# **Application of 4-color Management and Precise Elimination** in the Prevention and Control of PRRS in Boar Stud



### **CONTENTS**

- 1 Importance of PRRS prevention and control in boar stud
- Principles and points of 4-color management in boar stud
- Precise removal and elimination of PRRS in boar stud
- 4 Conclusion & Acknowledgements



# 1.1 Biosafety in boar stud

- ✓ Biosafety measures to prevent transmission of infectious diseases, parasites, and pests.
- ✓ Biosafety isn't just against ASFV;
- ✓ Biosafety is a philosophy or an attitude;
- ✓ Ensuring the biosafety of the boar stud is the basic guarantee of the health of the herd.



# 1.1 Biosafety in boar stud

Highlights of biosafety in boar stud:

- A. Site Selection;
- B. Personnel;
- C. Boar (semen);
- D. Supplies, vehicles;
- E. Air Filtration.

Potier found that 45% of pigs about 500m from a PRRS outbreak were infected with PRRSV through transmission, while only 2% of pigs about 1km from the outbreak became infected (Potier, 1997).









### 1.2 Diseases transmitted by boar semen

#### Major semen-transmitted diseases of breeding boars:

Classical Swine Fever Virus (CSFV): Studies have shown that the virus can be detected and isolated from boar semen in both experimental and natural infections (Floegel et al, 2000);

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV): Prieto et al. found PRRSV titers of  $7 \times 10^2$  TCID<sub>50</sub> /mL in the semen of infected boars (Prieto et al, 1996);

**Japanese Encephalitis Virus (JEV)**: 7 positive cases were detected in 797 porcine semen from 16 prefectural and municipal cities in Yunnan Province, with a positivity rate of 0.88% (Li Shishi et al., 2012);

Foot-and-mouth Disease Virus (FMDV): FMDV can be extracted from the semen of boars infected for up to 9 days after natural infection;

Porcine Circovirus Type 2 (PCV-2): naturally infected boars intermittently disperse the virus in semen for up to 27.3 weeks (Mc Intosh et al, 2006);

**Porcine Pseudorabies Virus (PRV)**: it has been found that boars can continue to disperse virus in semen for up to 12 days after infection with PRV (He Qigai et al., 2015);

**Porcine Parvovirus (PPV)**: PPV has been found to be predominantly present in semen and non-semen cell fractions (KIM et al, 2003);

**Porcine Epidemic Diarrhea Virus (PEDV)**: it was found that PEDV was present in semen at  $5.06 \times 10^2 \sim 2.44 \times 10^3$  copies/mL (Sarah et al, 2018);

**African Swine Fever Virus (ASFV)**: artificial insemination has been shown to transmit ASFV; can ASFV actively infect semen to transmit disease?

Others: Swine Vesicular Disease Virus (SVDV), Porcine Endogenous Retrovirus (PERV), Porcine Blue Eye Disease Virus (paramyxovirus, BEDV), etc.

# 1.3 Transmission of PRRSV by boar semen

Boar Test 0 1 3 5 7 9 11 14 16 18 21 23 25 29 31 35 38 42 45 49 52 56 59 63 67 71 74 77 80 84 87 92 98

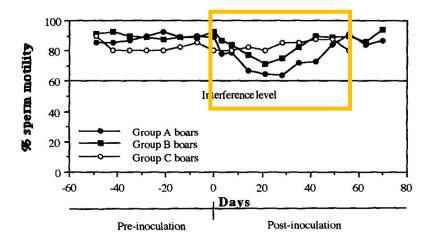
94X27 Serum VI
Serum PCR
Semen PCR
Semen PCR
Semen PCR
Serum VI
Serum PCR
Semen PCR
Semen PCR
Semen PCR
Serum VI
Serum PCR
Semen PCR
Semen PCR
Semen PCR
Semen PCR
Semen PCR
Serum VI
Serum PCR
Serum PCR
Serum VI
Serum PCR
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum VI
Serum PCR
Serum P

Table 1. Detection of PRRSV or PRRSV RNA in boar serum and semen by virus isolation (VI) and polyermase chain reaction (PCR).

Semen viability of boars before and after PRRSV infection

Science-driven solutions

PRRSV can be detected in boar semen even in the absence of viremia and neutralizing antibodies, demonstrating that the virus persistently replicates in one or more tissues of the reproductive system and that PRRSV can be detected later in semen than in blood (Christopher Hennings et al, 1995).



Semen viability of boars before and after PRRSV infection

The semen viability of boars was significantly reduced after PRRSV infection, which affected the quality of boar semen for about 2 months; it was also found that PRRSV titers of infected boar semen could reach 7×10<sup>2</sup> TCID<sub>50</sub>/mL (Prieto et al, 1996).

<sup>\* +</sup> VI indicates detection of PRRSV antigen following serum inoculation on MARC-145 cells suggesting infectious virus.

<sup>†</sup> Seroconversion as indicated by indirect fluorescent antibody titer.

<sup>#</sup> Seroconversion as indicated by virus neutralization titer

<sup>§ +</sup> PCR indicates presence of nested 236 bp PCR product in serum.

<sup>+</sup> PCR indicates presence of nested 236 bp PCR product in either whole semen or cell fraction of semen.

<sup>- =</sup> No detection of virus in serum by virus isolation or PCR products in serum or semen.

<sup>··· =</sup> Not determined.

# 1.4 Economic benefit of achieving PRRSV-negative production

This was found by analyzing four PRRS outbreaks from 2014 to 2017:

- Sow farms, with a levelized cost of ¥822 per sow in increased losses;
- Nursery fattening farms, with a levelized cost of ¥ 601 per sow in increased losses;
- Sow herds + nursery fattening herds, totaling ¥ 1,424 per sow in increased losses.
- Main influencing factors: reduction in the number of weaned piglets (reproduction phase) and reduction in feed conversion ratio (nursery fattening phase).

,	Farm	Production Model	Sow Size
Onset:	A	Two-site production sow farms	5000
November 2014	В	Two-site production sow farms	2450
February 2017	C	Two-site production sow farms	3800

One-site production sow farms

1750

Farm	Breeding herds losses	Fattening herds losses	Whole herds	
多曼州	(¥)/percentage (%)	(¥)/percentage (%)	losses (¥)	
A	668.14/63.65	381.64/36.35	1049.78	
ce Bdriven so	olutio 1672.56/41.78	937.14/58.22	1609.70	
C	1004.43/64.89	543.50/35.11	1547.93	
D	945.85/63.48	544.20/36.52	1490.05	
Mean	822.75/58.45	601.62/41.55	1424.37	

Whole herd economic losses after an outbreak of PRRSV equally distributed to each sow (Xiangyang Qu et al, 2022)

# 1.5 Health management objectives of boar stud

- ✓ Daily biosafety checks.
- ✓ Negative for important infectious disease pathogens.
- ✓ PRRS:

The minimum standard is to ensure PRRS antigen negativity;

Ultimately, a naïve state was maintained.

✓ Provide competent and healthy semen.

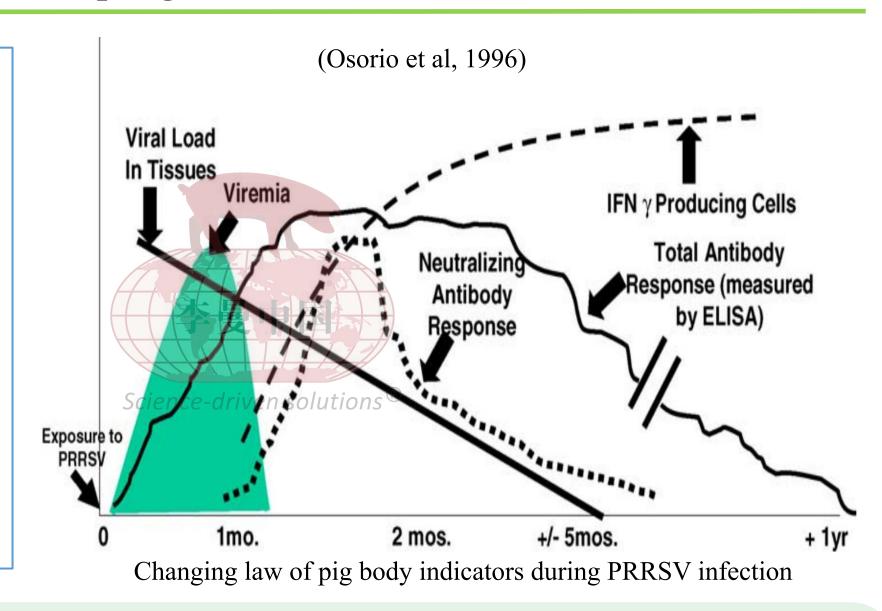


Checklist	Pivot	Do	Inspectorate	Note			
	Both sides of the shower can stay dry						
Orange to	Adequate toiletries						
yellow shower.	Top spray of nozzle						
	Shower rooms cleaned and sanitized daily						
	Fan cover complete and undamaged						
Environment	Fan cover 25cm or more above the ground						
outside	No breakage of water curtains (back-up)						
	No cracks in the walls						
	Doors should be airtight, no gaps						
	No water on the floor, floor drains should be plugged to prevent backdrafts						
	No breaks in the triple defense network						
	The triple defense net should be clean and not blocked by dust						
	Filter installed correctly without air leakage						
	Filter not broken						
THE AN	Primary filters are changed once in 8-12 months; intermediate filters are						
Air filtration	changed once in 18-24 months.  Red oil differential pressure gauge value is normal (reference value 15-						
room	30Pa)						
量由	The floor is clean and tidy with no visible dust						
X 11 1	Piping, valves, pumps are normal						
	Regular folds of waterproof paper / no spikes / no debris on the surface						
	Water curtain pools are regularly cleaned of debris and are free of leaks						
driven so	Make-up water float valve is normal						
1111001130	Roller shutters are functioning properly						
	Cleaning and disinfecting triple defense net and floor once a week						
Fan louvers	Weekly/sampling monitoring						
Deodorization							
booth	Depending on whether it is configured or not						
Yellow into Green	Both sides of the shower can stay dry						
	Adequate toiletries						
Bathroom	Top spray of nozzle						
	Bathrooms cleaned and sanitized daily						
Office/seminal	All the windows can't be opened.						
sorting room,	No air leakage from pigsty ceiling						
inside pigsties	Pipes into pigsties should be sealed.						
	The sewer has to have a storage bend, it can't drain directly to the outside						



# 2.1 Why tail-tip blood sampling is used to monitor boar health

- ✓ ELISA antibody test: 7-9 days after infection;
- ✓ Time of neutralizing antibody production: about 1 month after infection;
- ✓ Time of gamma interferon production: about 1 month after infection;
- ✓ Time of viremia formation: 6-48h after swift infection, with the highest viral load of 4-14 days and duration of about one month;
- ✓ Time of PRRSV clearance in pigs: 150 days or more.



# 2.2 Principles of 4-color management in boar stud



#### Sampling notes:

- 1. Divide all pens into **red**, **orange**, **yellow** and **green** colors, boars are evenly spaced according to the 4 colors as above, pens without pigs are skipped directly;
- 2. Weekly sampling of boars in the same color pen and taking tail-tip blood swabs;
- 3. Ensure that all boars of one color are sampled within 1 week and all boars are sampled and tested once a month;
- 4. The samples are cryopreserved and transported to the laboratory, and the test item is PRRSV, and the result is issued on the same day.

# 2.3 Routine health monitoring - tail-tip blood sampling procedure



2

- ✓ Wear gloves to prevent cross-contamination
- ✓ Blood infiltrated swabs

Sampling operation



4

- ✓ Low temperature sealed storage
- ✓ Transport rapidly



Sample transportation

Preparation for sampling



- ✓ Sterile cotton swabs, sterilized needles, gloves, sample preservation boxes, etc.
- ✓ Sampling samples should be sterilized in advance



Sample processing

- Sample solution numbering and sealing
- ✓ Add appropriate amount of saline



Laboratory test



- ✓ Prevents cross contamination between samples
- ✓ Precision testing
- ✓ Rapid testing
- ✓ Feedback on test results

## 2.4 Abnormal health monitoring

No.	General detection time	Heads of general detection	Number of PRRSV positives	positivity rate	Mean CT value	Number of positive throat swabs	Mean CT value of throat swabs	Number of positive blood samples	Mean CT value of blood samples
1	2023/1/12	791	56	7.08%	34.18	51	34.32	6	32.92
2	2023/2/19	362	36	9.94%	32.42	33	32.51	6	31.92
3	2023/3/16	361	8	2.22%	34.19	8	35.71	3	32.33
4	2023/4/14	326	15	4.60%	34.54	15	34.73	2	33.12
5	2023/5/3	618	56	9.06%	33.08	53	33.09	5	33.00
6	2023/5/28	745	63	8.46%	35.56	63	35.56	0	-
7	2023/6/30	405	47	11.60%	34.44	45	34.49	4	34.40
8	2023/7/25	425	55	12.94%	36.06	54	36.06	3	34.80
9	2023/8/22	536	99	18.47%	34.67	98	34.67	2 //	34.00
10	2023/9/19	624	96	15.38%	34.84	94	34.85	/ / 2//	34.69



#### Analysis of test results:

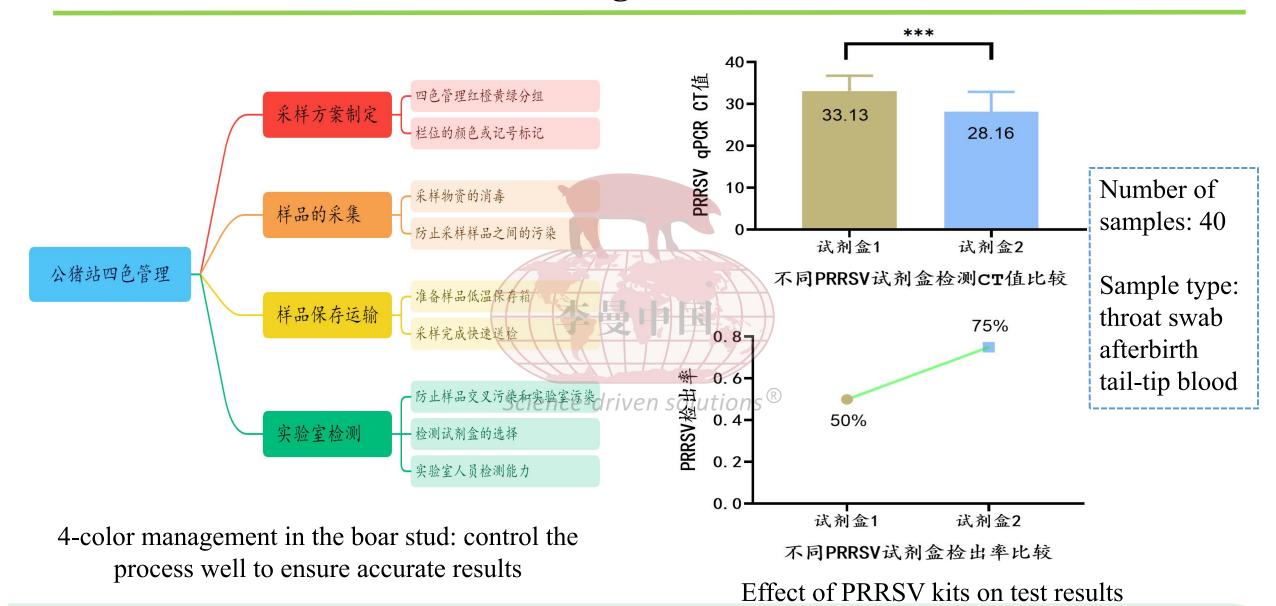
#### Science-driven solutions®

- 1. Time to throat PRRSV detoxification in PRRSV-positive pigs > time to viremia formation by PRRSV (\*\*\*);
- 2. Suspected PRRSV-positive pigs are monitored for health, with oropharyngeal swabs as the primary test and blood as a secondary test for each pig;
- 3. Early pathogen monitoring of PRRSV infection: blood swab sampling and testing (early and convenient detection).

Pathogen detection in the middle to late stages of PRRSV infection: testing throat swabs (long detoxification time).

When boars show abnormalities such as fever or not eating, throat swabs and environmental samples are immediately collected to test for PRRSV and ASFV; both results are negative, and the samples are re-sampled 1 and 2 weeks later for paired detection of antibodies to PRRS disease.

# 2.5 Considerations of 4-color management in boar stud





#### 3.1 Rapid and precise culling of PRRSV-positive pigs at boar studs - scheme formulation

Monitoring	Date	Sampling items	Contingency plan	Note
Definite diagnosis	D0	ABC	Confirmation of positives, specialized group meeting	
First round of monitoring	D1	ABC	Conduct the first round of 1357	A: throat swab; B: mouth and nose swab; C: fecal and urinary
	D3	ABC	sampling and testing, give feedback to specialized groups, hold a specialized	discharge area swab; D: blood swab; F: environmental swab; E: abnormal pigs
	D5	ABC	meeting when the results turn to be positive; 2 consecutive rounds of 1357	Instructions:
	D7	ABCD	sampling and testing to successfully achieve precise culling	<ul><li>B: including pig mouth and nose, pen door, trough;</li><li>C: including feces and urine, tailgate, ground and rails where</li></ul>
	D1234567	EF	Abnormal pigs and environmental monitoring	boar trips lying; F: including fans, water curtains, aisles and other samples;
Second round of monitoring	Ditto	Ditto	李曼中国	E: including samples of the four categories of A, B, C and D.

- ✓ Initiation of culling program: 4-color management or detection of PRRSV in abnormal pigs, and immediate initiation of the boar 1357 sampling and monitoring program upon confirmation of the diagnosis.
- ✓ Environmental samples are collected daily: fans, water curtains, walkways, and other samples.
- ✓ Abnormal pigs are sampled daily: mouth and nose, blood swabs, throat swabs, and fecal and urine discharge area swabs.
- ✓ After the second round of 1357 monitoring is negative, daily 4-color monitoring and abnormal pig monitoring will be restarted.
- ✓ Sperm-collecting boars are monitored for semen PRRSV antigen.

#### 3.2 Rapid and precise culling of PRRSV-positive pigs at boar studs - time target management

**Timeline**: 2nd 7 days, red, yellow and green management;

Target management: Achieve PRRSV elimination of boar studs within 2 weeks, aiming to complete it in 1 week.

First round of monitoring

第1个7天

红色: 危险期

Rapidly achieve sampling, testing and pig culling through 1357 days of full testing and daily testing of abnormal pigs.

Note: Sample and pig cull to avoid cross contamination.

Second round of monitoring

第2个7天

黄色: 交叉污染期后期

Science-driven solutions®

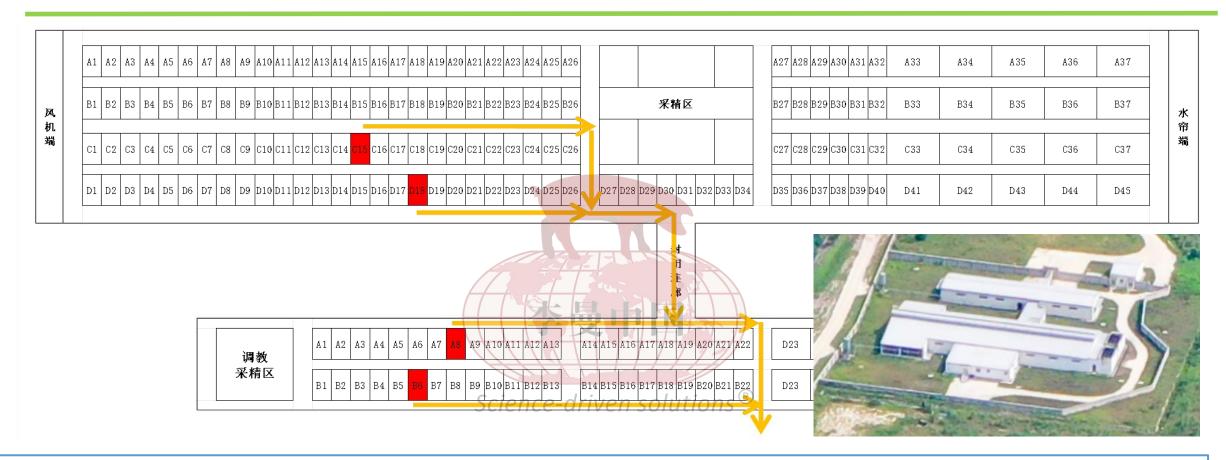
Uses the first round of culling to get rid of cross-contamination and rapidly enter a stabilization period.

Conversely, a large number of viruscontaining dust particles are formed and new positive sites are constantly added due to the fans. Safe production

绿色:安全期

The first and second rounds are consistently tested negative for PRRSV, and after two weeks the boar studs return to normal production operations and perform routine 4-color health management.

#### 3.2 Rapid and precise culling of PRRSV-positive pigs at boar studs - notes



- ✓ Laboratory: complete the sampling, delivery of samples for testing within the fastest possible time and get feedbak within 12 hours from the laboratory;
- ✓ Positive pigs will be eliminated from the farm within 24 hours after the test result, the specific operation is the same as ASF precision culling operation;
- ✓ According to the test results, update the PRRS combat map of boar barn on the same day and publish it in the specialized group.

#### 3.4 Case sharing of PRRSV precise culling - morbidity history investigation

#### History of PRRSV in a boar stud:

July 21, 2022: PRRSV antigen-positive boars were first detected in the monthly universal pickup;

July 22-August 13, 2022: The station continued abnormal pig testing with sporadic detection of PRRSV antigenpositive boars;

Treatment measures: The station removed PRRSV antigen-positive boars but did not match to make a systematic and comprehensive surveillance plan.

PRRSV elimination program implementation:

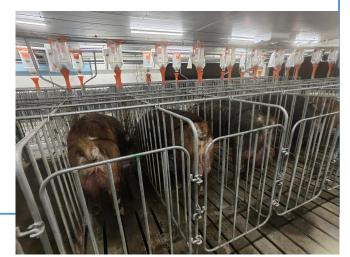
August 14, 2022: Strict implementation of the 1357 test precise culling program began.

#### PRRSV elimination goals:

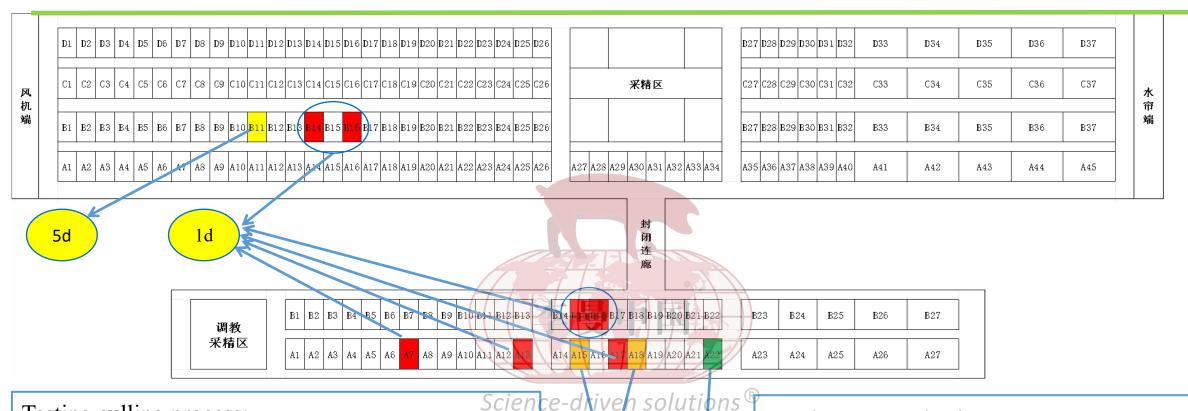
Science-driven solutions®

Complete PRRSV elimination of boar studs within 14 days (by August 28th ).

Achieve double negative PRRSV production at boar studs.



#### 3.4 Case sharing of PRRSV precise culling - Developing a precision culling program



3d

13d

Testing culling process:

August 15-August 27, 2022: 4 whole herd tests found PRRSV positive pigs, culling 3 farrowing gilts and 8 reserve boars for a total of 11 pigs.

August 28, 2022: Subsequent PRRSV continuous testing results were negative.

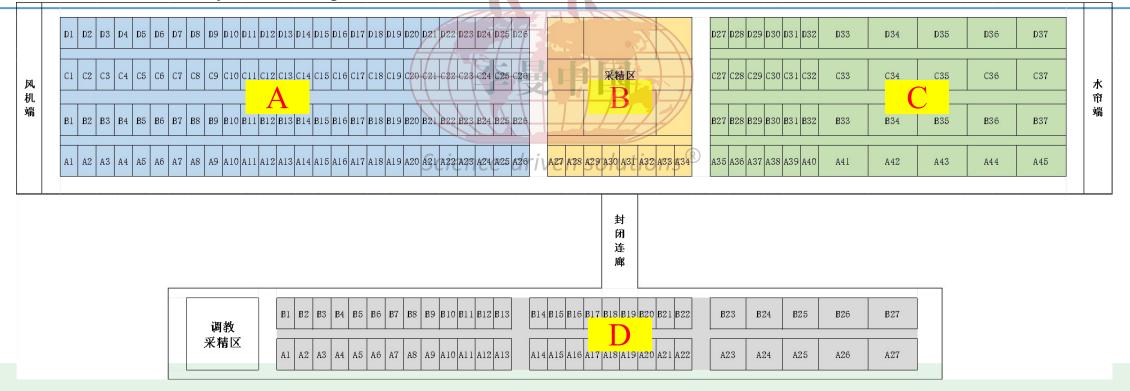
Continuous monitoring:

Continuous 4-color surveillance management and abnormal pig detection management at boar studs after culling is complete to maintain PRRSV antigen-antibody double-negative production at boar studs.

#### 3.4 Case sharing of PRRSV precise culling - regionalized management

#### Regionalized management:

- ① Building regionalized management: Production house were divided into three districts and reserve house consisted of another district: a total of four districts. Each district entrance and exit placed water shoes, 3% caustic soda disinfection basin. Staff were requied to change shoes and step over the basin beore get into the districts, to reduce the risk of cross-contamination.
  - 2 Personnel movement management: Reduced personnel cross-district movement, required flow hand washing, foot basin disinfection.
- ③ Management of the back-up house: Arranged for specialized personnel to manage the back-up house, including feeding, sanitation cleaning, sampling and other related work. All personnel left the barn after completing the day's work, and would not be allowed to re-enter the boar stud on the same day after leaving the barn.



#### 3.4 Case sharing of PRRSV precise culling - Disinfection management

#### **Disinfection:**

- **①Drinking water disinfection for pigs:** added potassium bisulfate (1:1000) into drinking water for 1 day.
- 2 Trough cleaning and disinfection: immersed the broom in potassium bisulfate (1:200) to disinfect it. Each through were epuipped with one broom, forbidding cross use.
  - ③Environmental pig disinfection: disinfected the pigs with potassium bisulfate (1:200) at 2-3 pm every day for 3 consecutive days.
- 4 Pig pen disinfection: used 3% caustic soda to pour on the original pen after transferring the pigs to remove faeces, cleaned and dried the pen with water, and then disinfected it with potassium persulfate (1:200).
- ⑤ **Disinfection of bathing room**: including orange yellow and yellow green bathing room, disinfected them with potassium bisulfate (1:400) every day to ensure that no live virus was brought out.
  - (7) Abnormal pig management: transferred abnormal pigs with fever or without feeding, etc. to the reserve house.
- 8 disinfection precautions: Management of all configure disinfectant containers must strictly implement the principle of two scale lines.



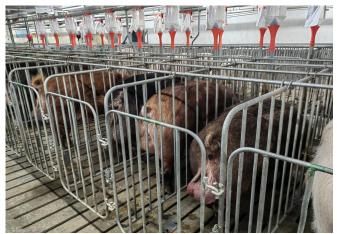






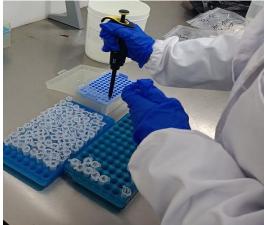
#### 3.4 Case sharing of PRRSV precise culling - Note

- ★Sample collection: At the initial stage of PRRSV elimination, strictly implemented 1357 whole herd sampling. Sampled according to the order of pig partitioning and rowing and the whole process was supervised by specialized personnel to ensure that the sampling was fully implemented, to reduce cross-contamination during the sampling process, and gave feedback on the implementation of the work in the specialized work group.
- **★Sample delivery**: Environmental swabs and abnormal pigs are sampled and delivered daily, and cryopreserved delivery/RNA sample protectant.
- ★Laboratory testing: After sample delivery, conducted 5-1 mixed sample testing for PRRSV antigen. Results should be available within 12 hours and reported in the special group.
- ★Kit selection: According to the PRRSV kit screening results, selected the kit with high sensitivity and good stability.
- ★Pig culling: The culling of PRRSV test positive boars should be completed within 24 hours.
- ★Herd health care: Continuously added Tavamycin in pig feed for health care at a dose of 6g/head/day in two additions.

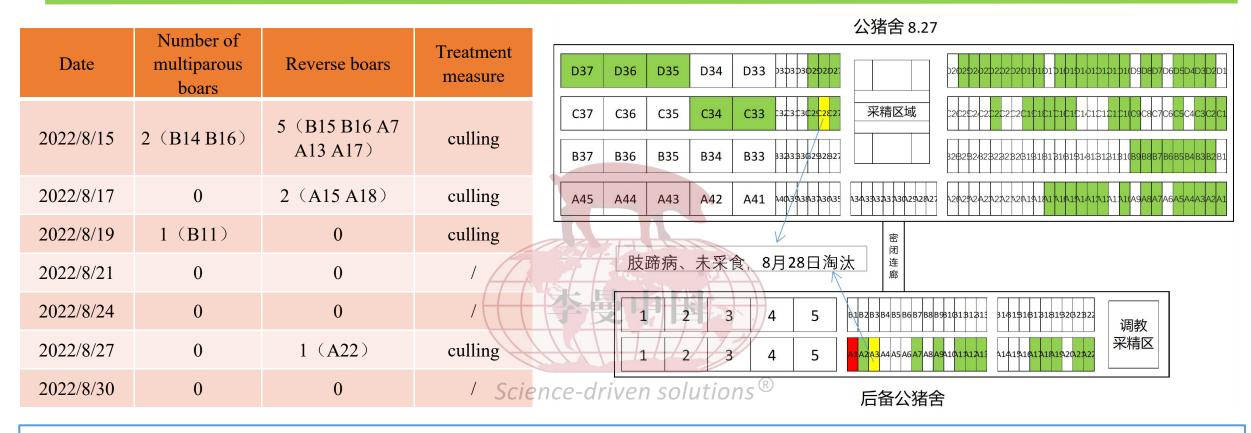








#### 3.4 Case sharing of PRRSV precise culling - PRRSV elimination results



- Last PRRSV-positive test results of boars: 13th day after starting implementation of herd-wide 1357 surveillance, totaling 11 culled boars (3 production boars, 8 reserve boars), followed by continued negative surveillance.
- ➤ PRRSV elimination of boar studs: PRRSV elimination of boar studs was achieved within 2 weeks after the start of implementation of herd-wide 1357 monitoring.
- > PRRSV elimination was achieved in a short period of time, minimizing the impact of PRRSV on normal production work including semen collection and breeding.



#### 4. Conclusion

Prerequisites for rapid PRRSV elimination in boar studs:

- ① Implement air filtration in the boar stud;
- 2 Do a good job of daily biosafety management in pig farms;
- ③ Strictly implement 4-color management and daily sampling and monitoring work in the boar stud.

#### PRRSV precise culling program:

- ① Detect PRRSV in time at the first time;
- 2 Define the target and make good organization and division of labor among personnel;
- 3 Adopt 1357 precise culling program;
- 4 Realize PRRS antigen conversion of boar studs within two weeks.

# Acknowledgment

- Mr. Zhijun Wu
- Dr. Shaoqin Wu
- Dr. Jason Yan
- Dr. Bob Morrison UMN
- Dr. John Deen UMN
- Dr. Frank Liu
- Guangdong Jingji Zhinong Team Members: Long

Guowei Chen Bin Huang Zhen Pan Keman, etc. solutions®

- New Channel Group
- Yongxin Animal Husbandry Group

In 2010, Liangqi Boar Station started to apply 4-color management.



# Thank you for your atttention

