtained in the Vietnamese market, and compared with Cevazuril® (Table 1). Viscosity, pH, granulometry and speed of sedimentation were determined following standard operation procedures at the Research and Development department of Ceva Animal Health. Products tested were Cevazuril® (Ceva Animal Health, batch 29A1), Baycox® (Bayer Animal Health, batch KP09JOE) and two regionally manufactured products (Product C: Vietnamese manufacturer, batch 031403723; Product D: Thai manufacturer, batch 25610256).

## Results

Cevazuril and the reference product Baycox 5% (Table 1) showed similar results for all parameters tested. However, products C and D were clearly different; product C especially for pH and product D especially for granulometry. Additionally, the speed of sedimentation was clearly higher for product C than for the rest of products, suggesting a lower stability in solution for this product.

*Table 1.- Pharmaceutics parameters for four medicinal products based on toltrazuril*

| Medicinal product | Ceva-zuril® | Baycox® 5% | Product C | Product D |
|---|---|---|---|---|
| **pH** | 4.2 | 4.5 | 7.0 | 4.2 |
| **Viscosity** | 49 cP | 42 cP | 37 cP | 78 cP |
| **Granulo-metry <10µm** | 82 % | 89 % | 69 % | 24 % |
| **Granulo-metry <30µm** | 100 % | 100 % | 94% | 36 % |

## Discussion

The relative bioavailability of toltrazuril sulfone suspended in water compared to in dimethylsulfoxide was 32% in horses (Dirikolu et al, 2009) suggesting that the pharmaceutics of toltrazuril-based products are critical to assure a good bioavailability. Our data demonstrate that for Cevazuril the parameters tested showed very similar results compared to those determined for the reference product, and clearly different from the two other products investigated.

Both larger particle size and a higher pH are expected to negatively impact bioavailability. Moreover, a lower stability of the solution might have a negative impact on the accuracy of dosing. An animal study in piglets would be required to confirm these differences in-vivo.

## References

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Dirikolu L. et al., Synthesis and detection of toltrazuril sulfone and its pharmacokinetics in horses following administration in dimethylsulfoxide. J Vet Pharmacol Ther. 2009; 32(4):368-378.

# Gilt synchronization with Altresyn®; a case study in Vietnam

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## Introduction

With the increased competitiveness and increase of average farm size, optimisation of use of resources becomes increasingly important in the Asian pig industry. To maximise the use of available farrowing crates and to reduce the cost of gilt rearing, a controlled influx of gilts into the farm's mating system becomes increasingly important.

Oral application of altrenogest is a feasible way to synchronise gilts (1, 2). This paper describes the results obtained after application of Altresyn® (Ceva Animal Health) to gilts prior to insemination in a large sow operation in Vietnam.

## Materials and Methods

In an integrated Vietnamese farm with hybrid sows, gilts were traditionally inseminated when seen in heat, thus following a "random pattern". To synchronize gilts, a group of 115 gilts aged 207 to 222 days received 5 mL Altresyn daily on their feed, during 18 days. On day 20 (2 days after the last Altresyn treatment), gilts were checked for signs of pro-estrus. From day 21 onwards, heat detection was carried out twice daily in presence of the boar.

First artificial insemination was performed 6-12 hours after the first observation of a standing reflex. A second insemination was performed 12 hours after the first. In the gilts still showing standing reflex, a third insemination was performed another 12 hours afterwards.

## Results

Out of the 115 gilts treated, 114 animals (99.1 %) showed first signs of estrus. The majority (97.4 %) of these gilts showed pro-estrus in between 4 and 7 days after ending treatment (figure 1), with 72.8 % of the animals showing first estrus at day 23 (5 days after completion of Altresyn treatment). Out of the 114 animals showing signs of pro-estrus 113 animals were inseminated.

Subsequent farrowing rate for the treated gilts was 91.2 %, with an average litter size of 11.0 piglets.


*Figure 1: Number of gilts with observed pro-estrus*

## Discussion

Altresyn was effective in synchronizing estrus in treated gilts, with 98% of the first estrus being observed in between 4 and 7 days after stopping the treatment. In a random distribution, 57 % (4/21) of all first estrus would occur in such a timeframe. The top of first occurrence of estrus was at 5 days after stopping the Altresyn treatment. At that day, 73% of the animals showed signs of estrus, while in a random distribution this is expected to be 5 % only (1/21).

The farrowing rate was slightly higher than the historical control (89.0 %) in primiparous sows, and the litter size was identical compared to the value historically seen in gilts on this farm.

Results observed are comparable with those found in studies performed outside of Asia (1, 2).

## References

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**Quantification of lethal effects against porcine respiratory and reproductive syndrome virus(PRRSV) using UV-C irradiation**

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## Introduction

Porcine respiratory and reproductive syndrome virus (PRRSV) as RNA virus causes devastating swine diseases with massive economic losses to the swine industry worldwide. In order to prevent economic losses from PRRS, many swine producers use UV-C light as a sterilizer for the workers, equipment, surface of farm units etc. The aim of the experiment was to measure levels of UV-C induced RNA damage by utilizing the property that damaged RNA of PRRSV can inhibit PCR.

## Materials and methods

PRRSV strain, ATCC VR2332, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150μW•sec/$Cm^2$, for 0.5, 1, 2, 4, 8, 16, and 32 min, respectively. RT-PCR amplified a 100bp region (1154-1253) (Table. 1) and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), Using regression normalization (a linear trendline correction).

**Table 1. Oligonucleotide primers used test**

| Primer | Primer Seq. | Product Size |
|---|---|---|
| PRRSV-f | 5'ACGGACCTATCGTCGTACAG3' | 100bp |
| PRRSV-r | 5'AGGAGGTCCTCAAACCCAGA3' | |

## Results

The inactivation ratio of PRRSV was showed relatively quantified results from ReTi-PCR. The Ct-value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999). The inactivation of PRRSV by UV-C light was dose-dependent (Table 2). Table 3 showed inactivation ratio of PRRSV using a linear trendline correction.

**Table 2. The result of Ct value and inactivation ratio by exposure time using ReTi-PCR**

| Exposure Time (min) | Ct value | Inactivation ratio(%) |
|---|---|---|
| 0.5 | 23.92 | 61.68 |
| 1 | 25,5 | 86.9 |
| 2 | 26.54 | 93.53 |
| 4 | 28.14 | 97.82 |
| 8 | 29.31 | 99.01 |
| 16 | 29.86 | 99.32 |
| 32 | 31.43 | 99.77 |

**Table 3. Calculated inactivation ratio of PRRSV**

| Virus | 1D* | 2D | 3D |
|---|---|---|---|
| PRRSV | 10.72 | 72 | 638.2 |

*D=1$log_{10}$, unit is mJ/$cm^2$ (Erwin *et al*., 2004)
* Inactivation ratio
1D=95.0% , 2D= 99.0%, 3D=99.9%

## Discussion

In Table 2&3, PRRSV was sensitive to UV light irradiation. The UV-C light is very effective and environment friendly for disinfection in pig farm units. It is thought that these results can be a useful data for sterilizing of PRRSV.

## Reference

[1] Scott D. et al. 2011. J Vetmic 150; 96-99
[2] Brouwer J. et al. 1994. Vet Q 16; 95-100

**Quantification of relative realtime RT-PCR of porcine epidemic diarrhea virus using UV-C irradiation**

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| Primer | Primer Seq. | Product Size |
|---|---|---|
| **PEDV-f** | 5`AATCCTGAAACTGACGCGCT3` | 90bp |
| **PEDV-r** | 5`TAGCGTTACACCAGTTGGGTC3` | |

**Introduction**

Recently, porcine epidemic diarrhea virus (PEDV) was detected in the USA. PEDV have continued to cause ongoing disease challenges for pork producers. In order to prevent economic losses from PED, many pork producers use UV-C light as a sterilizer. The purpose of this experiment was to measure levels of UV-C induced RNA damage by using the property that damaged RNA of PEDV can inhibit PCR.

**Materials and methods**

The PEDV strain, P-5v, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV-C lamp (Enputech, Korea) with wavelength output at 254nm, 150μW•sec/$Cm^2$, for 0.5, 1, 2, 4, 8 and 16 min, respectively. RT-PCR amplified a 90bp of PEDV membrane protein (M) gene region(337-426) (Table. 1) and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. To evaluate effects of UV induced RNA damage of PEDV, regression normalization(a linear trendline correction) was used.

**Table 1. Oligonucleotide primer used test**

**Results**

The inactivation ratio of PEDV was showed relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999) (Fig. 1).

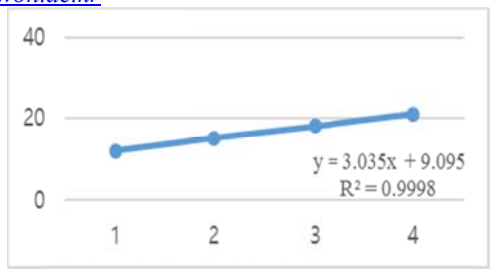


Fig. 1. Linear trendline correction of standard curve of serially diluted control samples. Y axis is Ct value and X acis is –Log (dilution ratio)-3.

The inactivation of PEDV by UV-C light was showed dose dependent (Table. 2).

Table 2. The results of Ct value and inactivation ratio of PEDV by exposed time using ReTi-PCR

| Exposure Time (min) | Ct value | Inactivation ratio (%) |
|---|---|---|
| **0.5** | 18.24 | 89.89 |
| **1** | 18.57 | 92.13 |
| **2** | 19.22 | 95.19 |
| **4** | 21.45 | 99.11 |
| **8** | 22.04 | 99.43 |
| **16** | 22.90 | 99.71 |

**Discussion**

The UV-C light was highly effective to inactivation of PEDV. But substantial viral inactivation occurred after exposure of 4 min. Increasing wavelength output of the UV lamp can be an effective way to reduce the time of exposure. The data reported in this experiment suggest that PEDV is killed by UVC light when it has absorbed the required amount of radiant energy in the lethal range. It is thought that these results can be a useful data for sterilizing PEDV.

**Reference**

[1] Ayman AM. et al. 2008. New Microbiologica 31; 47-55

[2] Dea S. et al. 2000. Arch Virol 145; 659-688

**Quantification of lethal effects against classical swine fever virus (CSFV) by UV-C irradiation**

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## Introduction

Classical swine fever virus(CSFV) as RNA virus is a highly contagious disease of pigs. It is classified as List A-grade disease determined by OIE. Because of its extremely high mortality with severe symptoms, UV light is used by many pork producers. However, it is very little known about actual effects of UV-C induced RNA damage. The aim of this experiment was to measure levels of UV-C induced RNA damage of CSFV.

## Materials and methods

CSFV, LOM strain, was used to assess the analytical performance of the reverse transcriptase PCR (RT-PCR) and real time-PCR (ReTi-PCR) protocols. This virus was treated by UV-C lamp (Enputech Co., Ltd., Korea) with wavelength output at 254nm, 150μW•sec/cm$^2$, for 1, 2 and 4 min, respectively. RT-PCR amplified a 99bp region(7824-7922), oligonucleotides CSFV-f (5'ACT ATC AAG GAA AAA GCC AAA CAG3') and CSFV-r (5'CGA ACA AGG GGG TCA GGT3'), and the amplicons were diluted $10^{-5}$-fold and analyzed by using SYBR® Green 1 Method for ReTi-PCR. Statistical evaluation was performed by Excel (Microsoft, USA), using regression normalization (a linear trend line correction).

## Results

The inactivation ratio of CSFV was showed relatively quantified results from ReTi-PCR. The Ct value of serially diluted positive control samples showed the linear correlation ($R^2$=0.999) (Fig. 1). The inactivation of CSFV by UV-C light exposure time was dose-dependent(Table1&2).

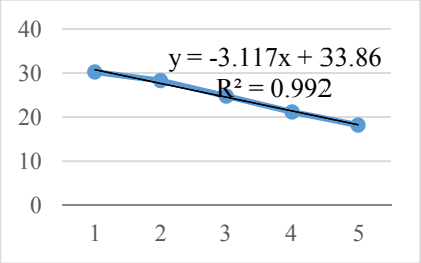


Fig. 1. Standard curv of serially diluted control samples Y axis is Ct value and X axis is Log(dilution ratio)+8.

Table 1. The results of Ct value and inactivation ratio of CSFV by exposed time using ReTi-PCR

| Exposure Time (min) | Ct value | Inactivation ratio (%) |
|---|---|---|
| **0** | 25.74938 | 0 |
| **1** | 26.5385 | 44.17158 |
| **2** | 27.25156 | 67.03028 |
| **4** | 30.4959 | 96.99835 |

Table 2. Calculated inactivation ratio of CSFV

| Virus | 1D* | 2D | 3D |
|---|---|---|---|
| CSF | 23.89894 | 101.3937 | 430.1734 |

*D=1log$_{10}$, unit is mJ/cm$^2$ (Erwin *et al*., 2004).
*Inactivation ratio:
1D=95.0% , 2D=99.0%, 3D=99.9%.

## Discussion

The UV-C light is effective to inactivate CSFV. As described in Table 1, it is required the longer than 4min to certainly inactivate CSFV. The UV light is convenient to use, as well as environment-friendly for disinfection of CSFV in pig farm units. These data can be thought to be useful for sterilizing of CSFV.

## Reference

[1] Hoffmann B et al. 2005. J.Virol.Methods 130; 36-44
[2] Erwin D et al. 2004. Appl Environ Microbiol 70; 4538-4543

# Parameters contributing to improve carcass weight per sow per year in 2011-2013, Japan

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## Introduction

In Japan, swine production benchmarking system (PigINFO) was developed for pig producers by collaboration between Japan Association of Swine Veterinarians (JASV) and the National Institute of Animal Health (NIAH), and provides useful information for their management. In PigINFO, carcass weight per sow per year (kg) (CW) which reflects both reproductive and growth performance is one of the most important index to evaluate the performance of farrow-to-finish farm. In this study, we analyzed PigINFO data and evaluated parameters that contributed to the improvement of CW.

## Materials and Methods

In this study, we used 59 farrow-to-finish farm data which contained complete data of 2011-2013, out of more than 100 farm data that were collected in to the PigINFO system each year. We classified these 59 farms by CW in 2013 as top 25% farm, middle 50% farm and bottom 25% farm. We then compared the following productive parameters in these three categories for each year: marketed pigs per sow per year (MP), pigs weaned per mated female per year (PWMFY), litters per mated female per year (LMFY), daily gain (g) (DG), feed conversion rate (FCR), average weight of marketed carcass (kg) (AWMC), and post weaning mortality (%) (POWM), in accordance with PigINFO definitions [1].

## Results

Only in the top 25% farm, CW increased significantly between 2011 and 2013(Figure 1) and the MP, PWMFY and LMFY increased significantly between 2011 and 2013 (Table 1). CW of middle 50% farm didn't increase significantly, though AWMC increased significantly in this class. In comparison with these three classes in each year, FCR, DG and POWM were not significantly different between the top 25% farm and the middle 50% farm. When we compared the top 25% farm performance with the middle 50% farm ones, CW, MP, and PWMFY only in 2013 were significantly higher than those of the middle 50% farm ($p< 0.01$). On the other hand, CW, MP, PWMFY, LMFY and DG of the bottom 25% farm were significantly lower than the other two classes over the three years ($p<0.01$-$0.05$) .

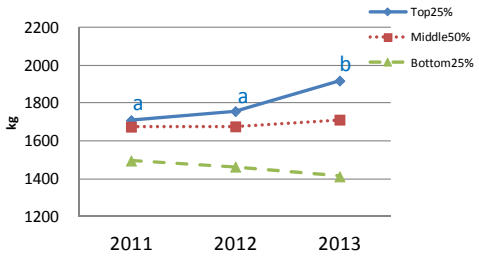


*Figure1 The trend of CW in 2011-2013 (n=59)*

*Table1 The top 25 % farm performance in 2011-2013 (n=15)*

| Production parameter | 2011 | 2012 | 2013 |
|---|---|---|---|
| | Mean | Mean | Mean |
| Carcass Weight (kg) (/sow/year) | 1710.41 a | 1758.06 a | 1919.46 b |
| Marketed Pigs (/sow/year) | 22.48 a | 23.16 a | 25.20 b |
| Pigs Weaned (/mated female/year) | 23.85 a | 25.09 b | 26.23 c |
| Litters (/mated female/year) | 2.36 a | 2.40 ab | 2.44 b |
| Average Weight of Marketed Carcass (kg) (/pig) | 76.15 a | 76.03 a | 76.20 a |
| Feed Conversion Rate | 4.86 a | 4.78 a | 4.74 a |
| Daily Gain (g) | 627.07 a | 625.49 a | 640.00 a |
| Post Weaning Mortality (%) | 4.96 a | 4.40 a | 4.53 a |

Mean values with different superscript are significantly different ($p<0.05$)

## Discussion

Only CW of the top 25% farm increased significantly between 2012 and 2013. It was because of the improvement of reproductive performance, not because of the growth performance. The trend of improvement of PWMFY in the top 25% farm was 1.19 pigs/mated female/year in those three years. On the other hand, some major breeding companies have said that their genetic trend of PWMFY was 0.375-0.45 pigs/mated female/year. So it seems that the improvement of PWMFY of the top 25% farm was affected by introduction of high reproductive performance genes in recent years. Moreover, considering the improvement of LMFY of the top 25% farm, it seems that there was a progress of breeding management in this top class as well.

## Acknowledgement

This study was supported by collaborative research grants from the NIAH and JASV.

## Reference

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# Small Producer Cooperation

The 7th Asian Pig Veterinary Society Congress
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# Investigation of pig farms productivity using post-cervical catheter

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## Introduction

Post-cervical artificial insemination is a method to inject sperm into the uterine body (Gil et al., 2000: Watson and Behan, 2002; Gil et al., 2004; Roberts and Bilkey, 2005) [1][3] and is knows as an artificial insemination with less technical problems or side effects (Roberts et al., 2005)[2]. In Korea, the post-cervical catheter has been expanded and distributed in full scale since 2012 and the breeding records before and after the use of the post-cervical catheter by farms were taken to proceed with the effectiveness analysis on the use of the post-cervical catheter.

## Materials and Methods

The total litter sizes of 12 farms after delivery that have consistently used the post-cervical catheter for more than 3 years between 2012 and 2014 were compared. The sows of the farms were a total of 27,000 heads and for data processing, farms that used the pig plan web version for more than 3 years were selected. As for the post-cervical catheter, Magapo integral post-cervical catheter was used and PPE (polyethylene + polypropylene) was used for the catheter that goes into the uterine body. For artificial insemination, 2 billion 100 ㎖ sperm produced by the Dodram Gene Center was used.

## Results

Table 1. Comparison of Total Litter Sizes after Use of Post-cervical Catheter

| | Calculation Basis : January ~ December | | |
|---|---|---|---|
| | 2012* | 2013** | 2014** |
| **A** | 12.2 | 11.8 | 13.2 |
| **B** | 10.9 | 12.5 | 12.3 |
| **C** | 11.5 | 13.2 | 13.7 |
| **D** | 12.2 | 12.1 | 13.0 |
| **E** | 10.7 | 12.0 | 11.3 |
| **F** | 11.8 | 12.0 | 12.4 |
| **G** | 12.5 | 12.4 | 12.2 |
| **H** | 11.7 | 12.0 | 12.5 |
| **I** | 12.3 | 12.5 | 12.6 |
| **J** | 11.7 | 12.5 | 12.4 |
| **K** | 11.4 | 12.4 | 13.0 |
| **L** | 11.9 | 13.3 | 13.0 |
| **Total Production/ Average** | 11.7 | 12.4 | 12.7 |
| **Average of EDP Farms*** ** | 11.6 | 12.1 | 12.3 |

* : Use of post-cervical catheter
**: After the use of post-cervical catheter
***: 102 farms using pig plan web

The average total production of 12 farms that used the post-cervical catheter for more than 3 years increased 1.0 head from 11.7 heads in 2012 to 12.7 heads in 2014 and the average total production of 102 heads confirmed through the data records during the same period increased 0.7 head from 11.6 heads in 2012 to 12.3 heads in 2014 and the total production of farms that used the post-cervical catheter increased 0.3 head compared to the average of the total farms that used electronic data processing.

## Conclusions and Discussion

In the investigation of abnormal meats by FMD
As confirmed in the data of Pig Plan Web for 3 years, it was found that after the use of the post-cervical catheter, farms showed increase of 0.3 head in the average total production compared to the time before its use so that the use of the post-cervical catheter gave a certain influence on breeding records. In case that the average total production of farms with 100 sows increases 0.3 head, it is presumed that the sow turnover ratio increases 2.4, the raising ratio after the weaning period does 85%, the average weight does 110kg and based on 5,500 Won (July 2013 ~ June 2014), approximately 20 million Won/year increases arithmetically. The use of the post-cervical catheter seems to be consistently increasing for the improvement of stable breeding records in the future.

## References

[1] Gil J, Tortades J, Alevia A. Post-cervical insemination. Asociación Nacional de Porcinocultura Científica 2001, 209, 150-156.
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[3] Watson PF, Behan JR. Intrauterine insemination of sows with reduced sperm numbers: Results of a commercially based field trial. Theriogenology 2002, 57, 1683-1693.

# Oral administration of a fermented potato protein product reduces early pre-weaning mortality in low birth weight piglets

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## Introduction

With the high prolificacy of the commercial sows, pre-weaning mortality remains an unsolved problem in pig industry. Among the different causes of early piglet pre-weaning mortality, insufficient colostrum intake is one of the most important. The smaller the piglet is, the higher risk of pre-weaning mortality is observed. Lianol® Colostro, a pro-metabolic regulator derived form fermented potato protein, have shown promising results in enhancing newborn piglet's performance [1,2]. Therefore, the aim of the experiment was to study the effect of Lianol® Colostro supplementation on newborn piglet's survival and growth during early lactation period.

## Materials and methods

The experiment was performed in a commercial swine herd in the western region of Thailand and included 36 multiparous sows and their litters (423 piglets). Litters were distributed to one of the two treatments: CONTROL, no management intervention to newborn piglets; or LIANOL, small piglets (SP: birth BW ≤ 1.35 kg) received 3 ml of Lianol® Colostro within 4 h after birth and 8 h after the first administration. Piglets were weighed shortly after birth, at 24 h and at day 10 of life. Piglet's rectal temperature was recorded shortly after birth and at 24 h. Cross-fostering was performed 24 h after farrowing and litters were fixed at 12 piglets. Each litter contained 4 or 5 SP. Mortality was recorded differentiating between the first 24 h of life (before cross-fostering) and after cross-fostering up to day 10. Data was analyzed using SAS 9.2. Litter was considered the statistical unit in all the situations.

## Result

Results showed a reduced total mortality rate at 24 h in LIANOL litters compared to CONTROL (2.1 vs 7.1 ± 1.1 %; $P = 0.024$) and a tendency for lower SP mortality rate (4.5 vs 11.1 ± 2.5 %; $P = 0.067$). Total mortality rate and SP mortality rate from cross-fostering to day 10 did not differ between groups ($P > 0.10$). Mean litter BW and SP BW results are presented in Table 1. At day 10, SP in the LIANOL group had a tendency for increased BW (2.65 and 2.78 kg, $P = 0.073$). Rectal temperature was not influenced by treatment ($P > 0.10$).

***Table.1*** The effect of Lianol® Colostro administration on mean litter BW and on small piglets BW.

| | CONTROL | LIANOL |
|---|---|---|
| Number of litters | 20 | 16 |
| Mean birth BW, kg | 1.44 ± 0.02 | 1.43 ± 0.02 |
| Mean BW 24h, kg | 1.54 ± 0.02 | 1.51 ± 0.03 |
| Mean BW d10, kg | 3.26 ± 0.06 | 3.35 ± 0.07 |
| SP birth BW, kg | 1.14 ± 0.02 | 1.12 ± 0.02 |
| SP BW 24h, kg | 1.2 ± 0.02 | 1.18 ± 0.02 |
| SP BW d10, kg | 2.65 ± 0.07[a] | 2.78 ± 0.09[b] |

[a,b] *differ at P < 0.10 within group*

## Discussion

Lianol® Colostro helped to increase SP survival during the first day of life, contributing to reduce total litter mortality at 24h. Moreover, piglets administered with Lianol® Colostro had a moderate increase of BW at day 10. Results from this study suggest that Lianol® Colostro administration to piglets with low birth BW increases survival without the risk of maintaining in the litter weak piglets with lower growth capacity.

## Reference

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# Longevity of boar semen extended in two types of extender

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## Introduction

Ageing of boar semen influence fertilizing ability of the spermatozoa. Loss of ATP and cAMP during storage decrease of sperm motility, an important parameter for fertility. The lipid peroxidation also damage the plasma membrane [1]. Selecting optimal semen extender for boar semen preservation is therefore important. The aim of the present study was to evaluate the longevity of semen extended in two different commercial semen extender under tropical conditions.

## Materials and methods

A total of 94 ejaculates of boar semen were prepared in two commercial AI studs and send to the laboratory within 24 h. Each ejaculates of the sample was split into two doses. The split semen were extended in either Beltsville Thawing Solution (BTS) (n=94) or Duragen® (Megapor®, Spain) (n=94) making a final dilution of 35 x $10^6$ sperm/ml. The diluted semen were evaluated for the viability and subjective motility on Days 1, 2, 3 and 5. For the sperm viability, 10 µl of semen were smeared on slide and was stained by eosin-nigrosin. In total, 200 sperm were examined for viability under light microscope at the 1000x magnification. The motility was estimated under light microscope at 200x magnification. A drop of 1:1 extended semen was placed on 37 °C warmed slide covered with cover slip. The subjective motility was evaluated under light microscope with 400 magnification and scored in percentage from 0% to 100% [2]. The repeated measure ANOVA and paired *t* test were used to analyze the difference within group and between groups of the semen traits, respectively. $P<0.05$ was regarded to be statistically significant.

## Result

The percentage of sperm viability, subjective motility were present in Table 1 and 2, respectively.

**Table.1** Percentage of sperm viability (means ± SEM) in BTS and Duragen ® for 5 days

| Day of preservation | Sperm viability (%) | |
|---|---|---|
| | BTS | Duragen® |
| 1 | 75.5±1.2 $^{a,A}$ | 75.8±1.0 $^{a,A}$ |
| 2 | 69.9±2.2 $^{b,A}$ | 67.1±2.1 $^{b,B}$ |
| 3 | 72.5±2.3 $^{ab,A}$ | 69.9±2.6 $^{b,B}$ |
| 5 | 51.0±13.8 $^{c,A}$ | 63.5±3.0 $^{b,A}$ |

**Table.2** Percentage of sperm subjective motility (means ± SEM) in BTS and Duragen ® for 5 days

| Day of preservation | Subjective motility (%) | |
|---|---|---|
| | BTS | Duragen® |
| 1 | 67.7±1.6 $^{a,A}$ | 64.1±2.2 $^{a,B}$ |
| 2 | 47.8±3.4 $^{b,A}$ | 47.7±3.5 $^{b,A}$ |
| 3 | 28.2±3.9 $^{c,A}$ | 25.0±4.4 $^{c,A}$ |
| 5 | 22.6±4.6 $^{c,A}$ | 24.4±4.9 $^{c,B}$ |

$^{A,B}$ Different capital letter superscript between column differ significantly ($P<0.05$); $^{a,b,c}$ Different small letter superscript within column differ significantly ($P<0.05$)

## Conclusions and Discussion

The present study revealed that both semen extenders had a similar decreasing pattern of sperm viability and motility during preservation. Furthermore, the longevity of the semen kept in Duragen® (i.e., a long term extender) was unexpected too low. This may cause by the fluctuation of the temperature during preservation or hot and humid climates during transportation. These results indicated that not only the semen extenders, but also the quality of the initial fresh semen used and the preservation procedure are important for longevity of extended boar semen preserved under tropical climates.

## Reference

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# Effects of lipid-encapsulated zinc oxide supplementation on colibacillosis, growth and intestinal morphology in weaned piglets challenged with enterotoxigenic *Escherichia coli* K88

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## Introduction

Enterotoxigenic *Escherichia coli* (ETEC), which frequently causes post-weaning diarrhea, proliferates in the small intestine and also is shed into feces after weaning of the piglets. Dietary supplementation of 1500–3000 ppm zinc oxide (ZnO) has been widely used to prevent porcine colibacillosis. Shield Zn® is a proprietary ZnO product which is encapsulated (coated) with lipid to allow the active component to reach the intestine without being ionized in the stomach. The present study was therefore initiated to investigate the effects of dietary supplementation of 100 ppm of the coated ZnO relative to those of 2500 as well as 100 ppm of native ZnO in weaned piglets with colibacillosis induced by the ETEC K88 challenge.

## Materials and methods

Thirty-two 35-day-old weaned piglets were orally challenged with $3 \times 10^{10}$ colony forming units of ETEC K88 while eight piglets received no challenge (control). Each eight challenged piglets received a diet containing 100 ppm ZnO (low ZnO), 2500 ppm ZnO (high ZnO) or 100 ppm of lipid (10%)-coated ZnO (coated ZnO) for 7 days; control pigs received the low ZnO diet. Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, villus height in the jejunum and ileum, fecal consistency score, serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum were measured by the coated ZnO, high ZnO and low ZnO groups.

## Results

The results of body temperature of challenged pigs are shown in Figure 1 and fecal consistency score of challenged pigs are shown in Figure 2.

Daily gain, goblet cell density in the villi of the duodenum, jejunum and ileum, and villus height in the jejunum and ileum, which decreased due to the challenge, were equally greater in the coated ZnO and high ZnO groups versus low ZnO group. Serum interleukin-8 concentration, subjective score of fecal E. coli shedding, and digesta pH in the stomach, jejunum and ileum, which increased due to the challenge, were equally low in the coated ZnO and high ZnO groups versus low ZnO.

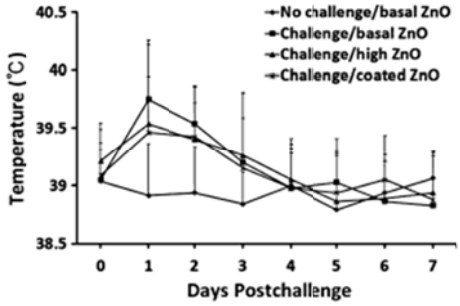


**Figure 7. Body temperature of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88.**

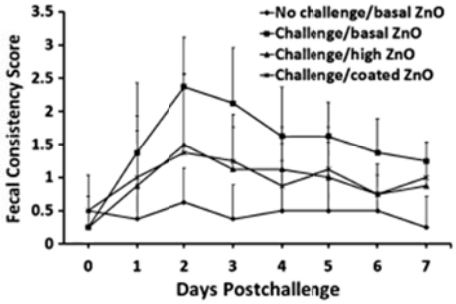


**Figure 8. Fecal consistency score (right) of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88. (0, normal;1, soft feces; 2, mild diarrhea; 3, severe diarrhea).**

## Discussion

Dietary supplementation of 100 ppm of lipid-coated ZnO (72 ppm Zn) can effectively alleviate colibacillosis caused by an oral challenge with ETEC K88 in weaned piglets. Moreover, the effect of 100 ppm of the coated ZnO on colibacillosis was equal to that of 2500 ppm of native ZnO (2000 ppm Zn) in almost all the measures associated with colibacillosis examined in the present study.

## Reference

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[2] Lee, CY, J. Ani. Sci. and Tech. 53, 227-235

# Effects of dietary supplementation of bacteriophages in treatment of infection in post-weaning pigs challenged with enterotoxigenic *Escherichia coli* K88 and K99

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## Introduction

The post-weaning diarrhea or colibacillosis is a most costly disease causing substantial mortality as well as growth retardation in swine production. Bacteriophages or phages have recently received reemerging attention as alternatives to antibiotics because of several merits as feed additives including their high stability within the feed and digestive tract as well as their high specificity of transfection. The present study was therefore initiated to evaluate the efficacy of dietary phages on treatment of colibacillosis induced by an oral challenge of ETEC K88 and K99 in post-weaning pigs.

## Materials and methods

Eighteen 35-d-old post-weaning pigs were allotted to three groups, after which two groups were orally challenged with $3.0 \times 10^8$ cfu of each of ETEC K88 and K99. The unchallenged group and one challenged group were fed a typical nursery diet (Control and Chal/Basal, respectively) while the remaining challenged group received the same diet supplemented with $1.0 \times 10^9$ cfu of each of ETEC K88- and K99-specific phages per kg (Chal/Phage). All animals were killed after a 7-d feeding trial and subjected to necropsy.

## Results

The results of body temperature of challenged pigs are shown in Figure 1 and fecal consistency score of challenged pigs are shown in Figure 2.

The ETEC K88 and K99 were detected in all feces samples obtained on d 1, 3, and 7 only in the Chal/Basal and Chal/Phage groups. The log cfu values of ETEC K88 per g feces on d 1 and 3 and per g tissue in the ileum and cecum at necropsy were less in the Chal/Phage group vs. the Chal/Basal group whereas in ETEC K99, neither the fecal excretion nor intestinal adhesion was influenced by the phage therapy.

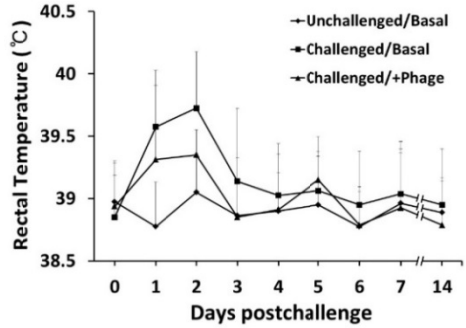


**Figure 9. Body temperature of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88 and K99.**

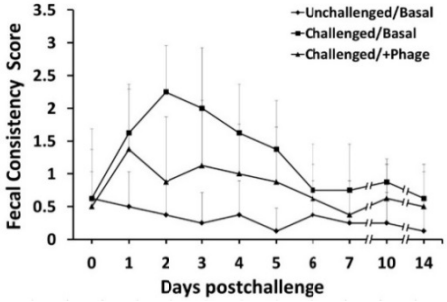


**Figure 10. Fecal consistency score (right) of weaned piglets after an oral challenge with enterotoxigenic Escherichia coli (ETEC) K88. (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea).**

## Discussion

The effect of the phage therapy appears to be significant in the ETEC K88 infection, but not in the ETEC K99 infection, in terms of suppression of intestinal adhesion and fecal excretion of the pathogens. Future studies are therefore needed to be focused on the effects of the ETEC K88-specific phage on the pathophysiological measures to further evaluate the phage as a therapeutic or prophylactic agent against porcine colibacillosis.

## Reference

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# Assessment of SUISENG® efficacy under field conditions in Korean farm

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## Introduction

Recently, *Escherichia coli*(*E. coli*) and *Clostridium perfringens* type C(CpC)-associated diarrhea in preweaner causes massive economic losses to the swine industry worldwide. In order to prevent economic losses from E.coli and CpC, many swine producers use inactivated vaccine.
Commonly, diarrheas induced by these pathogens are prevented through sow vaccination that are booster vaccinated 2 times before farrowing. The aim of this study is to assess the serological efficacy of SUISENG®(Hipra, Spain) in commercial farm located in Korea.

## Materials and methods

The study was carried out in a farm with 300 sows in Korea showed 15% occurrence of diarrhea and around 10% mortality during lactation. The experimental groups consisted of 6 vaccinated sows and 4 control sows (30 piglets of vaccination group and 20 piglets of control group). Sera from sows, vaccinated with SUISENG® and with a placebo, and their preweaners were used in this work. Tested by using in-house ELISA provided from Hipra HQ. Mann-Whitney test and T-test($p<0.05$) of SPSS statistics 20 (IBM Corp., USA) were used for statistical significance.

## Results

The mean of antibody titer against each antigens (IRPC) of the ELISA are represented in the Tables 1 and 2. Tables showed clear distinction between vaccination group and control group. Sows and their piglets of vaccination group were showed higher antibody titer against all fimbrial antigens and toxin. Statistical differences between vaccinated and control group were observed.

**Tables 1. Antibody titer against all fimbrial antigens and toxins in sows(mean IRPC) on each group**

| | 2nd shoot | | Farrowing | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 50.6 | 39.0 | 51.8 | 29.7 |
| F4ac | 63.4 | 41.5 | 72.3 | 29.9 |
| F5 | 26.5 | 47.6 | 62.2 | 19.9 |
| F6 | 30.2 | 10.4 | 60.3 | 13.5 |
| LT | 24.0 | 11.1 | 16.6 | 3.8 |
| CpC | 69.9 | 47.6 | 50.5 | 22.9 |

**Tables 2. Antibody titer against all fimbrial antigens and toxins in preweaners(mean IRPC) on each group**

| | 2-3 days | | 1 week | |
|---|---|---|---|---|
| Antigen | SUISENG | control | SUISENG | control |
| F4ab | 54.4 | 41.6 | 58.5 | 32.8 |
| F4ac | 85.7 | 42.0 | 48.4 | 41.1 |
| F5 | 32.2 | 36.4 | 43.2 | 13.4 |
| F6 | 64.3 | 29.2 | 48.4 | 18.1 |
| LT | 33.3 | 17.8 | 20.8 | 7.8 |
| CpC | 74.2 | 50.8 | 60.6 | 21.1 |

## Discussion

There was statistical significance in F4ac, F5.antibody titers of farrowing sows. And there was significant difference in antibody titers against CpC, F4ab, F5, F6 and LT of 1week-old-piglets. The results clearly demonstrate that SUISENG® induces the production of specific antibodies the LT toxin and fimbrial antigens of *E. coli* and toxoid of CpC.

## Reference

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# Field efficacy of a new *Mycoplasma hyopneumoniae* vaccine (Sprintvac MH®) in a swine herd in Thailand

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## Introduction

*Mycoplasma hyopneumoniae* are associated with many respiratory diseases such as enzootic pneumonia and porcine respiratory disease complex (PRDC)[1]. Generally, pneumonia caused by *M. hyopneumoniae* consists of a chronic lesion with dark red to grey areas of cranio-ventral consolidation. A sporadic, dry and non-productive cough is a characteristic clinical sign of *M. hyopneumoniae* infection.

Vaccination against *M. hyopneumoniae* has been applied for disease prevention and control. The aim of this study was to assess the efficiency of a new *M. hyopneumoniae* vaccine (Sprintvac MH®) to prevent enzootic pneumonia in swine herd.

## Materials and methods

A study was performed in the endemically *M. hyopneumoniae*-infected farrow-to-finish herd in the central part of Thailand. In total, 523 piglets were divided to 2 groups based on type of vaccines, group A with Sprintvac MH®; Merial (Thailand) Ltd. (258 piglets), group B with Ingelvac®MycoFLEX; Boheringer Ingelheim Animal Health (265 piglets). Piglets from both groups were vaccinated at 3 weeks old and kept inside the same barn but in separated pens until slaughtered. Nasal swab samples of 52 piglets in each group were serially collected at 3, 7, 10, 15 and 21 weeks of age for mycoplasmal culture and PCR assay. At slaughter, 149 lungs from each group were randomly evaluated for lung lesion scores using a method modified by Straw et al[2].

## Result

Percentages of slaughter pigs in each categorized lung lesion score were shown in figure 1. The average score of mycoplasma-like lesion of 2.4 and 2.7 in groups A and B, respectively, showed no significantly statistical difference ($p = 0.50$).

High percentages of pigs in both groups were found positive at 15 weeks of age by mycoplasmal culture and PCR assay.

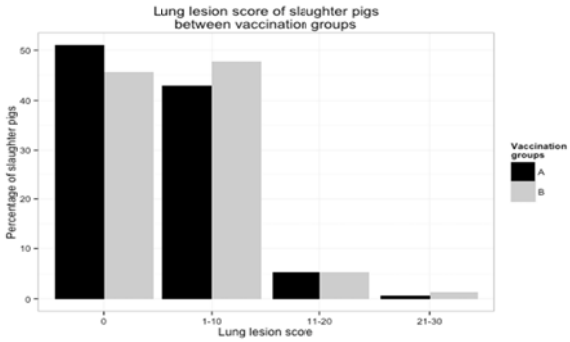


*Figure 1 Lung lesion scores of slaughter pigs in both vaccination groups*

## Discussion

Sprintvac MH® vaccine showed a good efficiency in reduction of mycoplasma-like lesion since more than 90% of lung samples showed lung lesion scores that were lower than 10. Moreover, the average lung lesion score is very low. Pigs in both groups developed a clinical sign of dry cough at the 14-16 weeks of age that was correspondent with mycoplasmal culture and PCR results.

## Reference

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# Implementation of a single fixed-time AI in weaned sows

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## Introduction

The use of a single, fixed time artificial insemination (AI) for swine has long been desired in order to increase efficiency of batch management. In this study, the reproductive outcomes following induction of ovulation using buserelin, in combination with a single, fixed-time, post-cervical AI (PCAI), were assessed.

## Materials and methods

Sows were weaned following an 18-23 day lactation period (average 20.5 days), and weaned between 8 and 14 piglets (average of 11.0 piglets). Sows (n=70; primiparous n=14, multiparous n=56) were randomly assigned to either the treatment group (n=35) or the control (n=35). Groups were balanced for parity and weight loss during lactation.
Buserelin 10 µg (Porceptal®; 2.5mL) or a control injection (saline; 2.5ml) were administered intramuscularly 93±1 hrs following weaning.
Transrectal Real-time Ultrasonography was performed every 24 hours beginning 94 hrs post-weaning and continuing until ovulation was confirmed. All sows were bred using PCAI; treatment sows were bred 31-33 hrs post-buserelin injection (irrespective of estrus), and control sows, following detection of estrus, and every 24 hrs subsequently until no longer expressing standing reflex. Inseminations were performed with pooled semen from 2-3 mature boars of known fertility, no more than 72 hrs following semen processing.

## Results

Farrowing rate, born alive and piglet weight for the treatment and control groups are shown below, (Table 1)

| | Treatment | Control |
|---|---|---|
| Submission to AI | 100 % | 91.2 % |
| Conception rate | 97.0 % | 100 % |
| Pregnancy rate | 97.0 % | 91.2 % |
| Farrowing Rate | 97.0 % | 91.2 % |
| Born alive | 14,3 | 14,19 |
| Piglet weight | 1,42 | 1,39 |

Treated sows tended to ovulate earlier than control sows (128.78±4.33 and 136.36±4.25 hrs post weaning respectively; p=0.07).
Control sows were bred following observed estrus, leading to a submission rate of 91.2%, all of which conceived from the subsequent multiple AIs, leading to a pregnancy rate of 91.2%. In the treated group 88.2% of sows expressed estrus on the day of fixed time insemination. All treated sows were AI'd at the designated time (100% submission rate) leading to a conception and pregnancy rate of 97%. In view of the animal numbers (n= 35 per group) statistical significance of the difference in farrowing rate was not reached.
Control sows received an average of 2.63 inseminations, contrasted with the single insemination each treated sow received. None of the control sows ovulated prior to insemination; however 20 of them received inseminations that were performed greater than 12 hrs after ovulation. In contrast, 5 treated sows were found to have ovulated prior to insemination, but only 4 inseminations were performed greater than 12 hrs after ovulation. This difference was significant ($p<0.01$). The average number of piglets born alive and the piglet birth weight were similar for the two groups, with a slight numerical advantage in the treated group.

## Discussion

Ovulation induction with buserelin followed by a single fixed-time post-cervical AI may enable more efficient batch management of sows. Potential benefits include reduced labour costs for heat checking and breeding, and more efficient use of semen from genetically superior boars, whilst maintaining equivalent reproductive performance.

## Reference

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# Use of buserelin in nulliparous and multiparous sows: Effect on the reproductive system and weight variability of the resulting offspring

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**Introduction**

Increasing prolificacy in pig farms is a challenge for swine production, because selecting for increased litter size has a direct impact on the variability of the piglet´s weight.

The present study includes two objectives: to determine how buserelin affects the reproductive tract and to evaluate the relationship between the administration of buserelin and piglet weight and litter uniformity.

**Materials and Methods**

In the first experiment, reproductive structures were compared between two gilt groups: Treated group (n=19) was administered a dose of 10µg of buserelin in gilts 120 hours after the last dose of altrenogest "Regumate®" and compared with non-treated Control (n=18). In the second, treated sows were administered 10µg of buserelin (2.5 ml "Porceptal®"), 85 hours after weaning (n=30) vs non-treated Control group (n=30). Treated sows were inseminated with a single fixed time AI, compared to the untreated group that was inseminated twice. In both cases the semen was from the same ejaculate. Birth weight and homogeneity of the resulting litter was evaluated.

**Results**

Progestogens were found to be an effective method of oestrus synchronization in gilts. An injection of was effective in reducing variability in follicle size and significantly increased the number of follicles above 6mm (P=0,05). Furthermore, the size (Buserelin=306cm Vs Control=238cm) and weight (Buserelin=912g Vs Control=685g) of the uterine horns was significantly greater in the treated group than the control (Size-P=0,001 & weight-P=0,026). Similarly, the buserelin sows had a significantly greater average piglet weight at birth (1,63g), regardless of size, (P=0,001) than the control group (1,49g). This difference remained in favor of buserelin group throughout lactation and nursery with an average daily weight gain in lactation and nursery that was higher in the buserelin group (Lac=215g/Nurs= 311g) than the control group (Lac=198g/Nurs= 288g).

**Conclusions and Discussions**

1. The use of progestogens is an effective synchronization of oestrus in gilts method.
2. The size and weight of the uterine horns was statistically higher in the group treated with buserelin group than in the control group.
3. The application of buserelin in gilts was effective in reducing variability follicle size and to increase the number of follicles with above 6mm.
4. The application of buserelin in weaned sows had significant differences with the control group on the average birth weight of piglets and Buserelin group had higher average weight at 21 and 60 days after birth.

# Insemination and heat detection cost in Spanish sow farms

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## Introduction

When we consider time consuming areas in a farm, gestation takes up 40% to 60% of the time. Insemination and heat detection are by far the most time consuming tasks for people in sow farms (**1**). Often when the cost of performing one insemination is evaluated, the emphasis is only on semen cost, whilst the importance of labor cost is overlooked.

The aim of this study was to evaluate the labor cost of insemination and heat detection.

## Materials and Methods

Forty (40) Spanish sow farms representing almost 70,000 sows, were included in this study to evaluate the time spend per sow on insemination and heat detection. Time Spend on heat-checking and insemination per sow and per day (TSD) was calculated considering the total daily time spend on these activities divided by the number of sows per batch. Cost per hour (CH) was calculated by dividing the total annual cost by the total number of working hours per year. Cost per insemination and heat detection (CIHD) was calculated taking into account the cost per hour (CH) divided by 60 minutes and then multiplied by TSD.

CIHD = (CH/60) x TSD

Finally Gestation Labor cost per pregnant sow (GCPS) was calculated considering a farrowing rate of 84.5 % (2) and 5 days expend doing Heat detection and insemination.

Farms were divided in three different groups according to size; 300-1000 sows as small (SF) (n=13), 1000 to 2000 as medium (MF) (n=12) and higher than 2000 as large (LF) (n=15).

## Results

Farm size affects the time that people spend per sow per day: larger farms have less time, 7.15 to 7.25 minutes, compared to small farms 9.12 minutes (almost two minutes difference). At the same time, cost per hour is lower in larger farm systems than in smaller ones, ie 9.1 Euros versus 9.8 euros respectively. These two characteristics result in a higher labour cost per insemination and heat detection per sow in small farms, 1.46 Euros, compared to medium ones, 1.12 Euros, and large ones, 1.08 euros. As a summary Gestation labor cost per pregnant sow (GCPS) varies betwen 8.43 Euros to 6.24 Euros. (See Table 1)

Table 1.Insemination and heat detection cost per sow and farm size.

| | TSD (minutes) | CH (Euros) | CIHD (Euros) | GCPS (Euros) |
|---|---|---|---|---|
| SF <1000 | 9.12 | 9.8 | 1.46 | 8.43 |
| MF 1000-2000 | 7.25 | 9.3 | 1.12 | 6.47 |
| LF >2000 | 7.15 | 9.1 | 1.08 | 6.24 |

## Conclusions and Discussion

Insemination and heat detection of a pregnant sow on Spanish farms costs a minimum of 6.24 Euros. Large farms (LF) and Medium farms (MF) are more efficient in terms of time spend doing heat detection and insemination. On the other hand, small farms (SF) tend to pay better than large farms and medium farms (LF, MF). The cost difference for insemination and heat detection (CIHD) between large (LF) and small farms (SF) is 0.42 Euros per sow. The fact that Large Farms have more inseminations compared to small farms makes them more efficient in terms of time and cost.

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BPEX, Agriculture and Horticulture Development Board 2011 Interpig Report

# Utilizing a progesterone ELISA in gilts to augment the use of P.G. 600® and MATRIX™

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## Introduction

Progesterone (P4) is an ovarian steroidal hormone produced by corpora lutea (CL) and is required for maintenance of pregnancy. Following ovulation, ovarian follicular tissue is rapidly luteinized to form CL and P4 production begins.[1] Pre-pubertal gilts are defined as those which have not yet had their first estrous cycle and therefore have low P4 levels due to the lack of CL. The estrous status of an individual gilt may be established by determining the P4 level and her history.[2] Sows that do not exhibit estrus within 7-10 days post-weaning and have low P4 levels are considered to be acyclic.[3] Animals with high P4 levels have CL on their ovaries and therefore are not considered to be pre-pubertal (gilts) or acyclic (gilts or sows).

Animals with normal estrous cycles have low P4 during their follicular phase and high P4 levels during their luteal phase. Animals with no history of detected estrus and high P4 levels either had a "silent" heat cycle or had a normal heat cycle that was missed by farm staff.

P.G. 600 is a combination of PMSG and HCG and is labeled in the US for the induction of estrus in pre-pubertal gilts and for the treatment of weaned sows experiencing delayed return to estrus. MATRIX (altrenogest 2.2%) is labeled in the US for estrus synchronization in gilts. MATRIX is fed orally for 14 days to gilts that have previously cycled; most will recycle within 4-9 days after the final dose. MATRIX is contraindicated in pre-pubertal animals. Conversely, P.G. 600 is contraindicated for post-pubertal gilts.

Herd A was the gilt developer for a 10,000-sow, parity segregated system experiencing an extended entry-to-service interval in gilts despite intensive boar exposure for at least 5 weeks. Herd B was a batch farrowing operation that needed to synchronize gilts to fit a specific breeding period. Due to labor and facility constraints, observed heat detection in gilts was not possible in Herd B. MATRIX was used to synchronize gilt groups, with variable success.

## Materials and Methods

Herd A- Serum was collected from 29-week-old gilts that had not been detected in heat after 5 weeks of boar exposure. A total of 116 gilts were sampled over a period of 2 weeks.

Herd B- Serum was collected from 33 gilts one day prior to MATRIX use (day 0). Only gilts with high P4 levels (N=21) were placed on MATRIX. For those given MATRIX, P4 levels were also determined on day 14 of Matrix treatment.

All serum was assayed with a semi-quantitative ELISA to determine P4 levels (Ovucheck® Premate Porcine, Biovet, Inc., St Hyacinthe, Quebec, Canada).

## Results

Herd A- Low P4 levels were detected in 113 of 116 gilts tested. Gilts with low P4 levels were injected with P.G. 600 within 24 hours of P4 testing; 107/113 gilts (94.7%) exhibited standing estrus within 8 days of P.G. 600 treatment.

Herd B- For the 21 gilts treated with MATRIX, 20/21 gilts (95.2%) had exhibited standing estrus within 8 days of the last treatment.

## Conclusions and Discussion

Herd A- P4 testing indicated that most of the 29-week-old gilts with no history of observed estrus were pre-pubertal. The post-treatment estrus response to P.G. 600 was excellent.

Herd B- P4 testing was used as a proxy for pre-breeding estrus detection in developing gilts since facility and labor constraints prevented farm staff from gathering heat-no-service data. MATRIX response in gilts with high P4 levels was excellent. The historical subpar response rate was most likely due to its use in prepuberal gilts.

Timely onset of estrus in replacement gilts is crucial for all herds, especially those with non-continuous breeding programs and in parity segregated operations where gilts are the only source of animals available for meeting breeding targets. For herds without the ability to monitor estrus in developing gilts, P4 testing can help verify the estrous status of individual animals, aid in the selection of proper hormonal interventions and improve the outcome following hormonal therapies such as MATRIX or P.G. 600.

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# Efficacy of a novel Enteropathogenic *Escherichia coli*/Clostridium vaccine: Induction of protective antibodies

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## Introduction

Sows and gilts are routinely vaccinated to protect their offspring against neonatal diarrhea caused by Enteropathogenic *E. coli* (ETEC). Because *C. perfringens* is often also involved in neonatal disease, combination vaccines have been developed against ETEC and Clostridium spp.. For these vaccines, it is crucial that sufficient vaccine-induced antibodies against the virulence factors of both organisms reach the progeny via the colostrum. In earlier challenge studies with *E. coli* strains expressing the virulence factors F4ab, F4ac, F5, F6 and LT, titer levels in ELISA were related to the reductions in mortality and severe diarrhea, and minimum protective titers were determined[1]. Also for *C. perfringens* type C, it has previously been shown that vaccination can induce antibodies in colostrum that protect against hemorrhagic enteritis[2]. In the present study, the functionality of the colostrum antibodies after vaccination with the new combination vaccine Porcilis® ColiClos was further characterized.

## Materials and Methods

Pregnant sows and gilts (n=23) were vaccinated twice with Porcilis® ColiClos or with placebo (n=4). All animals were vaccinated 6 and 2 weeks before farrowing and colostrum samples were taken. Samples were tested by ELISA for antibodies against ETEC virulence factors F4ab, F4ac, F5, F6 and LT and *C. perfringens* β-toxin that are included in the vaccine. Antibody functionality was determined by haemagglutination inhibition (fimbrial antigens), neutralization of the cytopathic effect on Vero cells (LT) and inhibition of mouse toxicity (β-toxin).

## Results

*ELISA results (Fig 1)*

Colostrum antibody levels to *E. coli* fimbrial antigens, LT and *C. perfringens* β-toxin were determined by ELISA using the purified antigens as coat.

*Haemagglutination inhibition results (Fig 2)*

Colostrum pools were tested for their ability to neutralize agglutination of guinea pig (F4), bovine (F5) or chicken (F6) erythrocytes by wild-type *E. coli* strains expressing the respective antigens. D-mannose was added to the tests to prevent agglutination by *E. coli* type 1 fimbriae.

*Figure 1- Elisa Result*

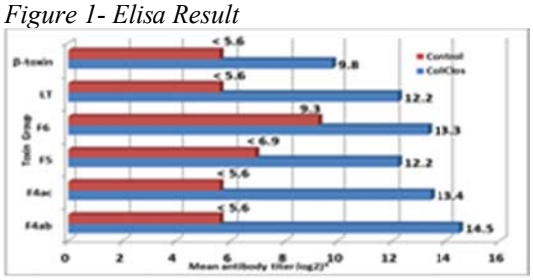
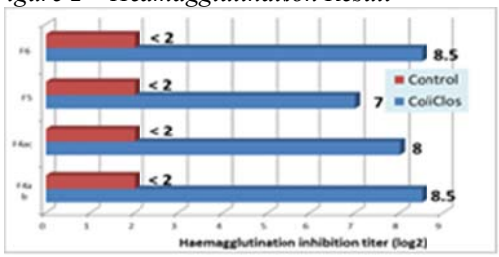



*Figure 2 – Heamagglutination Result*




*Toxicity neutralization results*

| Group | LT neutralization titer ($log_2$) | β-toxin neutralization level* (IU/ml) |
|---|---|---|
| ColiClos | 8.5 | 35 |
| Control | <2 | <4 |

*: protection against necrotic enteritis in piglets demonstrated for colostrum level of 10 IU/ml[2].

## Conclusions and Discussions

Porcilis® ColiClos induces *E. coli* antibody levels in colostrum that are well above published values of 11.9 (F4ab), 11.9 (F4ac), 10.2 (F5), 12.4 (F6), 10.4 (LT) [1] as well as antibody levels that are well above published[2] protective antibody titers for *C. perfringens* β-toxin. Antibodies induced by Porcilis® ColiClos inhibit the binding activity of *E. coli* fimbriae and neutralize toxic activity of *E. coli* LT and *C. perfringens* β-toxin.

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# Comparative study of using metal amino acid chelates and metal methionine complexes on culling rate of sows

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## Introduction

Trace mineral are essential to the diets of pigs because they participate in the biochemical processes required for normal growth, reproductive performances and development of bone (1). Chemical form of trace minerals effects their bioavailability and tissue deposition which may cause negative effect to farm animals (2). However, earlier report showed that inorganic forms and organic forms of trace mineral were equally effective in maintaining growth performance of pigs [3]. In addition, Metal-Amino Acid Chelates and Complexes could promote growth of weaned pig equally [3]. Due to the requirement for nutrients of gestating and lactating sows is very high to support their reproductive performances and health and the information regard this is still lack. This study aim to compares the effects of metal amino acid chelates (Complemin®) and metal methionine complexes on performance of sows which was showed as their culling rate.

## Materials and methods

Thirty two crossbred sows (Large White x Lanrace) from commercial pig farms in Loei province were used in this study. Only sows of the parity 1-5 were randomly assigned to 4 treatment groups:
Control group (n=8) received gestation diet with 1000 ppm metal methionine complexes
250 ppm group (n=8) received gestation diet with 250 ppm of metal amino acid chelates
500 ppm group (n=8) received gestation diet with 500 ppm of metal amino acid chelates
750 ppm group (n=8) received gestation diet with 750 ppm of metal amino acid chelates
The duration of the study was 211 days. Culling rate of sows and cause thereof were recorded. Fisher Exact test and chi-square analyses were carried out compare between group differences in observation data.

## Result

Culling rate of control group was 50% (4/8) which was more than in 500 and 750 ppm groups ($p<0.05$) which was 0% (0/8) and 0% (0/8) respectively. However, 12.5% (1/8) of 250 ppm group was not different from control group, 500 and 750 ppm group ($p>0.05$).

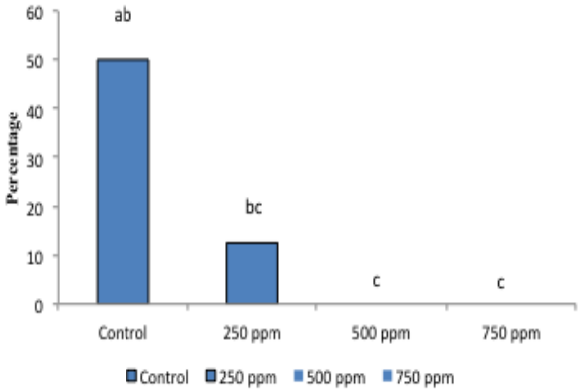


*Figure 1: culling rate of sows from each group (different letter mean differ significantly ($p<0.05$)*

## Discussion

The result of this study confirms that metal amino acid chelates have positive effect to health of sows. Lameness and/or reproductive problems were the main cause of culling which was unintentional. We could recommend from the result of this study that the appropriate dose of metal amino chelates is 500 ppm.

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# ALTRESYN® EFFICIENTLY SYNCHRONIZES ESTRUS IN SOWS AFTER WEANING AND INCREASES THE LITTER SIZE

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## Introduction

In modern pig farming there is often a need to manipulate the time of estrus in sows after weaning (e.g. loss of litters due to PED, completing the batches, second litter drop, etc.). The most convenient way to synchronize estrus of sows with other batch-mates is delaying heat by using synthetic progestagen - altrenogest [1]. The aim of this study was to demonstrate the efficacy of Altresyn® (Ceva) on the synchronisation of oestrus of sows after weaning.

## Materials and methods

Sows were randomized according to parity, age and back fat and allocated into 3 treatment and 3 control groups with 30 individuals in each group. Treated sows received Altresyn® 5ml for 3, 10 and 18 consecutive days, respectively, starting 1 to 2 days prior to weaning. After detection of the first standing reflex, the sows were inseminated twice (12 hours apart). Between days 28 to 34 after insemination, the sows were tested for pregnancy.

## Results

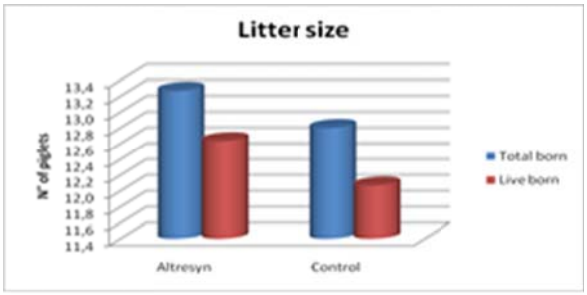
In treated group the total of 94.4 % of sows exhibited estrus, with 93.3% of sows within 4-6 days after the end of treatment. In the control group 95.6% of sows exhibited estrus (P>0.05). The mean interval between treatments and detection of oestrus was 4.2 to 4.6 days in the Altresyn® groups (p>0.05) and 4.5 to 4.9 days in the control groups. The pregnancy rate of all sows in trial was the same in the Altresyn® group and control group with 86% of pregnant sows. The pregnancy rate of served sows was 90.6% and 89.6% respectively (p>0.05).

***Table 1.*** *Estrus and pregnancy rates in Altresyn® treated and the control sows*

| | Altresyn | Control |
|---|---|---|
| Estrus rate (%) | 94.4 | 95.6 |
| Pregnancy rate (%) | 90.6 | 89.6 |

The N° of total born piglets per litter was in Altresyn® group 13.3 and in the control group 12.8 (p>0.05) with from 0.4 to 0.7 more piglets born alive in the treated groups than in the control groups.

***Fig.1.*** *Litter size in Altresyn® treated and the control sows*




## Conclusions

This study demonstrated that Altresyn® treatment from 3 to 18 days of duration synchronized efficiently the estrus of sows. Altresyn® didn't influence negatively the incidence of estrus after treatment neither the reproductive performance, on contrary the litter size was higher in treated sows, yet the difference was not statistically significant.

## Reference

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# Fertipig® improved the return to reproduction in primiparous hyper-prolific sows.

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## Introduction

Meeting breeding targets is the essential element influencing the pig flow in the breeding herd and piglet output. Weaned sows failing to return to oestrus within 7 days after weaning contribute to missed breeding targets and increased non-productive days (NPD) (Patterson 2010). Longer interval between weaning and insemination together with real anoestrus increase the NPDs and contribute to the inability to fill completely the vacant farrowing places. The use of gonadotrophins after weaning may reduce the long weaning–oestrus interval (WOI), prevent anestrus and reduce the number of NPDs.

Primiparous sows used to have the tendency to longer WOI and lower ovulation rate due to extensive utilization of body reserves during lactation (Kemp 2004). The aim of the study was to verify if gonadotrophins (Fertipig®, Ceva) can improve the return to reproduction of primiparous sows of current hyper-prolific breeds.

## Materials and methods

A conventional repopulated 900 sow farm practicing 2 weeks batch farrowing system was selected for conducting the trial. In total 177 P1 sows of LW x Landrace were used as non-treated control and 214 P1 sows were randomized according to the backfat thickness and treated with Fertipig® within 24 h after weaning. Heat was detected twice daily within 9 days after weaning. The following litter size was evaluated.

Result

There was no significant difference between the groups as regards to the estrus rate. Fertipig® treated sows had on average 14,2 hours shorter WOI (Table 1).

***Table.1*** *Average WOI and proportion of sows in heat within 6 or 5 days*

| | **Mean WOI (h)** | **Heat in 6 days (%)** | **Heat in 5 days (%)** |
|---|---|---|---|
| **Control** | 122,0[a] | 87,92[a] | 71,81[a] |
| **Fertipig** | 107,8[b] | 99,49[b] | 94,9[b] |

[a,b] represents statiscically significant difference ($p<0.001$)

This difference was higher in sows of poorer body condition with $p<0.0001$ for slim &thin (table 2).

***Table 2.*** *WOI (in hours) in relation to body condition at weaning*

| | **Normal** | **Fat** | **Slim** | **Thin** |
|---|---|---|---|---|
| **Control** | 120,05 | 118,72 | 126,13 | 126,14 |
| **Fertipig** | 110,01 | 114,87 | 104,85 | 107,45 |
| **Difference** | **10,04** | **3,86** | **21,29** | **18,69** |

Sows treated with Fertipig® had on average 0,68 total born and 0,48 live born piglets more than non-treated sows in the following litters (table3).

***Table 3.*** *The size of consequent litters*

| | **Total born** |
|---|---|
| **Control** | 13,5[a] |
| **Fertipig** | 14,18[a] |

[a]not statiscically significant, however a clear tendency ($p=0.088$)

## Discussion

It was confirmed that in the current high prolific breeds already selected for a short WOI, Fertipig® treatment of primiparous sows can reduce the mean WOI and reduce the number of NPDs. The benefit of lower cost of NPD and 0,48 piglet more calculated using published standards (Ifip 2013) was 27,44€ per sow.

## Reference

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# Transboundary Disease Transmission and Regional Cooperation

The 7th Asian Pig Veterinary Society Congress
October 25-27, 2015

# Co-infection of *Toxoplasma gondii* and PRRSV in Suckling Piglets in Jeju

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## Introduction

*Toxoplasma* (*T.*) *gondii* infection is common in humans and animal. Generally, cats are main sources of *T. gondii* infection, but also pigs, as an intermediate hosts, can transmit to human by contaminated meats [2]. In swine, abortion and weak newborns are main clinical sign of toxoplasmosis by transplacental infection so that can link to loss of productivity and potential risk to human's health. Porcine reproductive and respiratory syndrome virus (PRRSV) infection can effect to reproductive and respiratory systemic disorder including abortion, stillbirth and interstitial pneumonia, so called "swine infertility and abortion syndrome" or "porcine epidemic abortion and respiratory syndrome" in the past [1]. Toxoplasmosis and PRRV infection are can severely damage to farms, especially *T. gondii* is zoonosis, so it is essential to control both diseases. This study reports the case of *T. gondii* and PRRSV co-infection in two suckling piglets in Jeju Island.

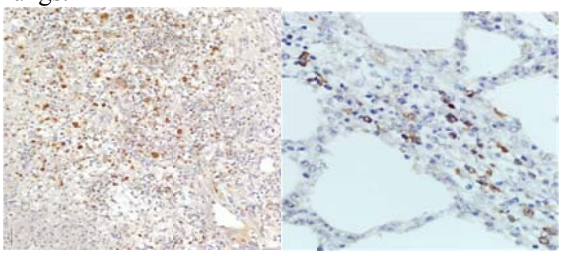
## Materials and methods

Two suckling piglets, 4-day and 10-day-old, were examined by necropsy at the Pathology Department of Veterinary Medicine, Jeju National University. Collected tissue was fixed in 10% buffered formalin, embedded in paraffin, sectioned at 2 ㎛ and stained with hematoxylin & eosin. Immunohistochemical stain (IHC) with *T. gondii* antiserum (PAB-TOXO, 1:5000, VMRD®, Pullman, Washington) was used to detect the antigen of *T. gondii* [3]. IHC for PRRSV was also performed with Mouse anti-PRRSV (SDOW17, 1:5000, South Dakota State University USA) in the lungs and lymph nodes.

## Result

In gross finding, the lung was non-collapsed and interlobular connective tissues were expanded. Affected pulmonary parenchyma was diffusely mottled dark-red and firm and rubbery. Multifocal pale foci with irregular string pattern were presented in pericardium and heart muscle. Histopathologically, diffuse interstitial pneumonia, necrosis of alveolar macrophage, hemorrhage and multiple necroses with protozoan tachyzoites were observed in lungs. Severe multifocal to diffuse necrosis with many tachyzoites were observed throughout the heart. In brain, necrotic encephalitis with intralesional protozoan tachyzoites was present. According to IHC many tachyzoites of *T. gondii* were detected in heart, brain and adrenal gland (**figure 1**). And also IHC revealed PRRSV antigens in the cytoplasm of macrophages in the lungs (**figure 1**).

**Figure 1.** Positive reactions for tachyzoites of *T. gondii* (left) in adrenal gland and PRRSV (right) in lungs.



## Discussion

Based on the histopathologic features, two piglets are diagnosed as toxoplasmosis and PRRSV infection. In our best knowledge, PRRSV may induce the characteristic diffuse interstitial pneumonia and infected tachyzoits of *T. gondii* may lead the necro-hemorrhagic lesion in lung, heart, and brain. Finding of protozoan tachyzoites in internal organs such as lung, heart, brain and adrenal gland was the key for the diagnosis toxoplasmosis. Also IHC was very useful method to prove the presence of antigens including *T. gondii* and PRRSV. In our best knowledge, this study is first report about co-infection of *T. gondii* and PRRSV in Jeju.

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# Safety of a new PCV2 and *Mycoplasma hyopneumoniae* combination vaccine: laboratory and field studies

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## Introduction

The majority of piglets in the pig industry are vaccinated against PCV2 and *M. hyopneumoniae*. Most vaccines are monovalent, require one or two vaccinations or have to be mixed before injection. A safe and efficacious ready-to-use one dose combination product is desirable from a practical and animal welfare perspective. Here, the safety of such a new combination vaccine - Porcilis® PCV M Hyo - is described.

## Materials and Methods

Safety studies were done according to the Ph.Eur in 17-24 day old piglets. In the GCP laboratory study 2 groups (Porcilis® PCV M Hyo or PBS control) of 12 SPF piglets were used and the field study was done in 3 farms (at least 28 pigs per group). The health of the piglets was checked daily and rectal temperatures were measured for 4 days post-vaccination (dpv). The injection site was palpated for local reactions for 14 days. In the laboratory study, all piglets were sacrificed to examine the injection site and in the field study animals were weighed before vaccination and at the end of the study (21 dpv). Furthermore, in the ten GCP field efficacy studies that were performed with the vaccine (total: ± 3100 pigs per group) safety parameters (general health/local reactions/average daily weight gain (ADWG) until the end of the nursery period) were recorded. The ADWG was compared using a mixed model ANOVA.

## Results

In the laboratory study, none of the animals developed local or systemic reactions and no macroscopic abnormalities were observed at the injection site at necropsy. A transient rise in temperature ($p<0.05$), well below the limit allowed by the Ph.Eur, occurred 4h post vaccination. In the field safety study, treatment resulted in local reactions with a maximum diameter of 1 cm in vaccinates and 0.3 cm in controls (observed at 4h post vaccination only). Local reactions were only in one of the three farms. Percent piglets with a deviation from the normal general health were similar in both groups (vaccinates: 6%; controls: 4.7%). A difference in rectal temperature ($p<0.05$) was again only measured 4h post vaccination. ADWG was not different between groups during the observation period. In the field efficacy studies, more than 3,000 pigs were observed in each vaccinated and control groups. Local reactions were observed in approximately 0.1% of the pigs in both groups (max. size: 2 cm) with a maximum duration of one day. A deviation from normal general health was found in 1.3% of vaccinates and 1% of controls. There were no significant differences in ADWG during the nursery phase.

## Conclusions and Discussion

The frequency of systemic reactions following vaccination with Porcilis® PCV M Hyo was very low and as these reactions were also observed in the control group that was injected with saline, they appear to be more treatment related than a result of vaccination. Vaccination also did not have a negative effect on the growth of pigs during the nursery phase and the local reactions were small and transient. An average increase in the rectal temperature of approximately 1°C was observed at 4 hours post vaccination. However, as the temperature returned to normal the following day and as furthermore neither the general behavior nor the feed intake of the animals was affected, this transient increase of rectal temperature can be considered an acceptable vaccine related finding. This notion is supported by the fact that an average increase of 1°C is well within the limit of 1.5°C that is allowed according to Ph.Eur monograph 2448 (Porcine enzootic pneumonia vaccine (inactivated)). The data therefore support that the new Porcilis® PCV M Hyo vaccine can be administered safely to 3 week old piglets.

# Sero-neutralizing antibody response after vaccination with an inactivated EU-typed PRRS vaccine in a Korean farm

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## Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disease in young pigs. Prevention of PRRS neonatal infections by passive colostrum and lactogenic immunity transferred from the sow has been shown to be dose dependant[1]. The aim of this study was to assess the PRRS-specific SN response after sow vaccination with an inactivated EU-typed PRRS vaccine and its passive transfer to piglets, by serum neutralization (SN) test in Korean field conditions.

## Materials and methods

The study was carried out in a 420-sow EU-typed PRRSV positive farm located in Dang-jin city, South Korea. Eight sows randomly chosen were vaccinated (V) with PROGRESSIS® (Merial, Lyon, France) 9 weeks before farrowing and revaccinated 3 weeks later. As a non-vaccinated control group (NV), 8 other sows were injected with saline according to the same schedule. From each of the 16 litters, 5 piglets per sow were selected to be SN tested. All sows were bled on day D-63, D-42, D0 (farrowing day) and D26, and 5 of their newborn piglets were bled on day D7, D14 and D26 after birth. Antibody titres of all sera were analyzed using an SN test implemented according to Jusa[2], with PRRSV Lelystad strain, and MARC-145 cells. T-test and Mann-Whitney U test of SPSS statistics 21 (IBM Corp., USA) were used for statistical significance.

## Results

Table 1. Results of average PRRSV-specific antibody titres of the sows and piglets by SN test.

| Sows | Day -63[a] | Day -42 | Day 0 | Day 26 |
|---|---|---|---|---|
| V | 0.50[b] (±0.64[c]) | 1.38 (±0.82) | 2.75 (±0.49) | 1.75 (±1.10) |
| C | 0.63 (±0.82) | 0.63 (±0.82) | 0.50 (±0.64) | 0.50 (±0.64) |
| *P* value | 1.000 | 0.279 | 0.001 | 0.130 |

| Piglets | Day 7[a] | Day 14 | Day 26 |
|---|---|---|---|
| V | 1.95[b](±0.34[c]) | 0.98(±0.33) | 0.05(±0.10) |
| C | 0.88(±0.35) | 0.30(±0.22) | 0(±0) |
| *P* value | 0.000 | 0.002 | 0.317 |

a: Day of sow blood sampling (1st vaccination, 2nd vaccination, farrowing and weaning).
b: Average $\log_2$ neutralized antibody titer of each group.
c: Confidence interval(CI) in confidence level(CL) of 95%.
d: Day of piglet blood sampling(1-wk-old, 2-wk-old and weaning).

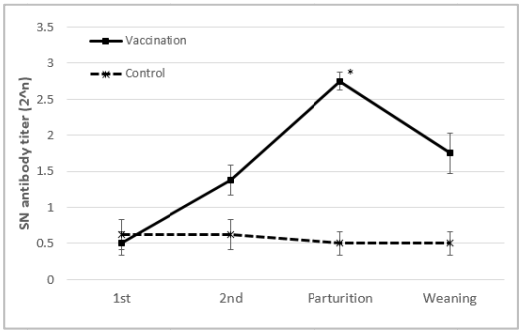


Figure 1. PRRSV-SN antibody titres in V and NV sows

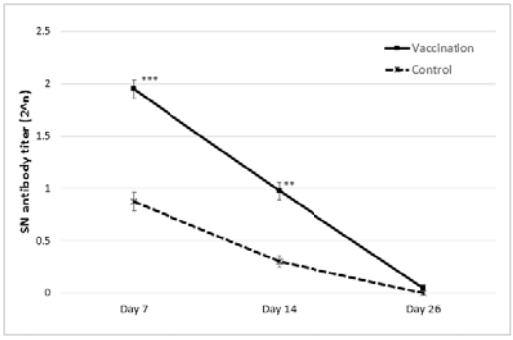


Figure 2. PRRSV-SN antibody titres in piglets born from V and NV sows

## Discussion - Conclusion

SN antibody titres of PROGRESSIS clearly increased in vaccinated sows although titres in control group remain stable, with a significant difference at farrowing. There were also significantly higher antibody titres in 7-day-old and 14-day-old piglets from vaccinated sows indicating that the passive colostrum immunity from vaccinated sows was well transferred to the newborn piglets. These results show that PROGRESSIS sow vaccination improved SN immunity to help preventing infections during pregnancy and to be efficiently transferred to their progeny.

## Reference

[1]Lopez O.J.,Clin. Vaccine Immunol. 2007, 14: 269-275
[2]Jusa E.R., J. Vet. Med. Sci. 1996, 58: 749-753

# Comparison of PCV2 vaccines in two commercial farms in the Philippines

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## Introduction

In this study, we compared the production performance of PCV2 vaccinated animals in terms of weight gain, average daily gain and mortality in two commercial farms. One farm vaccinated with Product A and the other with Product B at 3 weeks of age and these vaccination programs were compared against Product B. The farms, which are located in the northern part of Luzon, use conventional housing in farrow to finish operation.

## Materials and Methods

One hundred eighty (180) pigs in each farm were included in the study and were divided into two groups. Vaccination was done at 3 weeks of age. In Farm A, 90 pigs were vaccinated with Product A given IM and the other 90 pigs with Porcilis PCV 2 mL, IM. In Farm B, 90 pigs were vaccinated with Product B 2 mL, IM and the other 90 pigs with Porcilis PCV 2 mL, IM. Body weights and mortality data were obtained. Observation was done from 3 weeks of age until harvest time. Pigs were weighed at time of vaccination and harvest. Mortalities were recorded for the duration of the study. The data were analyzed using Mann-Whitney Test for comparison on a group basis.

## Results

Among the parameters, mortality rate were significantly lower ($P<0.05$) in Porcilis PCV vaccinated group in Farm B compared to Product B vaccinated group. However, this difference was not significant in Farm A compared to Product A vaccinated group. For the ADG, no significant difference was observed in Farm A. However, there was a significant difference of 0.33 kgs found in Farm B. Porcilis PCV vaccinated group yielded higher harvest weight of 5.10 kgs difference from that of Product B vaccinated group. All data are summarized in Table 1. Data of the causes of mortality are also shown in Table 2.

**Table 1**. Data comparison of Porcilis PCV and other commercially used PCV vaccines in farm A and B.

| FARMS | Pre-Vax (Kgs) | Harvest | Gain (K | ADG (K | % Mort |
|---|---|---|---|---|---|
| **Farm A** | **3 wks** | **26 wks** | | | |
| **Porcilis PCV** | 5.64 | 99.05 | 93.41[a] | 0.519[a] | 6%[a] |
| **Product A** | 4.94 | 98.06 | 93.11[a] | 0.517[a] | 3%[a] |
| **Farm B** | **3 wks** | **25 wks** | | | |
| **Porcilis PCV** | 6.4 | 94.3 | 87.9[a] | 0.571[a] | 8%[a] |
| **Product B** | 6.50 | 89.3 | 82.8[b] | 0.538[b] | 14%[b] |

a, b: value with different superscripts within a Farm in each column represent statistically significant differences ($p<0.05$)

**Table 2**. Causes of Mortality.

| Farms | Respiratory | Enteric | Anemia / Runt / Weak | Total |
|---|---|---|---|---|
| **Farm A** | | | | |
| **Porcilis PCV** | 1 | 0 | 4 | 5 |
| **Product A** | 0 | 0 | 3 | 3 |
| **Farm B** | | | | |
| **Porcilis PCV** | 2 | 2 | 4 | 8 |
| **Product B** | 4 | 2 | 7 | 13 |

## Conclusion

The study demonstrated that Porcilis PCV vaccination significantly reduced mortality and improved ADG compared to Product B (Farm B), while such differences were not measured when comparing Porcilis PCV against Product A (Farm A). In summary, Porcilis PCV vaccination at 3 weeks of age improved production parameters under the conditions tested in one of the farms.