Novel Detection and Elimination Schemes for Attenuated Strains of African Swine Fever Virus in China

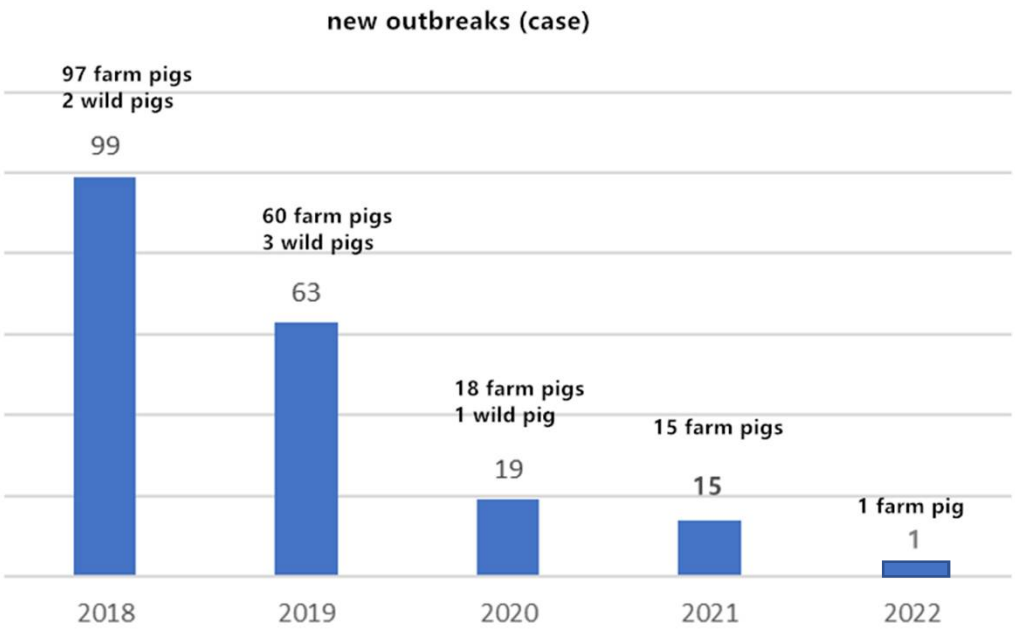
非洲猪瘟弱毒株检测与剔除思路

Leon li

IDvet China

RRENT ASFV PREVALENCE IN CHINA
当前非洲猪瘟流行状况

cial reports of ASF in China from 2018 to 2022



situation of ASF in China (2021)

Red: present ASF
Green : non-presen

Based on official data, the overall situation of ASF in China is stable

However, prevention and control pressure of ASF is still very severe!!

从官方数据来看，我国非瘟疫情防控形势整体平稳

实际情况……依然严峻，防控压力依旧很大！！

RRENT ASFV STRAINS IN CHINA
目前流行毒株

eorgia 2007 strain (genotype Ⅱ, serogroup 8) （Aug 2018）

latural" deletion attenuated strain: CD2v deletion (since Aug 2020)

owadays, at least 4 different types of “natural” mutations or deletion in EP402R gene from the isolate in China

前我国至少存在4种以上低致死率非洲猪瘟基因Ⅱ型"自然变异株"（源自国家非瘟参考实验室）

enotype Ⅰ： Henan strain（HeN/ZZ-P1/21）(Jun 2021)

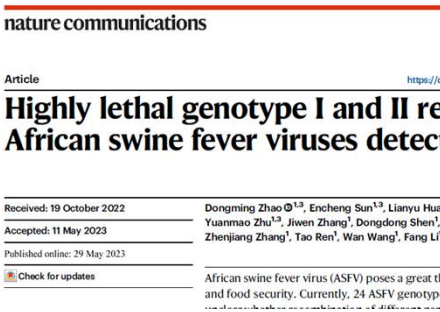
Shandong strain （SD/DY-I/21）

68（1968) and OURT88/3（1988）

enotype I and II recombinant virus: Jiangsu strain (JS/LG/2021） (since OCT 2022)

Henan strain （HeN/123-14/22）

Inner Mongolia strain (IM/DQDM/22)


nature communications

Article

Highly lethal genotype I and II re
African swine fever viruses detec

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Check for updates

Dongming Zhao[1,3], Encheng Sun[1,3], Lianyu Hua
Yuanmao Zhu[1,3], Jiwen Zhang[1], Dongdong Shen[1],
Zhenjiang Zhang[1], Tao Ren[1], Wan Wang[1], Fang Li

African swine fever virus (ASFV) poses a great t
and food security. Currently, 24 ASFV genotyp

PS: 1, B646L(p72) belong to gene type I; MGF505/360 and EP402R(CD2V) belong to gene type II.

2. The virulence is strong, and the attenuated live vaccine (HLJ/18-7GD) has no protective effect.

EMIOLOGICAL CHARACTERISTICS OF ATTENUATED ASFV

株”流行病学特点

Long incubation period ——（asymptomatic infection）

潜伏期更长———（无症状感染）

- High virulent strain：3~7 days
- Attenuated strain：28 days (cohoused infection test by natural attenuated strain HLJ/HRB1/20-Chin
  45 days（horizontal transmission by natural attenuated strain NH/P68-spain）

Weak pathogenicity and low mortality—— (atypical clinical symptoms)

致病性弱、死亡率低——（非典型临床症状）

- High virulent strain: anorexia, depression, fever, high mortality, detected immediately

不食、精神沉郁、体温升高、病死率高；当天异常，当天检测出；

- Attenuated strain: normal feed intake and body temperature, mainly arthritis, phyma and necrosis, continuously

吃料正常或少食、体温正常，以关节炎、肿块、溃疡为主；存在连续异常才检测出；

Ct value

Ct值变化

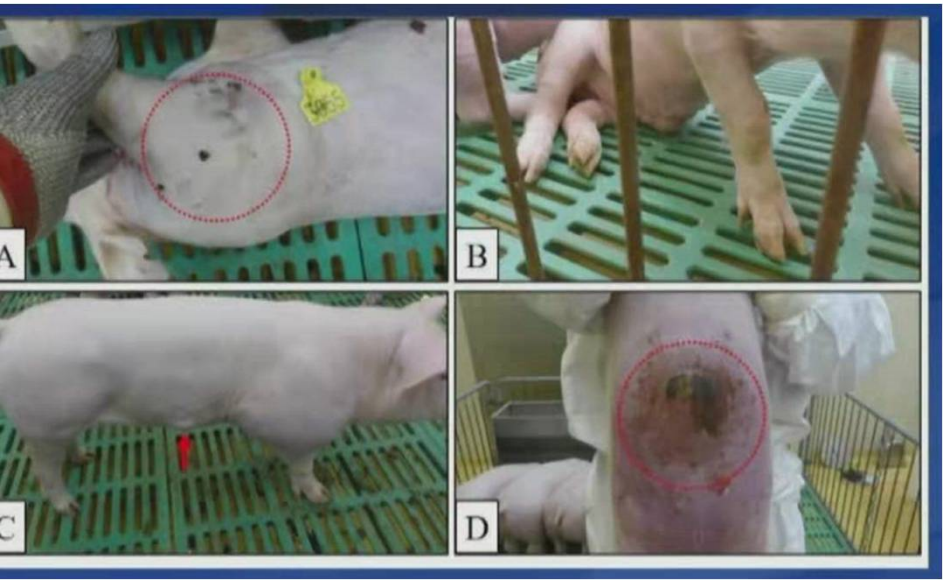
- High virulent strain：Ct value less than 35
- Attenuated strain：Ct value higher，more than 36, intermittent positive 呈间歇性阳性

NICAL CHARACTERISTICS OF ATTENUATED ASFV
临床特点

Weak pathogenicity and low mortality, mainly arthritis, phyma and necrosis

致病性弱、致死率低，临床症状以关节肿大、皮肤丘疹/坏死等为主

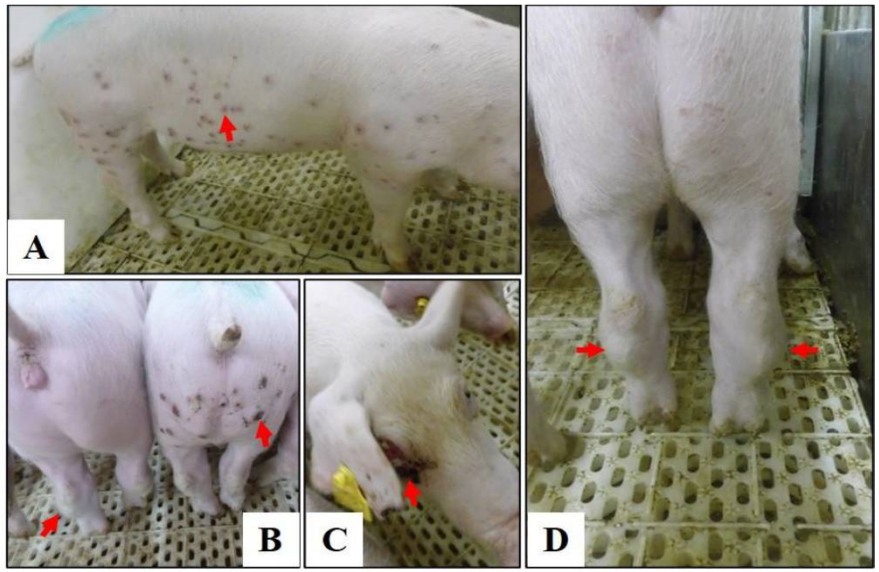
nfected with genotype Ⅱ attenuated ASFV



（A）arthroncus 关节肿大（B）paralysis 瘫痪
（C）phyma 皮肤肿块（D）necrosis 皮肤坏死

Bu zhigao 2021.2

Infected with genotype I attenuated ASFV



（A,B）papules 皮肤丘疹
（B,C）cutaneous necrosis 皮肤坏死
（B,D） arthroncus 关节肿大

Bu zhigao 2021.1

ECTION OF DIFFERENT VIRULENCE STRAINS

Table 1. Experimental settings summary.

Trial Trial Period)	Number of Animals	ASFV Strain (Genotype)	Strain Characteristics	Route of Infection	Dose per Animal
T1 (0–8 dpi)	n = 6	Arm07/CBM/c2 (II)	highly virulent	i.m.	10^3 $TCID_{50}$
T2 (0–21 dpi)	n = 7	Pol18_28298_O111 (II)	moderately/highly virulent	i.n.	10^3 HAD_{50}
T3 (0–28 dpi)	n = 6	NH/P68 (I)	attenuated	i.m.	10^3 $TCID_{50}$

i.m.—intramuscular, i.n.—intranasal.

viruses

Article

Non-Invasive Sampling in the Aspect of African Swine Detection—A Risk to Accurate Diagnosis

Marek Walczak [1,*], Anna Szczotka-Bochniarz [1], Jacek Żmudzki [1], Małgorzata Juszkiewicz [1], Krzesimir Szymankiewicz [1], Krzysztof Niemczuk [1], Daniel Pérez-Núñez [2], Lihong Liu [3] and Yolan

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[2] Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Microbes in Health and Welfare c/Nicolás Cabrera 1, 28049 Madrid, Spain
[3] National Veterinary Institute, SE-756 51 Uppsala, Sweden
* Correspondence: marek.walczak@piwet.pulawy.pl

ALUE AND ANTIBODY OF ATTENUATED ASFV

Table 2. The detection of ASF DNA in relevant matrices or specific anti-ASFV antibodies in the respective trial period.

Trial	Blood	Blood Mean Ct (±SD)	Oral Swabs	Oral Swab Mean Ct (±SD)	Rectal Swabs	Rectal Swab Mean Ct (±SD)	Seropositive Animals	Maximum Antibodies Titer (log_{10}/mL)
T1 Arm07)	6/6	26.6 (±7.4)	5/6	35.1 (±2.3)	5/6	34.1 (±3.5)	1/6	4.0
T2 Pol18)	6/7	23.7 (±5.0)	4/7	36.2 (±2.2)	7/7	34.3 (±3.3)	1/7	4.11
T3 NH/P68)	4/6	38.3 (±1.2)	5/6	36.6 (±2.4)	3/6	38.0 (±1.4)	6/6	5.0

- ✓ Through qPCR detection of blood, oral swab and rectal swab, the Ct value of attenuated ASFV is higher than that of high virulence ASFV.
- ✓ The attenuated strain can detect 100% ASFV antibody (10-12 days).

T DETECTION AND POSITIVE RATE OF ATTENUATE ASFV

Table 3. Mean latent and incubation periods.

Trial	First Detection of Fever (Mean dpi (±SD))	First PCR-Detection in Blood (Mean dpi (±SD))	First PCR-Detection in Oral Swabs (Mean dpi (±SD))	First PCR-Detection in Rectal Swabs (Mean dpi (±SD))
T1 (Arm07)	3.3 (±0.7)	2.8 (±0.7)	4.2 (±1.2)	4.4 (±1.2)
T2 (Pol18)	8.6 (±3.6)	8.5 (±4.1)	12.0 (±2.5)	9.7 (±4.3)
T3 (NH/P68)	3.0 (±2.3)	14.0 (±4.0)	21 (±6.4)	11 (±3.1)

Table 4. Percentage of PCR-positive samples collected during experiments in whole trial periods.

Trial	Number of Relevant Sampling Timepoints (Blood and Swabs) in Respective Trial Periods (Trial Period)	Oral Swabs —Positive (%)	Rectal Swabs—Positive (%)	Blood—Positive (%)
T1 (Arm07)	16 (0–8 dpi)	6/16 (37.5%)	6/16 (37.5%)	11/16 (68.8%)
T2 (Pol18)	43 (0–21 dpi)	13/43 (30.2%)	16/43 (37.2%)	24/43 (55.8%)
T3 (NH/P68)	63 (0–28 dpi)	4/63 (6.3%)	3/63 (4.8%)	7/63 (11.1%)

DDING OF ATTENUATED ASFV
散毒规律

Article

Distinct African Swine Fever Virus Sheddi
Boar Infected with Virulent and Attenuated

Aleksandra Kosowska [1,2,*], Estefanía Cadenas-Fernández [1,2], Sandra Barr
Jose M. Sánchez-Vizcaíno [1,2] and Jose A. Barasona [1,2,*]

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* Correspondence: alkosows@ucm.es (A.K.); jbarason@ucm.es (J.A.B.)

数据源自马德里康普顿斯大学

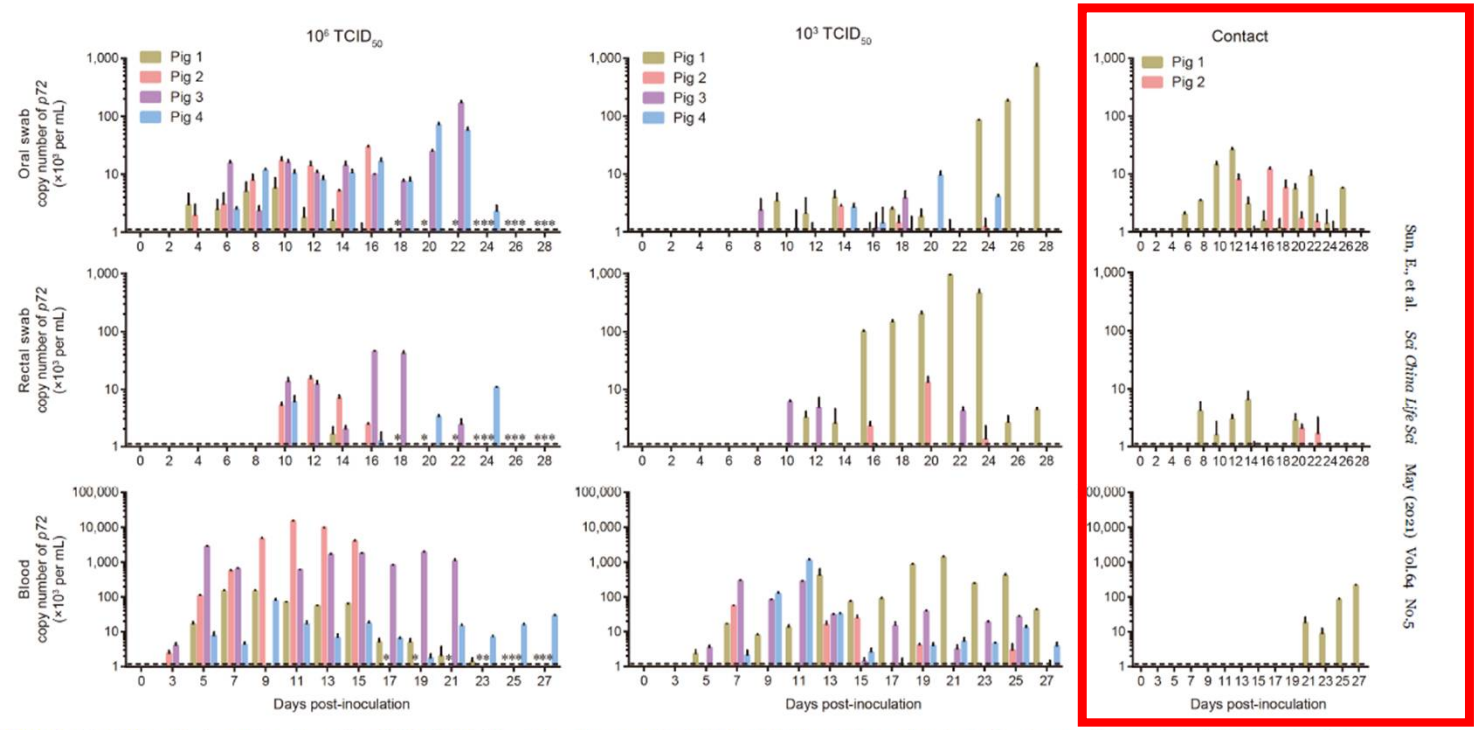


Figure 6 Viral DNA copies in samples collected from HLJ/HRB1/20-infected pigs and contact pigs. Samples, including oral and rectal swabs and blood, were collected at the indicated timepoints from pigs inoculated with HLJ/HRB1/20 and from contact pigs. Viral *p72* gene copies in the samples were quantified by using qPCR. The dashed black lines indicate the limit of detection with 1,000 copies per mL. The asterisks indicate that no samples were analyzed at the indicated timepoints because the pigs died.

After the infection of attenuated isolate, the shedding virus of contact pigs were irregu

Only rely on qPCR test, there is more difficult to find the positive pig

弱毒株感染后，含毒量变低，排毒不规律，呈现qPCR时有时无的状态，存在漏检的可能

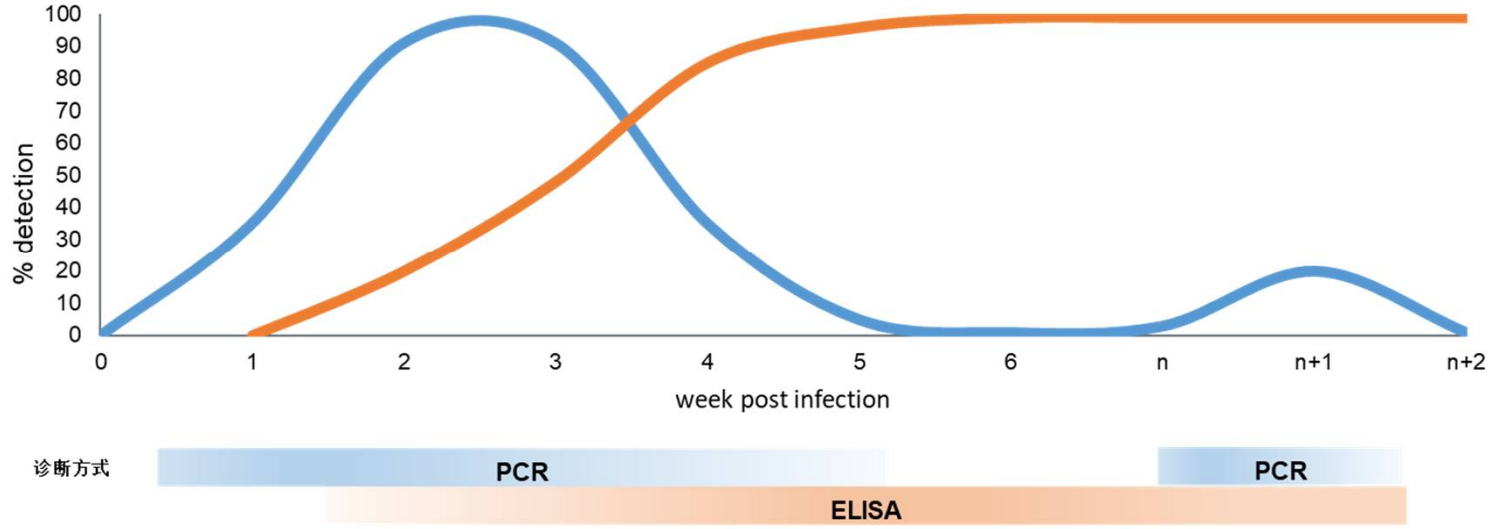
NITORING OF ASFV ANTIBODY

抗体监测的必要性

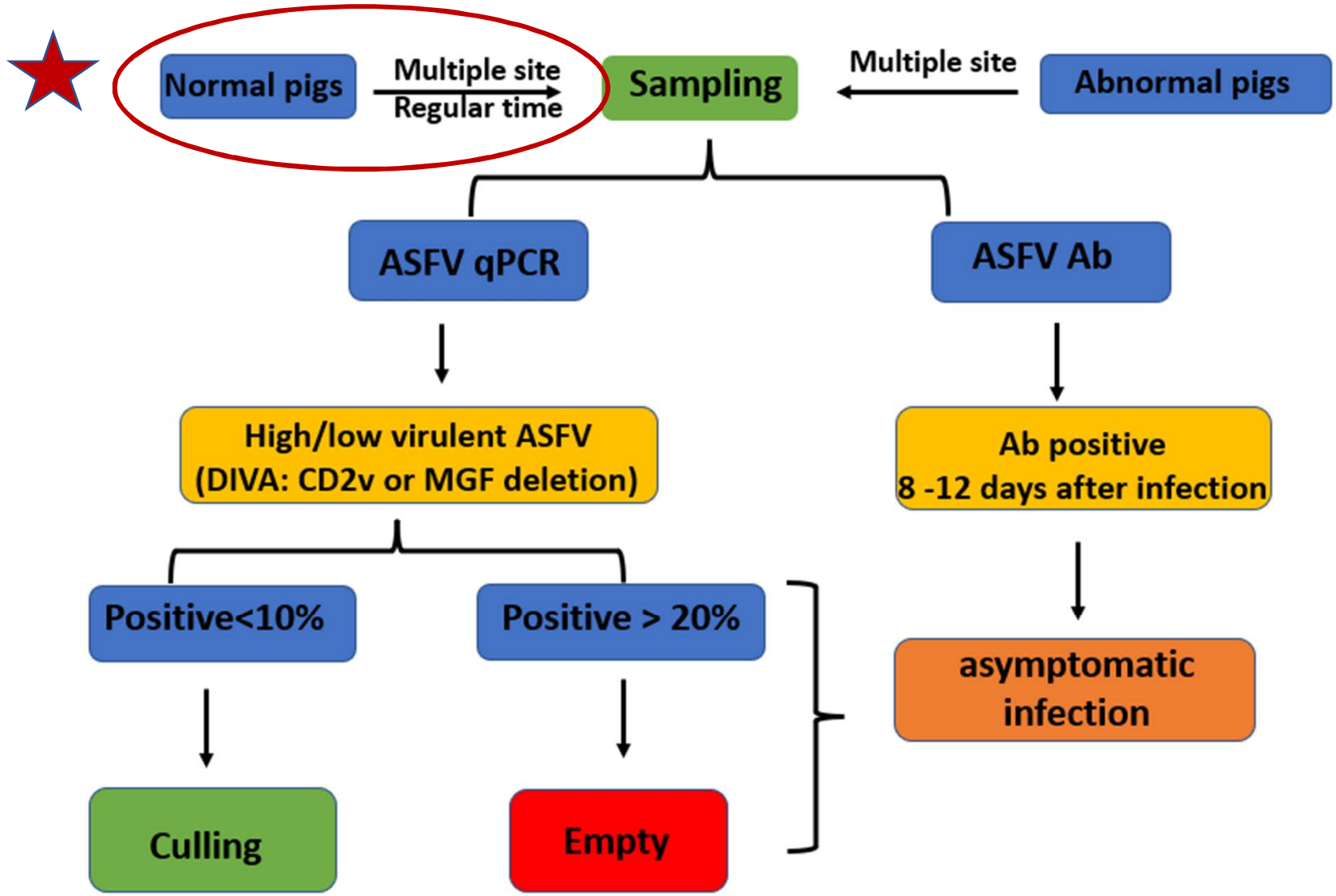
Long incubation period, atypical clinical symptoms, intermittent PCR positive; 弱毒株潜伏期长，临床症状不明显，核酸检出率低，间接性排毒；

After ASF pandemic, it was possible for the virus to mutate under the selection of antibody and immune function; ASF大流行过去，在抗体和免疫功能的选择下，非瘟病毒出现变异是可能的；

In areas where ASFV is endemic, the standard serological test (ELISA) is the best way to confirm suspicious cases (European Union reference laboratory for ASF) 欧盟非瘟参考实验室指出，在ASF呈地方流行的区域，确诊可疑病例最好用标准的血清学试验（ELISA）；



ONITORING IDEA OF ASFV IN THE PRESENCE OF ATTENUATED STRAIN
毒存在下，非瘟监测思路



S OF ABNORMAL SOWS:

ftover feed 有剩料的母猪

thargy/depressed 嗜睡/精神沉郁

ver (＞39℃) thermal imager 热成像仪异常体温39℃以上

rthritis 关节肿胀/关节炎

ecrosis 皮肤坏死

bortion 流产

outh/nosebleed, hemafecia 口鼻流血、便血

ath sow 死亡母猪

- The detection of abnormal sow is mainly to collect oral swab and bleed, and inguinal lymph node of dead sow
- 异常母猪监控以采集咽拭子+静脉血为主，死亡母猪采腹股沟淋巴结。

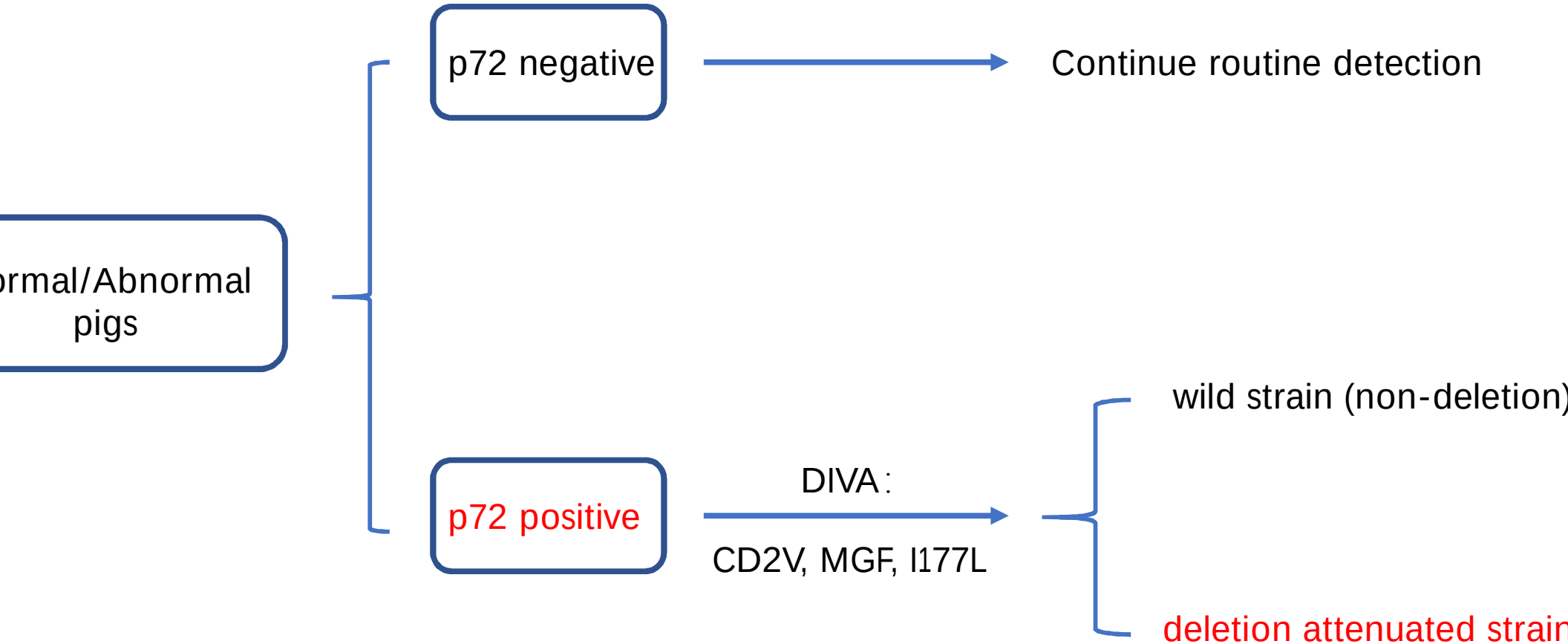
IVE ROUTINE DETECTION（ASYMPTOMATIC）

- **Purpose:** to find “asymptomatic” pigs as soon as possible, and improve the success ra of culling监测目的：尽早发现“无症状感染”猪只，提高精准剔除的成功率
- **Sampling pigs**: different production areas and stages of healthy pigs (focus on sows a gilts)采样对象：全场不同生产区、不同阶段的健康猪群（重点母猪和留种猪群）
- **Sample type**： oral swab (oral fluid), cord blood, blood swab/serum口咽拭子（口腔液）、脐带血、血液/血清（前腔静脉血或尾根血）
- **Item:** qPCR (pools of 5), ELISA (individual) 检测项目：qPCR（5合1混检）、ELISA（单检）
- **Frequency:** once a month 采样频率：每月一次
- **Proportion:** 5% - 10% 采样比例： 5%-10%

◆ PS: Avoid cross-contamination during blood collection

避免由于采血而出现交叉污染

V QPCR TEST FOR PIGS



ENGTHEN ENVIRONMENTAL SAMPLING

Purpose: ensure that the materials, vehicles and personnel entering the pig farm, and the environment in the production area are free of ASFV for qPCR test

- Gestation crates: pig trough, drinking spout, crate, ground……
- Pig pens: trough, floor……
- Environment: pig farm fan, door handle, floor

OLOGICAL METHOD

ning（by ELISA tests）

-iELISA

mmercial ELISA kit

ELISA (coated with p32, p62 and p72)

cELISA (coated with p32)

Confirmation

1、 Immunoblotting (免疫印迹)

2、IFA (间接免疫荧光)

3、IPT (免疫过氧化物酶试验)

No vaccine!

Antibody = infection (high or low virulent ASFV)

Antibody can be detected at the early stage of infection (7-12 days after)

ASFV Ab can persist for a long time (How long?)

OSE OF ASF ELISA KIT- DETECTION PURPOSE

Detection purpose (screening, confirmation) and target (introduction, routine screening)

ening: increase the detection rate of true positive, improve the diagnostic sensitivity

初筛：提高真阳性的检出率，即提高诊断的敏感性

cation: culling, early diagnosis, introduction, daily screening

适用场景：阳性拔牙场、早期诊断、从外引种、日常监测

firmation: increase the detection rate of true negative, improve the diagnostic specificity

复核：提高真阴性的检出率，即提高诊断的特异性

plication: middle/last infection (convalescent pig), daily screening, recheck suspicious sam

适用场景：感染中后期（耐过猪）、日常监测、可疑样本复检

OSE OF ASF ELISA KIT-PRODUCT PERFORMANCE

Specificity（Analysis/ Diagnostic Specificity ）

Sensitivity（Analysis/ Diagnostic Sensitivity）

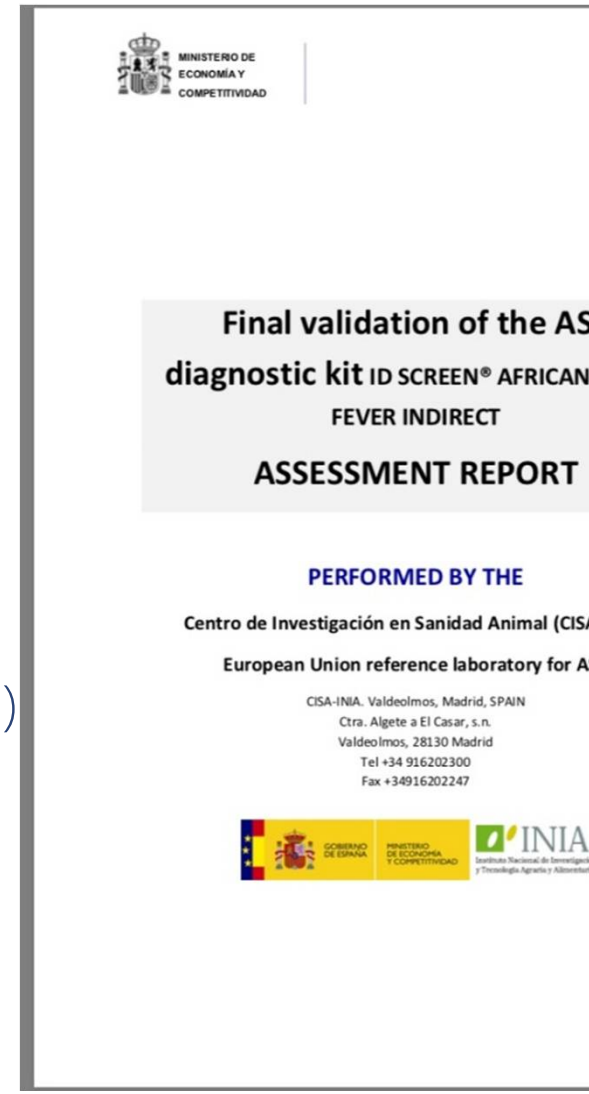
Repeatability (intra-plate/ inter-plate）

Brand

Coated protein（p30/32、p62、p72……）

Production process（prokaryotic/ eukaryotic expression原核或真核表达）

Detection purpose（Screening and/or confirmation）



MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Final validation of the AS

diagnostic kit ID SCREEN® AFRICAN

FEVER INDIRECT

ASSESSMENT REPORT

PERFORMED BY THE

Centro de Investigación en Sanidad Animal (CISA

European Union reference laboratory for A

CISA-INIA. Valdeolmos, Madrid, SPAIN
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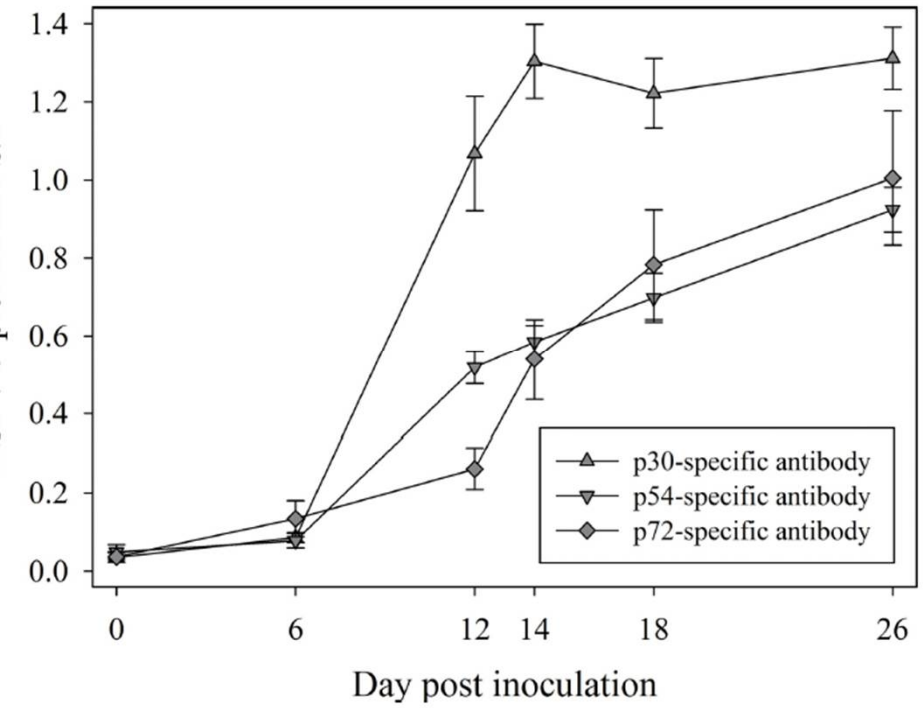
GOBIERNO DE ESPAÑA · MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD · INIA

PROTEIN

Serum antibody responses to RP vaccines by multiplex FMIA (Table 1)

Analysis of the multiplex FMIA protein-specific serum antibody responses in RP vaccine groups (1) p30 RP vaccine, (2) p54 RP vaccine, (3) p72 RP vaccine, (4) p30/54/72 RP vaccine,



1. ASFV multiplex fluorescent microbead-based immunoassay (FMIA) sample-to-positive (S/P) serum ibody response (mean, SE) against three recombinant antigens (p30, p54, p72) in 9 pigs inoculated with FV NHV/P68 (experiment 1).

10.1371/journal.pone.0161230.g001

PLOS ONE

RESEARCH ARTICLE

Detection of African Swine Fever V Antibodies in Serum and Oral Fluic Specimens Using a Recombinant F 30 (p30) Dual Matrix Indirect ELIS

Luis G. Giménez-Lirola[1]*, Lina Mur[2], Belen Rivera[2], Mark Mogler[3], Y Sergio Lizano[5], Christa Goodell[5], D. L. Hank Harris[3], Raymond R. R. Carmina Gallardo[7], José Manuel Sánchez-Vizcaíno[2], Jeff Zimmerma

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CrossMark

OPEN ACCESS

Data from Iowa State U

- p30 antibody was detected as early as 8 da infection;
- p30/p54 antibodies were produced earlier tha
- P30 antibody expression level higher than p54
- P30 protein is more suitable for early diagnosi

PROTEIN

p62 蛋白作为 ASFV p15 和 p35 的前体蛋白，病毒感染的晚期蛋白。对于 p62 蛋白作为诊断抗的研究，Gallardo 等[26]分别以 ASFV p62、p32 和 4 为研究对象，将重组蛋白作为 ELISA 诊断抗，研究结果显示，重组蛋白 p62 的特异性为 99%，2 和 p54 为 97%，三种蛋白的敏感性约为 97%。究检测 37 ℃破坏 1 个月的血清，p62 的敏感性和异性为 100%，优于另外两种重组蛋白，这一原因能是针对该蛋白的抗体比其他蛋白对应的抗体更定或表现出更强的亲和力，使得抗体在热处理后能与抗原蛋白结合，因此 p62 更适合用于检测保不当的血清样品[26-28]，具有作为诊断抗原的潜质。

- p62 protein is suitable for detection of poorly preserved and/or hemolytic serum

PROTEIN

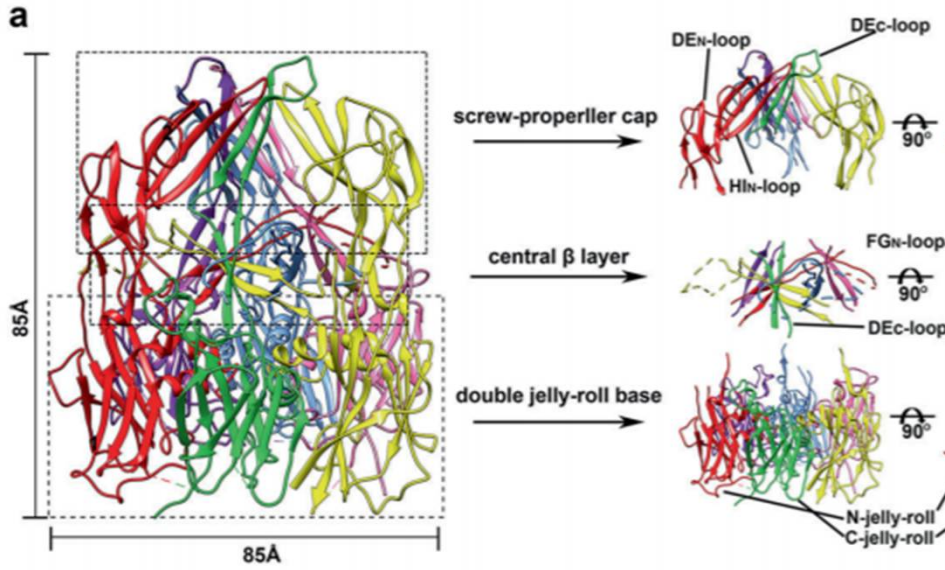
natural structure of p72 protein is a trimer structure;

s the main capsid protein of ASFV, belongs to a late

ein;

pared with other structural proteins (p54), p72 has a

er stability.



2019年，清华大学医学院向烨

LETTER TO THE EDITOR OPEN

Structure of the African swine fever virus ma
protein p72

Cell Research (2019) 29:953–955; https://doi.org/10.1038/s41422-019-0232-x

白可作为 ASFV 血清学诊断的一种理想抗原，用于世界上不同地区 ASFV 的

eije et al., 1993)。

OCONVERSION

40 samples from 8 domestic pigs inoculated with ASFV OURT 88/3 strain (genotype I) were analyzed using the ASF cELISA. Data were obtained by ANSES laboratory, Ploufragan, France.

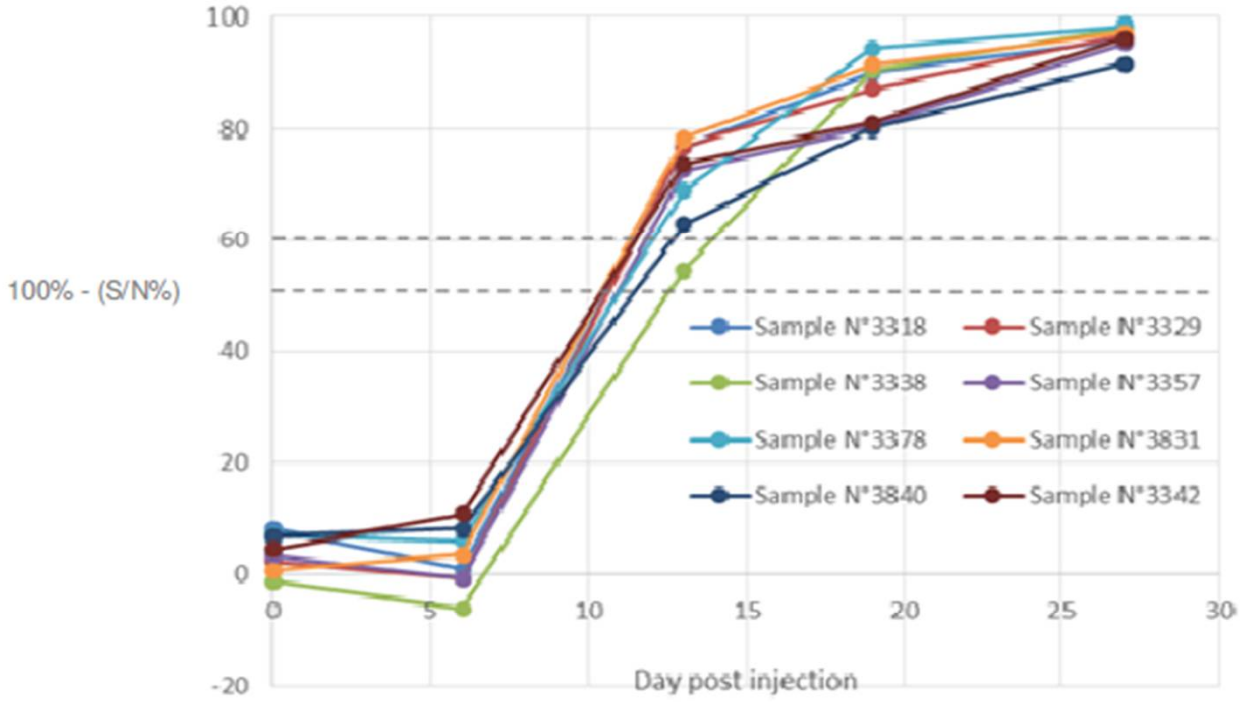


Figure 3: Seroconversion kinetics of 8 domestic pigs inoculated with ASFV OURT 88/3

Results (Figure 3):

- The [redacted] ASF cELISA detected seroconversion between 8 and 14 days post injection.

Seroconversion between 8 and 14 days post injection（p30）

OCONVERSION

OPEN

Deletion at the 5′-end of Estonian ASFV strains associated with an attenuated phenotype

d: 22 January 2018
d: 29 March 2018
ed online: 25 April 2018

Laura Zani[1], Jan Hendrik Forth[1], Leonie Forth[1], Imbi Nurmoja[2,3], Simone Leidenberger[1], Julia Henke[1], Jolene Carlson[1], Christiane Breidenstein[1], Arvo Viltrop[3], Dirk Höper[1], Carola Sauter-Louis[1], Martin Beer[1] & Sandra Blome[1]

Data from Friedrich-Loeffler-Institute, Germany（FLI） （2018）

ion of antibodies against ASFV. In trial A, 15 dpi four minipigs were tested positive for antibodies ASFV and at 21 dpi all but one of the recovered minipigs showed positive enzyme-linked immunosorbent LISA) results. At the necropsy on the end of the trial, all nine minipigs that survived the acute phase of the were still positive for ASFV-specific antibodies (see Fig. 3).

pigs of trial B showed the first positive ELISA results on 10 dpi and two weeks after the inoculation four ve pigs were tested clearly positive for antibodies against ASFV. From 21 dpi until the end of the trial at 36 animals were tested positive for antibodies (Fig. 3).

wild boar in trial C showed negative ELISA results in samples from 7 dpi. The sera taken from the two pig- e necropsy showed positive results (see Fig. 3), while the sera from the adult wild boar were still negative bodies at their endpoints.

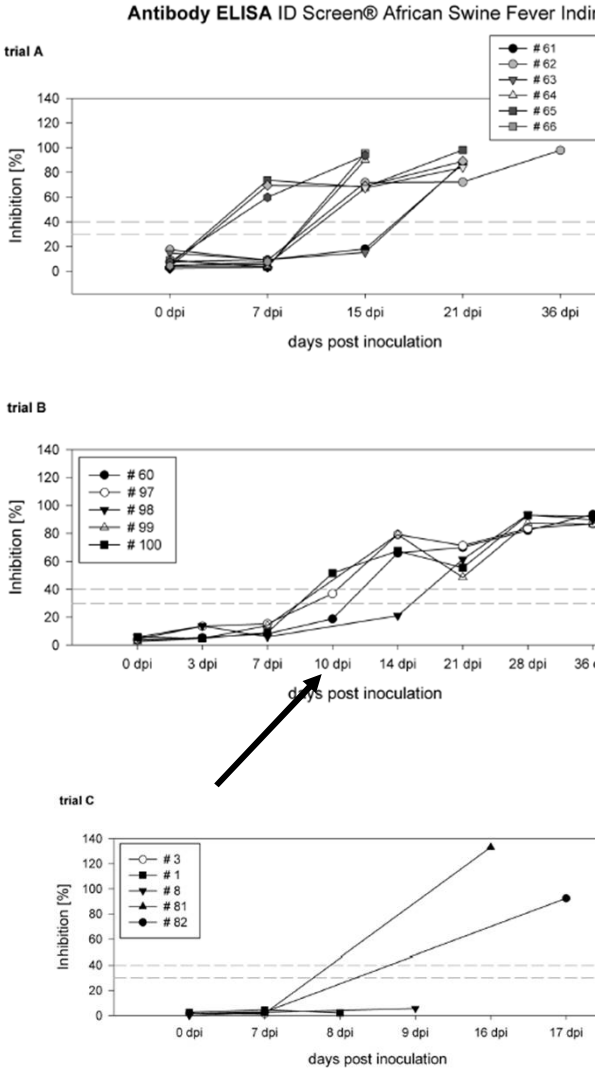
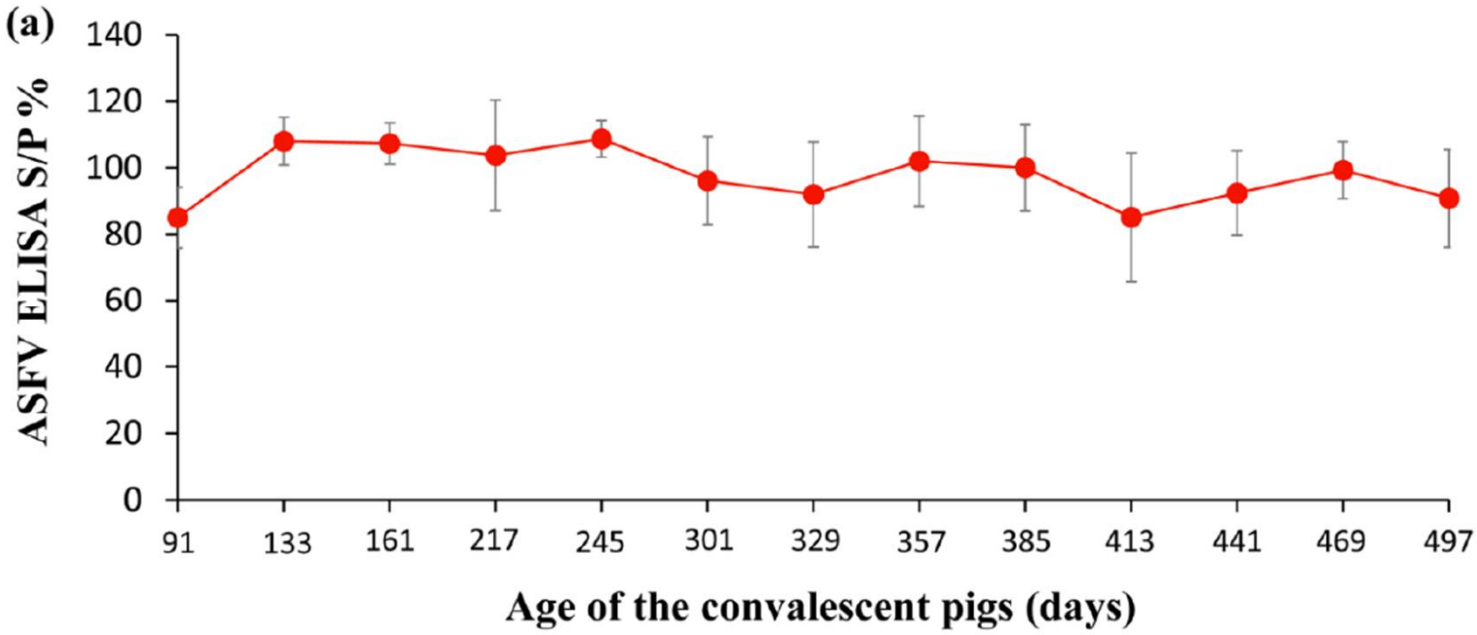


Figure 3. Antibody response trial A-C ELISA results in [%] inhibition graphed as

ASF-iELISA，showed positive results on 10 dpi post injection

ATION TIME OF ASFV ANTIBODY



V antibody remained for a long period in the convalescent pigs (14 months, from 91 day-old to 497

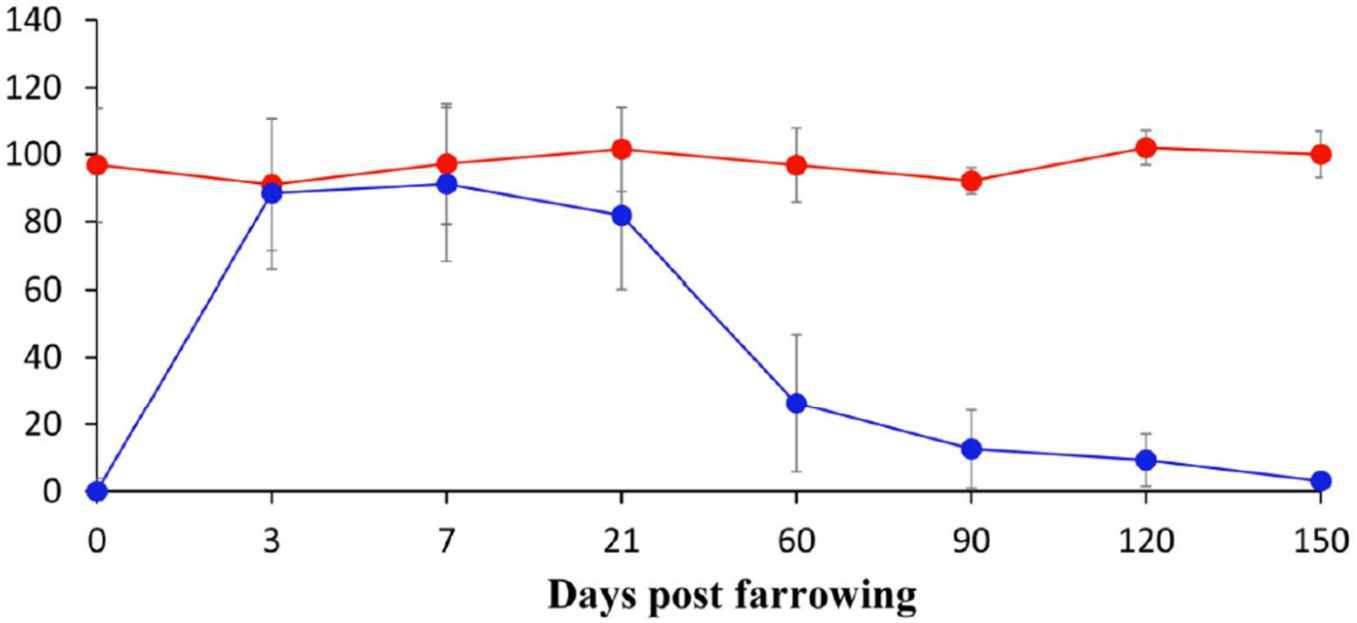
S/P % value did not change appreciably during the entirety of the study. (ASF-iELISA kit)

跟踪耐过猪，ASFV抗体阳性时间可持续14个月（选用-间接ELISA）

Data from :Long-term follow-up of convalescent pigs and their offs
after an outbreak of acute African Swine Fever in Vietnam (2021)

ORMANCE OF CONVALESCENT GILTS
D CHANGE OF ASFV ANTIBODY POST FARROWING

Red line: convalescent gilts
Blue line: their offspring



Parameter	n
Mating	14/14
Return to heat	2/14
Abortion	1/14
Farrowing	11/14
Gestation length	115.7 ± 2.0
Total born	9.73 ± 4.35
Born alive	8.45 ± 4.81
Stillborn	1.27 ± 2.53
Mummified	0.45 ± 0.89
Total weaned	7.00 ± 4.24

High levels in nursey pigs (21 days-old),

and decreased gradually after weaning (qPCR negative)

PS: 后备猪及其后代均未检测到核酸阳性

total born: 9.73 vs 12.6,
born alive : 8.45 vs 12,
total weaned piglets: 7 vs 10.2

Performance decrease more than 30%!!!

ESTION

- The workload of blood collection is huge in short time;

Manpower? Patience? Work quality?

- How to prevent cross-contamination of sampling?

Can we reduce the proportion of blood collection and realize the detection of nucleic acid and antibody through other sample types (such as oral fluid and blood swab)?

ECTION ANTIBODY FROM ORAL FLUID

- Some studies have confirmed that in oral fluid sample, more than 23 kinds of pathogens can be produced detectable levels of nucleic acids and antibodies (such as ASF, PRRS, FMD, PED and PCV-2).
- Advantages: non-invasive, easy to collect, less cross-contamination, and labor cost saving

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V ANTIBODY FROM SERUM AND ORAL FLUID

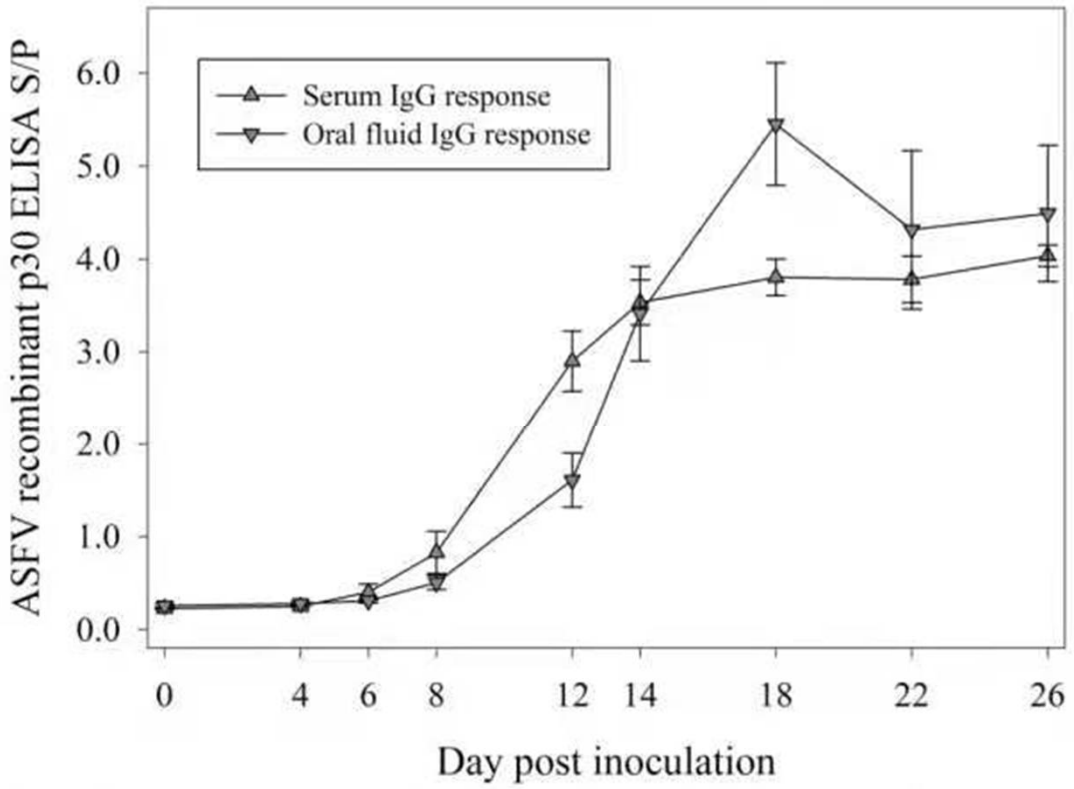

Fig 3. ASFV recombinant p30 antibody ELISA serum-to-positive (S/P) responses in serum (mean, SE) and oral fluid (mean, SE) samples from 17 pigs (experiments 1 and 2) inoculated with ASFV NHV/P68.

RESEARCH ARTICLE

Detection of African Swine Feve
Antibodies in Serum and Oral Flu
Specimens Using a Recombinan
30 (p30) Dual Matrix Indirect EL

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Data from Iowa State Univer

Seroconversion of ASFV

Serum Ab: DPI 6; Oral fluid Ab: DPI 8

RELATION RESULT BETWEEN SERUM AND ORAL FLUID SAMPLE

er	African Swine Fever-Indiret		
	Sample	S/P (%)	Result
	serum	105.06%	P
	serum	108.26%	P
	serum	103.92%	P
	serum	100.49%	P
	serum	93.56%	P
	serum	104.54%	P
	serum	108.67%	P
	serum	94.32%	P
	serum	103.61%	P
	serum	104.06%	P
	serum	117.34%	P
	serum	102.06%	P
	serum	89.59%	P
	serum	113.21%	P
	serum	107.43%	P
	serum	105.37%	P
	serum	125.18%	P
	serum	122.70%	P
	serum	125.80%	P
	serum	129.10%	P
	serum	121.05%	P
	serum	123.63%	P
	serum	125.59%	P
	serum	125.28%	P
	serum	106.22%	P

	African Swine Fever-Oral Fliud		
Number	Sample	S/P (%)	Result
1	oral fluid	166%	P
2	oral fluid	133.70%	P
3	oral fluid	50.20%	P
4	oral fluid	194.25%	P
5	oral fluid	276.70%	P
6	oral fluid	38.70%	P
7	oral fluid	125.50%	P
8	oral fluid	101.40%	P
9	oral fluid	69.00%	P
10	oral fluid	225.12%	P
11	oral fluid	40%	P
12	oral fluid	109.60%	P
13	oral fluid	130.00%	P
14	oral fluid	98.90%	P
15	oral fluid	63%	P
16	oral fluid	91.30%	P
17	oral fluid	326.10%	P
18	oral fluid	310.10%	P
19	oral fluid	307.40%	P
20	oral fluid	230.20%	P
21	oral fluid	208.40%	P
22	oral fluid	305.40%	P
23	oral fluid	335%	P
24	oral fluid	309.40%	P
25	oral fluid	159.00%	P

Collect serum and oral fluid from the asymptomatic pig to test ASFV antib showed that the detection rate of th type of sample were the same 无症状感

采集血清和口腔液进行非瘟抗体ELISA检测，结果发现样本的检出率相一致。

OD SWAB SAMPLE

etails and results. The status of the sample was defined by a p72 antibody ELISA (Ingezim PPA Compac, Ingenasa) of the corresponding orage time is depicted in month (M). DPI = days post inoculation; neg = negative according to the test criteria; dbt = doubtful according os = positive according to the test criteria; nd = not done; inact. = inactivated.

Animal ID	DPI	Storage	Virus	Status	Result swab	Result filter
HS1	0	21 M	–	neg	neg	nd
HS2	0	21 M	–	neg	neg	nd
HS3	0	21 M	–	neg	neg	nd
HS4	0	21 M	–	neg	neg	nd
HS5	0	21 M	–	neg	neg	nd
HS6	0	21 M	–	neg	neg	nd
HS7	0	21 M	–	neg	neg	nd
HS8	0	21 M	–	neg	neg	nd
HS9	0	21 M	–	neg	neg	nd
HS11	0	21 M	–	neg	neg	nd
HS12	0	21 M	–	neg	neg	nd
HS13	0	21 M	–	neg	neg	nd
HS3	28	21 M	Armenia08 inact.	neg	neg	nd
HS4	28	21 M	Armenia08 inact.	neg	neg	nd
HS6	28	21 M	Armenia08 inact.	neg	neg	nd
HS7	28	21 M	Armenia08 inact.	pos	pos	nd
HS8	28	21 M	Armenia08 inact.	pos	pos	nd
HS9	28	21 M	Armenia08 inact.	pos	pos	nd
HS11	28	21 M	Armenia08 inact.	pos	pos	nd
HS8	35	21 M	Armenia08 inact.	pos	pos	nd
HS12	28	21 M	Armenia08 inact.	pos	pos	nd
HS13	28	21 M	Armenia08 inact.	pos	pos	nd
HS4	41	21 M	Armenia08 inact.	dbt	neg	nd
HS6	41	21 M	Armenia08 inact.	dbt	pos	nd
HS7	41	21 M	Armenia08 inact.	pos	pos	nd
HS8	41	21 M	Armenia08 inact.	pos	pos	nd
HS9	41	21 M	Armenia08 inact.	pos	pos	nd
HS11	41	21 M	Armenia08 inact.	pos	pos	nd
HS12	41	21 M	Armenia08 inact.	pos	pos	pos
HS13	41	21 M	Armenia08 inact.	pos	pos	pos
HS1	29	1 M	OURT88/3	pos	pos	pos
HS2	29	1 M	OURT88/3	pos	pos	pos
HS3	29	1 M	OURT88/3	pos	pos	pos
HS4	29	1 M	OURT88/3	pos	pos	pos
HS5	29	1 M	OURT88/3	pos	pos	pos
HS6	29	1 M	OURT88/3	pos	pos	pos
HS7	29	1 M	OURT88/3	pos	pos	pos
HS8	29	1 M	OURT88/3	pos	pos	pos
HS9	29	1 M	OURT88/3	pos	pos	pos
HS10	29	1 M	OURT88/3	pos	pos	pos
HS1	0	1 M	–	neg	neg	neg
HS2	0	1 M	–	neg	neg	neg

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Alternative sampling strategies for passive classical and African swine feve surveillance in wild boar – Extension towards African swine fever virus antibody detection

- The results of blood swab and serum are basically consistent, expect for tw doubtful samples.

RELATION RESULT BETWEEN BLOOD SWAB AND SERUM SAMPLE

Date	1/14/2023	
Type / No	Serum	Blood swab
1	39.80%	39.30%
2	26.40%	30.20%
3	55.20%	48.60%
4	77.50%	72.40%
5	54.00%	49.50%
6	59.60%	61.20%
7	72.20%	70.50%
8	78.20%	60.70%
9	54.90%	49.40%
10	58.00%	57.70%
11	70.10%	63.00%
12	22.00%	25.50%
13	44.70%	38.90%
14	43.60%	44.20%
Average	54.00%	50.80%
Positive rate	85.70%	92.90%
cut-off	S/P≥40%, positive; 30% < S/P < 40%, doubt; S/P≤30%, negative	

- ✓ The result of blood swab and serum have a high coincidence rate.
- ✓ If the test of blood swab shows abnormal result, it can recheck by serum.

RELATION RESULT (BLOOD SWAB AND SERUM)

Date	1/16/2023	
Type / No	Serum	Blood swab
1	1.30%	4.50%
2	3.20%	5.10%
3	1.50%	5.90%
4	1.10%	8.50%
Average	1.80%	6.00%
Positive rate	0.00%	0.00%
cut-off	S/P≥40%, positive; 30% < S/P < 40%, doubt; S/P≤30%, negative	

h the S/P value of blood swab is a little

han serum, the result of both is just the

Date	1/30/2023	
Type / No	Serum	Blood swab
1	0.50%	3.90%
2	3.40%	9.20%
3	0.50%	1.90%
4	0.50%	2.00%
5	2.80%	8.50%
6	4.60%	29.40%
7	1.10%	5.70%
8	0.90%	5.40%
9	0.90%	4.00%
10	1.70%	9.10%
11	0.60%	2.40%
12	1.30%	6.40%
13	0.90%	4.60%
14	2.20%	6.80%
15	0.60%	4.20%
16	1.50%	5.90%
17	1.60%	3.40%
18	0.80%	2.00%
19	2.20%	5.30%
Average	1.50%	6.30%
Positve rate	0%	0%
cut-off	S/P≥40%, positive; 30% < S/P < 40%, doubt; S/P≤30%, negative	

IMIZATION OF ASFV DETECTION

- ✓ In the presence of attenuated strains, monitoring of nucleic acid and antibody is still the first choice to find asymptomatic pigs of ASFV.

- ✓ In the process of daily monitoring and accurate elimination, due to the heavy workload of blood collection and the need for many sampling personnel, the risk of cross-contamination is very high in the actual implantation.

- ✓ Many studies have confirmed that oral fluid and blood sample is suitable for early diagnosis, both virulent strain and attenuated strain. When the pressure of prevention and control is high, in addition of qPCR detection, oral fluid and blood swab sample can be used to detected ASFV antibodies at the same time, reduce the risk of cross-contamination, save more costs, and achieve better monitoring and elimination effect and

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