Cracking Pig Farming Complexity——Technical methods for PRRS detection and eradication

Tian Kegong

Science-driven solutions®

Member of Russian Academy of Engineering

Professor of Henan Agricultural University

Chief Expert of WOAH PRRS Reference Laboratory

Director of National Research Center for Veterinary Medicine (NVC)

普莱柯生物
PULIKE BIO-ENGINEERING

Contents

普莱柯生物
PULIKE BIO-ENGINEERING

01

Complexity of PRRS Prevention and Control

Complexity of PRRS Prevention and Control

Complex Clinical Virus Strains

- Multiple genotypes, lineages, and branches
- Recombination of different virus strains
- Various live vaccine strains

Diverse Routes of Transmission

- Direct transmission: infected pigs and their feces, urine, and semen
- Indirect transmission: aerosols, mechanical pathways
- Insect borne transmission: house flies and mosquitoes

Severe clinical harm

- Reproductive disorders in sows and respiratory symptoms in piglets
- Immunosuppression leads to secondary infection or mixed infection with other pathogens
- Persistent infection, where the virus persists in the tissue for a long time

- **PRRS is the number one killer affecting the pig farming industry.**

The classification of infection status in pig farms is not standardized

普莱柯生物 PULIKE BIO-ENGINEERING

Pig farm category

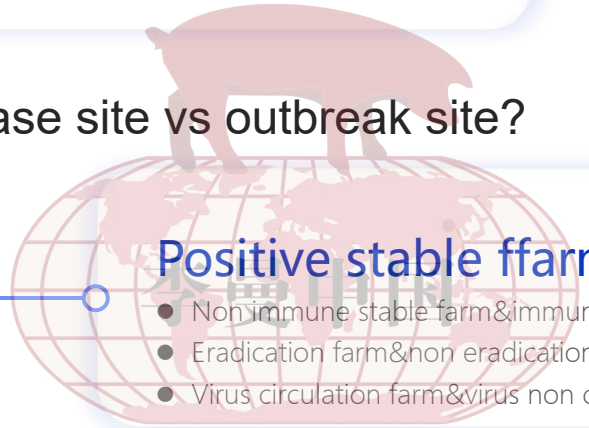
Positive unstable farm
- High prevalence&low prevalence?
- Disease site vs outbreak site?

Disease site vs outbreak site?

Positive stable ffarm
- Non immune stable farm&immune stable farm?
- Eradication farm&non eradication farm
- Virus circulation farm&virus non circulation farm?

Provisional Negative

Negtive farm
- Provisional negative farm?
- N antibody negative&antigen negative?
- N antibody negative&GP antibody negative&antigen negative?



- Lack of unified classification standards
- Unclear detection indicators
- Inconsistent interpretation of test results

普莱柯生物
PULIKE BIO-ENGINEERING

02

Classification of Breeding Herd PRRSV Infection Status

Classification Guideline by AASV

- Jointly revised by representatives from AASV and the National Pork Board in 2019
- Published in *the Journal of Swine Health and Production* in 2021

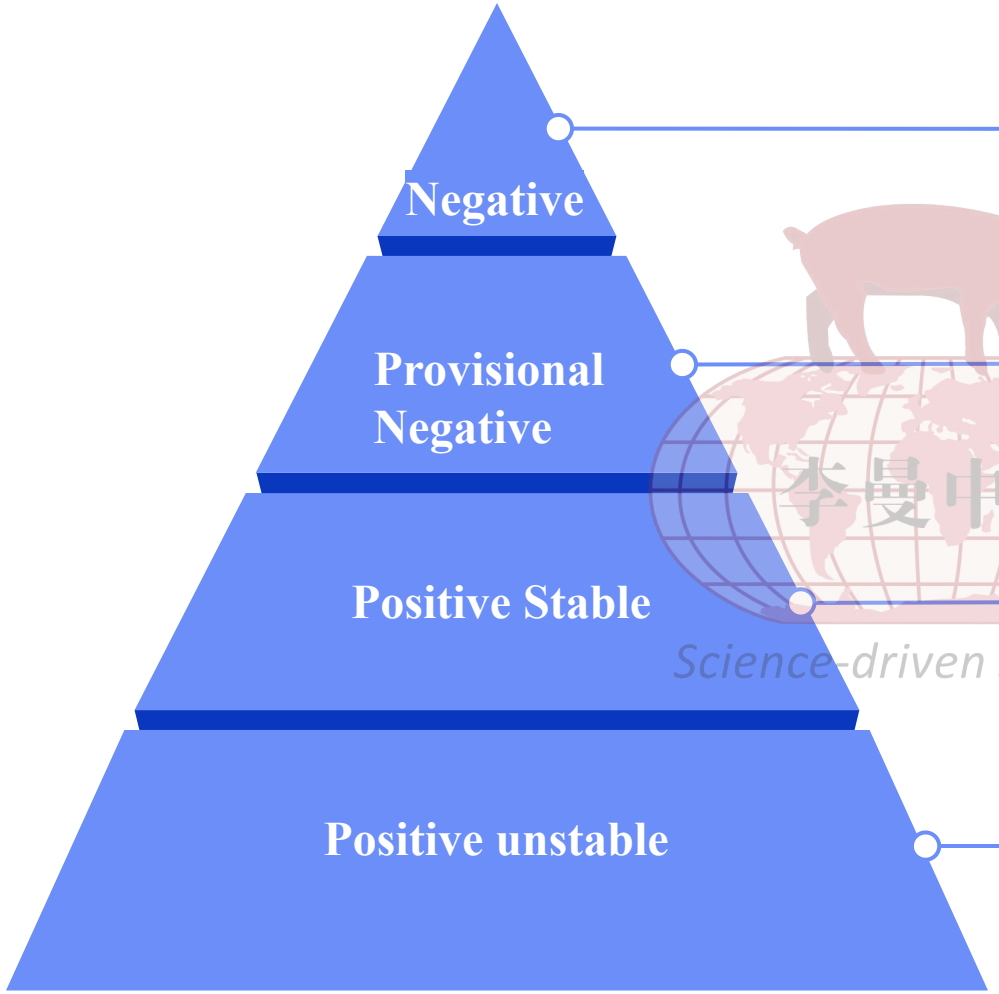
PEER REVIEWED

Proposed Modifications to PRRSV Herd Classification

Proposed modifications to porcine reproductive and respiratory syndrome virus herd classification

Derald J. Holtkamp, DVM, MS; Montserrat Torremorell, DVM, PhD; Cesar A. Corzo, DVM, MS, PhD; Daniel C. L. Linhares, DVM, MBA, PhD; Marcelo N. Almeida, DVM, MS; Paul Yeske, DVM, MS; Dale D. Polson, DVM, MS, PhD; Lisa Becton, DVM; Harry Snelson, DVM; Tara Donovan, DVM; Jeremy Pittman, DVM; Clayton Johnson, DVM; Carles Vilalta, DVM, PhD; Gustavo S. Silva, DVM, PhD; Juan Sanhueza, DVM, PhD

AASV Guideline -Classification (category standards) for breeding herds



- Category IV: Negative
- Category III: Provisional Negative
- Category II-vx：Positive stable (immunized with vaccine）
- Category II: Positive Stable
- Category I-B: Positive unstable; Low prevalence
- Category I-A: Positive unstable; High prevalence

AASV Guidelines - Classification Standards for Pig Farms

普莱柯生物 PULIKE BIO-ENGINEERING

Type	Clinical status	Main detection methods
Class I-A: Positive, unstable, high prevalence rate	● Sows loss appetite, miscarriage, stillbirth, mummified fetuses, etc ● Increased mortality rate of piglets in delivery rooms	**Piglet RT-PCR detection**
Class I-B: positive, unstable, low prevalence	● Mild or no Clinical symptoms ● Productivity recovery or approaching baseline levels	
Class II: Positive and stable	● Always produce piglets with negative PRRSV RT-PCR ● Very mild or no clinical symptoms of PRRSV infection ● Productivity restored to baseline level	
Class II-VX: Positive and stable (immune vaccine)		
Class III: Provisional negative	● Clearance PRRSV of Transition pig herds through lockdown and de-population	**Reserve or breeding pig antibody testing**
Class IV: Negative	● All animals in Class III that have been exposed to PRRSV have been cleared	

AASV Guideline - Universal Concept & Requirement

普莱柯生物 PULIKE BIO-ENGINEERING

Sample Type

- Serum
- Processing fluids (castration, tail docking and teeth clipping)
- Family oral fluids

- Piglet processing fluids can be used as a supplementary method for sera diagnosis, and it alone is insufficient in determining piglet virus shedding.
- Success in collecting family oral fluids can be variable. Family oral fluids alone can only be used as evidence to maintain in the category, **not for promoting into.**

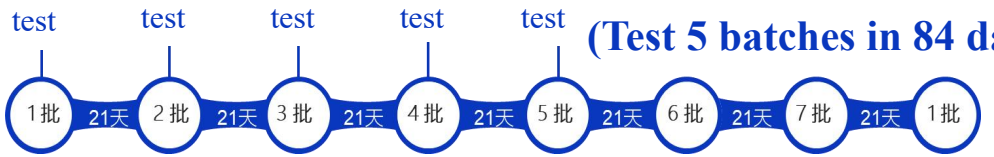
Sample Size

Category II/II-vx to promote into category III, category III to IV: serum samples to increase from 30 samples 5/pool to 60 samples 10/pool

The 60-pig sample size was based on the number of samples required to detect an **expected prevalence of 5%** with 95% confidence for any **population size greater than 1000** assuming a diagnostic test sensitivity greater than 95% from a population with an homogenous distribution of positive animals.

Testing Frequency

To promote into higher category: Test for 90 days or at least 4 batches

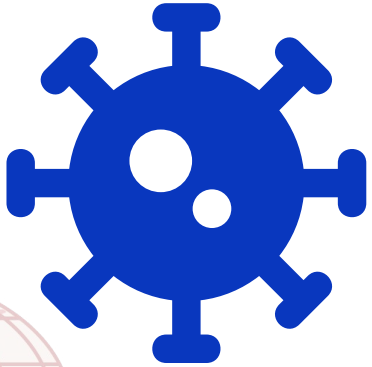


Classification and sampling plan for a 3-week batch-production system (5 tests over 84 days)

One pool positive for RT-PCR and ELISA means herd test is positive.

张择扬 *et al.* 2022

Class I-A: Positive, unstable, high prevalence rate



No supporting evidence belonging to other categories

or

Outbreak of pig farms

Recently, there have been pig farms experiencing outbreaks of PRRSV, as well as farms with consistently high shedding and infection rates for weaned piglets, Pig farms without other supporting evidence are classified as I-A

Class I-B: positive, unstable, low prevalence

普莱柯生物 PULIKE BIO-ENGINEERING

	Detection scheme				Result application	
	Sample	Sampling amount	Detection frequency	Testing Method&Result Requirements	Category determination	Category maintenance
Option 1	Weaned piglet serum	30 pigs，5 pool 1	Once per month, approximately 90 days Or at least 4 batches Once per month or by batch	RT-PCR Negative frequency ≥ 75%	√	√
Option 2	Piglet processing fluid	Processing fluid Combine 1 or more	1 time/week, 13 times for approximately 90 days or at least 4 batches Once per month or by batch		√	√
Option 3	Oral fluid of weaned piglet's litter	20 litter，5 pool 1	Once per month or by batch		/	√

If the number of negative RT-PCR results is less than 75%, it is determined as Class I-A.

Class II: Positive stability/Class II-vx: Positive stability (immune vaccine)

Class II-vx: pigs immunized with live vaccines are allowed to have a positive RT-PCR result within 2 weeks after immunization; If RT-PCR is still positive after 2 weeks, it is necessary to determine whether the sample is vaccine or wild virus.

	Detection scheme				Result application	
	Sample	Sampling amount	Detection frequency	Method&Result Requirement	Category determination	Category maintenance
Option 1	Weaned piglet serum	60 pigs，10 pool 1	Once a month, approximately 90 days or at least 4 batches	RT-PCR Negative frequency 100%	✓	
		30 pigs，5 pool 1	Once per month or by batch			✓
Option 2	①Weaned piglet serum + ②Piglet processing fluid	①30 pigs，5 pool 1 ②Processing fluid Combine 1 or more	① Once per month, 90 days or at least 4 batches ② 1 time/week, 13 times for approximately 90 days or at least 4 batches		✓	
			① Once per season ② Once per month or by batch			✓
Option 3	①Weaned piglet serum + ②Oral fluid of weaned piglet's litter	①30 pigs，5 pool 1 ②20 litters，5 pool 1	① Once per season ② Once per month or by batch		/	✓

If the number of negative RT-PCR results is less than 100%, it is determined as Class I-B or lower.

Class III: Provisional negative

普莱柯生物 PULIKE BIO-ENGINEERING

	Detection scheme				Result application	
	Sample	Sampling amount	Detection frequency	Testing Method&Result Requirements	Category determination	Category maintenance
Option 1	Entry ≥ 60 days Negative replacement Pig (Sentinel Pig) Serum	60 pigs, donot pool	Detect once	ELISA negative for antibodies	✓	
		30 pigs, donot pool	half a year			✓
Option 2	Entry ≥ 60 days Negative replacement pig Processing fluid of piglets	Processing fluid Combine 1 or more	half a year		/	✓
Option 3	Entry ≥ 60 days Negative replacement pigs and Litter piglets oral fluid	20 litters, donot pool	half a year		/	✓

If the six-month test result (one of the three options) is positive, it will be downgraded to Class I-B or lower.

Class IV: Negative

	Detection scheme				Result application	
	Sample	Sampling amount	Detection frequency	Testing Method&Result Requirements	Category determination	Category maintenance
Option 1	Breeding pig serum	60 pigs, donot pool	Detect once	ELISA negative for antibodies	✓	
		30 pigs, donot pool	half a year			✓
Option 2	Piglet processing fluid	Processing fluid Combine 1 or more	half a year		/	✓
Option 3	Oral fluid of weaned piglet's litter	20 litters, donot pool	half a year		/	✓

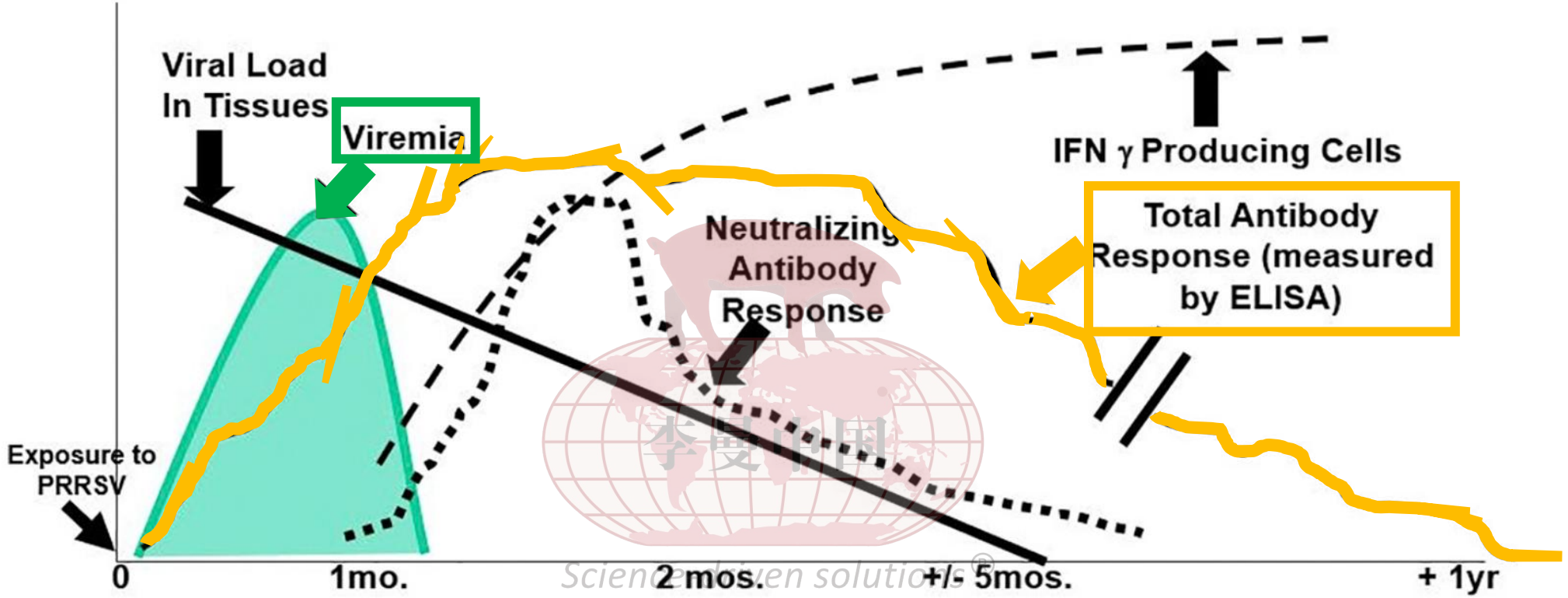
If the six-month test result (one of the three options) is positive, it will be downgraded to Class I-B or lower.

普莱柯生物
PULIKE BIO-ENGINEERING

03

Technical Methods for Detection of PRRSV

Dynamics of viruses and antibodies in the body after PRRSV infection



- Viremia can occur 6-12 hours after PRRSV infection; Viremia can last for more than one month.
- Under continuous infection, the virus mainly exists in lymphatic tissue and can be reactivated and transmitted through oral and nasal secretions under various stimuli.
- Specific antibodies can be produced 7 to 9 days after PRRSV infection,
- But early antibodies have no neutralizing activity.
- After PRRSV infection, the total antibodies in the body can be maintained for several months.

Alexandra Henao-Diaz et al., Res Vet Sci,2020

Diagnosis and detection methods of PRRSV

普莱柯生物 PULIKE BIO-ENGINEERING

Classification Guidelines for the American Association of Pig Veterinarians (AASV)

Category	I-B		II and II-vx	
Description	Positive Unstable, Low Prevalence		Positive Stable and Positive Stable With Vaccination	
Testing purpose	To promote into	To maintain in	To promote into	To maintain in
Animals and sample tested†	Serum from weaning-age pigs	Serum from weaning-age pigs	Serum from weaning-age pigs	Serum from weaning-age pigs
Minimum number sampled	30 pigs	30 pigs	60 pigs	30 pigs
Pooling recommendation	5 pigs/pool	5 pigs/pool	10 pigs/pool	5 pigs/pool
Test used	Test pools by RT-PCR	Test pools by RT-PCR	Test pools by RT-PCR	Test pools by RT-PCR

Category	III		IV	
Description	Provisionally Negative		Negative	
Testing purpose	To promote into	To maintain in	To promote into	To maintain in
Animals and sample tested	Serum from PRRSV naive re-placement breed-ing animals that have been in herd for at least 60 days	Serum from PRRSV naive re-placement breed-ing animals that have been in herd for at least 60 days	Serum from adult breeding animals	Serum from adult breeding animals
Minimum number sampled	60 animals	30 animals	60 animals	30 animals
Pooling recommendation	None allowed	None allowed	None allowed	None allowed
Test used	Test individual samples by ELISA	Test individual samples by ELISA	Test individual samples by ELISA	Test individual samples by ELISA

WOAH handbook

Table 1. Test methods available for diagnosis of porcine reproductive and respiratory syndrome and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[1]						
Virus isolation	–	++	–	+++	–	–
Conventional RT-PCR	+++	+++	+++	+++	++	–
Real-time RT-PCR	+++	+++	+++	+++	++	–
IHC	–	–	–	++	–	–
ISH	–	–	–	++	–	–
Detection of immune response[2]						
ELISA	+++	++	+++	++	+++	++
IPMA	++	++	++	+	++	+++
IFA	++	++	++	+	++	+++

+++: Recommended;++: Recommended, but with limitations;+: Suitable for very limited situations; -: Not recommended for this purpose

Nucleic acid testing (RT PCR, RT qPCR) and antibody testing (ELISA) are important detection methods for PRRSV.

…strial Manual.,

Nucleic acid detection of PRRSV

General detection — RT-PCR — Highly conservative ORF7/3'UTR region

Typing — RT-qPCR — PRRSV-1 and PRRSV-2 respectively ORF6/ORF7 conservative areas

PRRSV-2 branch — 测序 — Nsp2: Strain type; GP5：lineage analysis

Virus isolation+ Animal experiments

GBT 18090-2023

中华人民共和国国家标准

GB/T 18090—2023

代替 GB/T 18090—2008

猪繁殖与呼吸综合征诊断方法

Diagnostic techniques for porcine reproductive and respiratory syndrome

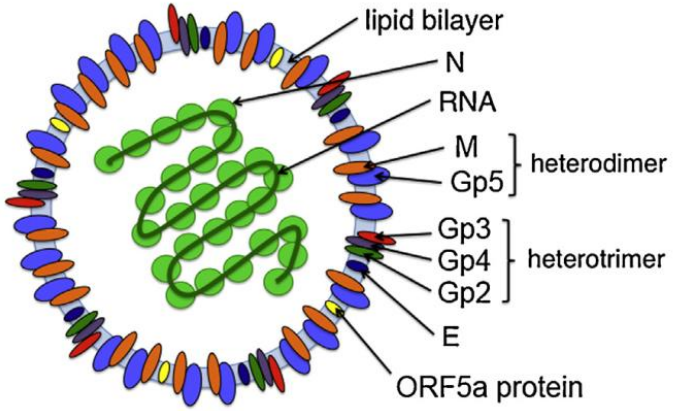
2023-03-17 发布

2023-10-01 实施

Implemented on October 1st, 2023

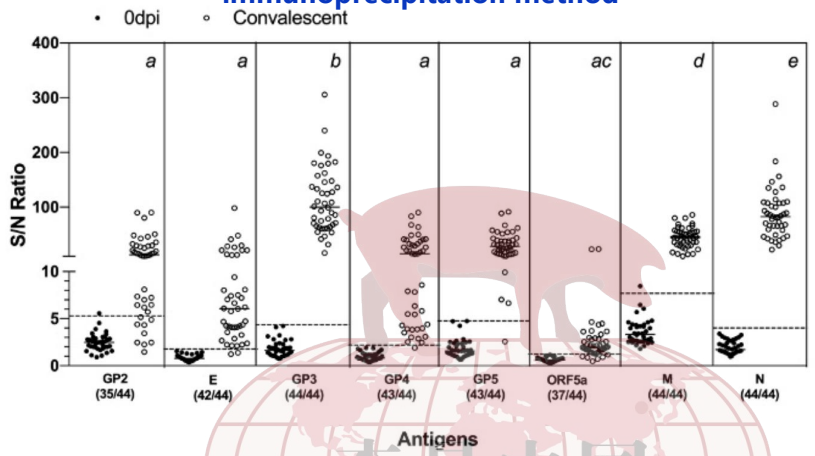
- Classification of infection status in pig farms: RT PCR for general inspection
- Clinical diagnosis requires further identification of infected strains: differential RT-qPCR or sequencing, virus isolation+animal experiments

PRRSV antibody detection



- Capsid protein: N protein
- Two main membrane proteins: GP5-M heterodimer
- 5 secondary membrane proteins: GP2-GP3-GP4 heterotrimer, GP2a, E

Fluorescence immunoprecipitation method



Among the eight structural proteins in 44 pig serum samples infected for 42-63 days, the three proteins with the strongest immunogenicity were GP3, M, and N.

Immunoblotting

Pig no.	Detection of viremia: First day	Detection of viremia: Last day	Appearance of SVN activity	Appearance of antibody against viral proteins: 15 kD	19 kD	23 kD	26 kD
1	3	9	9	5	11	15	9
2	3	11	11	5	11	15	11
3	3	11	11	5	15	15	11
4	5	13	21	7	21	28	15
5	5	13	21	7	21	28	15
6	5	15	28	7	28	42	21
7	5	15	28	7	28	35	28

* Number of days after inoculation with PRRS virus.

The order of antibody production for structural proteins is: 15kD (N protein), 19kD (M protein), 23kD, and 26kD GP proteins

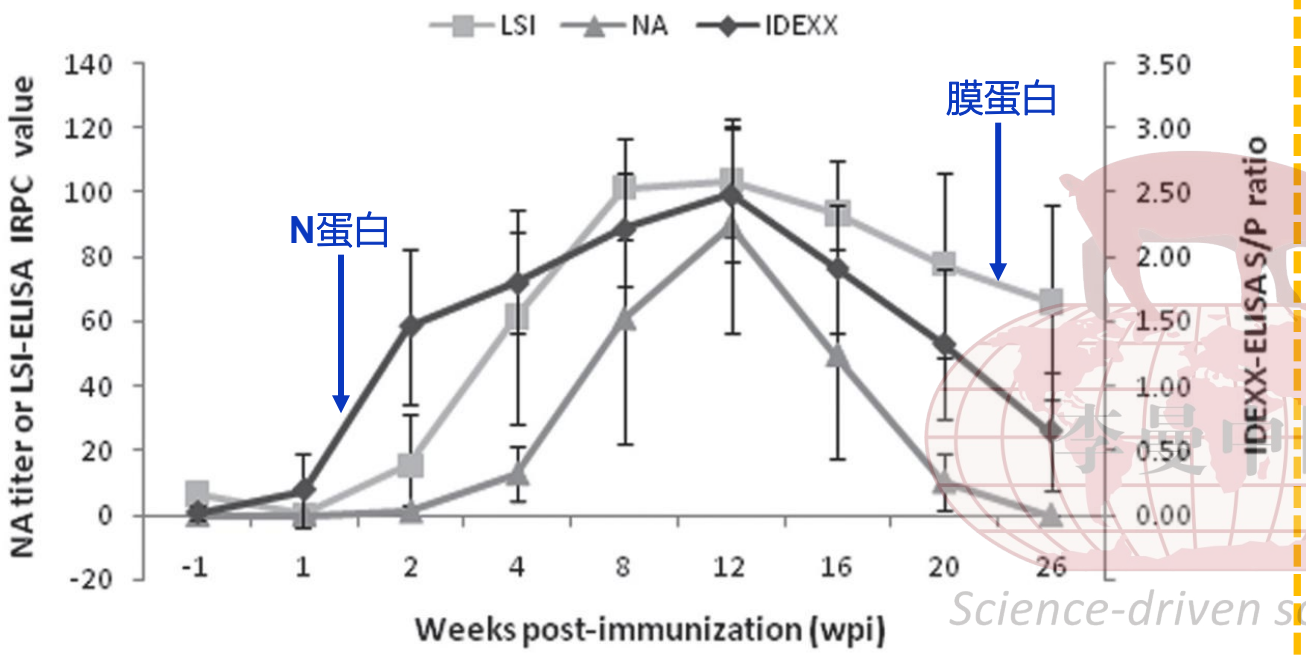
Hung Q. Luong etal., *Vaccines* 2020, 8, 533; Kyoung-Jin Yoon etal., *J Vet Diagn Invest* 7:305-312 (1995)

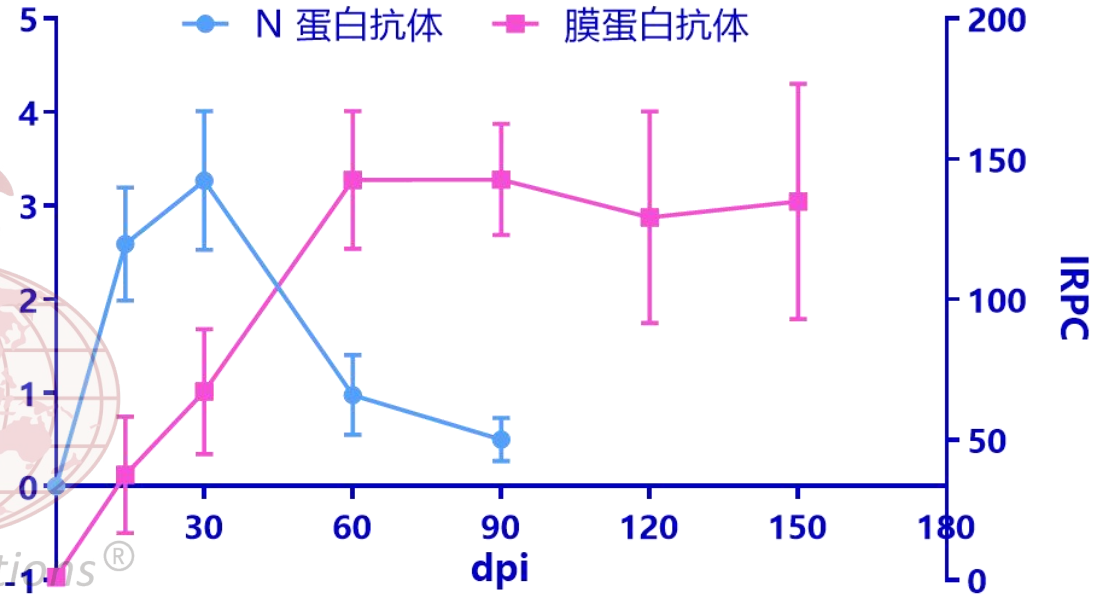
- **N protein - the protein with the most abundant content in virus particles (approximately 40%); The efficiency of membrane protein transcription or translation is low, and the content is low in viral particles.**
- **There are multiple antigenic epitopes on the surface of N protein, indicating strong immunogenicity; The glycosylation modification of glycoproteins blocks the antigenic epitopes on the surface of viral particles.**

N蛋白和膜蛋白均为抗体检测的主要靶标。

PRRSV antibody detection

Different ELISA kits detect: N protein antibodies are produced earlier and have a shorter duration; Membrane protein antibodies are produced relatively late but last for a long time.





- N-protein antibodies are produced earlier than membrane proteins
- At 12 weeks after infection, the levels of N protein antibodies and membrane protein antibodies reached their peak;
- After 12 weeks, N protein antibodies sharply decreased, and the decrease in membrane protein antibodies was less than
- N protein antibodies.

Meng GE *et al.*, *J. Vet. Med. Sci*, 2019

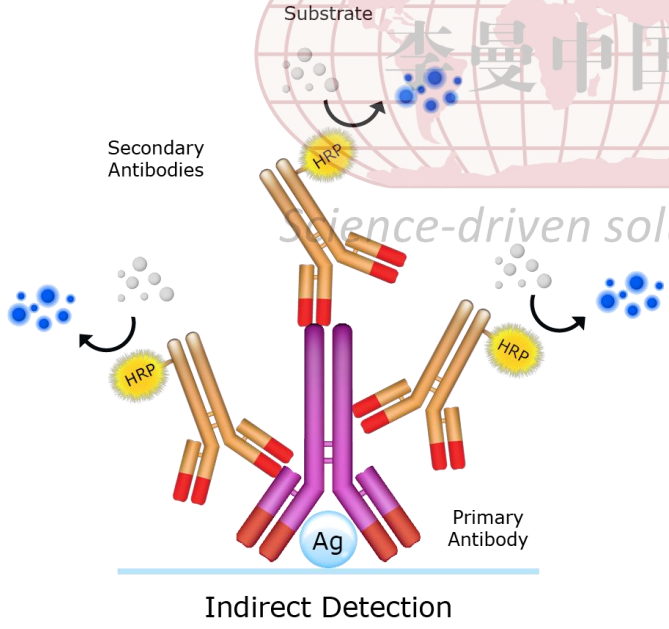
- The N protein antibodies have all turned positive in 14 days, reaching a peak in 30 days and then sharply decreasing; After 90 days, some pigs' antibodies have turned negative.
- The level of membrane protein antibody slowly increased, reaching a plateau period after 60 days, and remained at a high level until 150 days.

数据来源：WOAH PRRS参考实验室

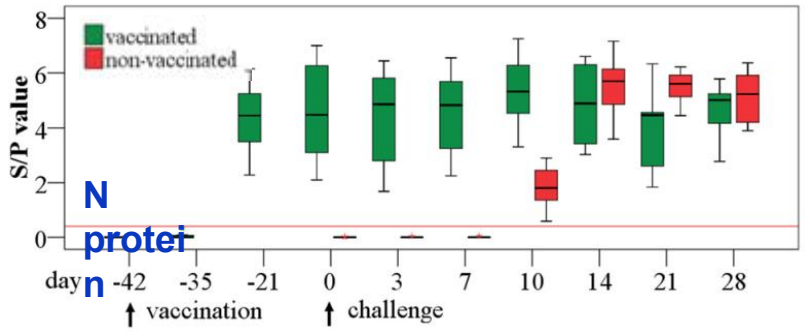
PRRSV antibody detection

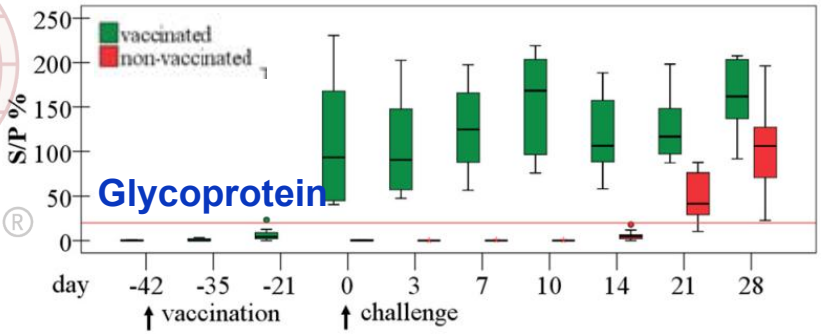
PRRSV antibody ELISA kit coating: In addition to N protein and membrane protein, whole virus can also be used

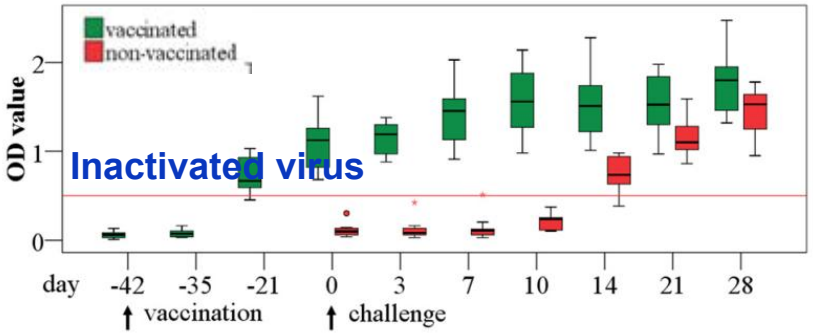
- **N protein**
  - IDEXX
  - INgenasa
  - QIAGEN
  - Thermo
  - ……
- **membrane protein**
  - HIPRA
- **Whole virus**
  - Analytik Jena



Indirect Detection







Sattler et al. Porcine Health Management,2015

Background detection and classification of PRRSV in clinical pig farms

普莱柯生物 PULIKE BIO-ENGINEERING

Clinical manifestations&production performance Preliminary classification

- Positive unstable field
- Positive stable field
- Provisional negative field
- Negative field

According to preliminary classification Develop sampling and testing plans

Fixed detection cycle

- Pig serum
- Piglet processing fluid
- Litter oral fluid

Nucleic acid and antibody testing

- Nucleic acid: RT-PCR, RT-qPCR
- Antibodies: N-protein antibodies, membrane protein antibodies

Classification of pig farms

- Positive unstable field
- Positive stable field
- Provisional negative field
- Negative field

Nucleic acid testing, N-protein antibody testing

- Can be used for category determination and category maintenance
- But both have a faster rate of turning negative

Membrane protein antibody

- Pigs that have been infected with PRRSV have a longer duration of membrane protein antibodies;
- At this point, the virus exists in lymphatic tissue and there is a risk of reactivation and reinfection.

When introducing and entering the reserve pig, it is necessary to simultaneously test for membrane protein antibodies to ensure true negative results.

The ultimate goal of clinical pig farms - Eradication

普莱柯生物 PULIKE BIO-ENGINEERING

Positive instability
High prevalence rate

Positive instability
Low prevalence rate

Weaned piglets

RT-PCR detection
≥ 75% (3/4)
negative

Positive stability (Class II, Class II-vx)

Weaned piglets

RT-PCR detection
100% negative

Provisional negative

Replacement gilts

ELISA detection
Antibody
negative

Negtive

Breeding pig

ELISA detection
Antibody
negative

Appropriate sample type

Sufficient sample quantity

Appropriate testing reagents

Sufficient detection frequency

On the premise of stable production performance, stable positive or tentative negative results can be considered as the goal of pig farms.

The eradication of pig farms must be negative for nucleic acid, and both N-protein antibodies and membrane protein antibodies are negative.

普莱柯生物
PULIKE BIO-ENGINEERING

Thank you!