Case study of PRRS eradication in newly built pig farms

From pre-eradication assessment of infection parameters to eradication evaluation

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Porcine Reproductive and Respiratory Syndrome Virus hereinafter referred to as "PRRSV"

Global PRRS Solutions

Contents

- Definition and criterion of PRRSV eradication
- Why to eradicate PRRSV?
- How to eradicate PRRSV?

PRRSV status classification system 2020 by American Association of Swine Veterinarians（AASV）

PRRSV status classification	Antibody detection-shedding status	Antibody detection-exposure status	Classification criteria	Supporting evidence required
positive unstable, high prevalence (I-A)	positive, high proportion of shed pigs	positive	Default Category I for herds that do not meet other criteria	None required
positive unstable, low prevalence (I-B)	positive, low proportion of shed pigs	positive	Weaned piglets remain antigen-positive for 90 consecutive days <25%	Monthly test Test serum from weaning-age pigs 30 samples（five samples mix for one） Weaned piglets remain antigen-positive for 90 consecutive days <25% Tested after 2 weeks of vaccination (if vaccinated)
positive stable (II)	uncertain	positive	Weaning piglets remain antigen-negative for 90 consecutive days	Monthly test Test serum from weaning-age pigs 60 samples（ten samples mix for one） Weaned piglets remain antigen-negative for 90 consecutive days
positive stable, immunity achieved through vaccination (II-vx)	uncertain	positive	Weaned piglets remain antigen-negative for 90 consecutive days	Monthly test Test serum from weaning-age pigs 60 samples（ten samples mix for one） Weaned piglets remain antigen-negative for 90 consecutive days Tested after 2 weeks of vaccination (if vaccinated)
provisional negative (lll)	negative, no shedding	negative	PRRSV positive pigs are removed from the herd and there's on infection since mixing the introduced PRRSV naïve gilts for 60 days. No vaccination.	PRRSV naïve gilts. Growing pigs confirmed ELISA negative.
negative (IV)	negative, no shedding	negative	All pigs that previously tested positive for infection are removed from the herd. Herd confirmed ELISA negative for one year.	Confirmed ELISA negative for one year. One year after classified as IV.

Holtkamp DJ, Polson DD, Torremorell M, Morrison B, Classen DM, Becton L, Henry S, Rodibaugh MT, Rowland RR, Snelson H, Straw B, Yeske P, Zimmerman J; American Association of Swine Veterinarians; United States Department of Agriculture PRRS-Coordinated Agricultural Project. Terminologie zur Klassifizierung des PRRSV-Status von Schweineherden [Terminology for classifying the porcine reproductive and respiratory syndrome virus (PRRSV) status of swine herds]. Tierarztl Prax Ausg G Grosstiere Nutztiere. 2011;39(2):101-12. German. PMID: 22138772.

Classification by China Animal Disease Control Center

中国动物疫病预防控制中心
（农业农村部屠宰技术中心） 文件

疫控监〔2021〕4 号

中国动物疫病预防控制中心关于印发《动物疫病净化示范场评估标准（试行）（2021 版）》《动物疫病净化创建场评估标准（试行）（2021 版）》的通知

I. **Eradication standards** are met if the requirements listed below are followed：

（1）The samples of boars, sows and gilts were all negative for antibody detection of PRRSV.

（2）Immunization ends for more than two years and there's no clinical cases.

（3）Pass the site comprehensive review.

II. Sampling requires experts of the assessment team to design plans and supervise the tasks and provincial disease prevention and control institutions should also work with them.

表 1-6 净化评估实验室检测方法

检测项目	检测方法	抽样种群	抽样数量	样本类型
抗体检测	ELISA	种公猪	生生产公猪存栏 50 头以下，100%采样；生产公猪存栏 50 头以上，按照证明无疫公式计算（CL=95%，P=3%）	血清
		生产母猪 后备种猪	按照证明无疫公式计算（CL=95%，P=3%）；随机抽样，覆盖不同猪群	血清

Project Farm's PRRSV Progression Schedule and Diagnosis

positive unstable

	PCR	ELISA
Breeding pigs	+	+
piglets	/	/

March 8th , 2023

positive stable

	PCR	ELISA
Breeding pigs	-	+
piglets	/	/

August 15th , 2023

Negative for piglets at 6-8 weeks

	PCR	ELISA
Breeding pigs	-	+
piglets	-	-

Projection in November, 2023

provisional negative（III）

	PCR	ELISA
Breeding pigs	-	+
piglets	-	-
setinal pigs	-	-

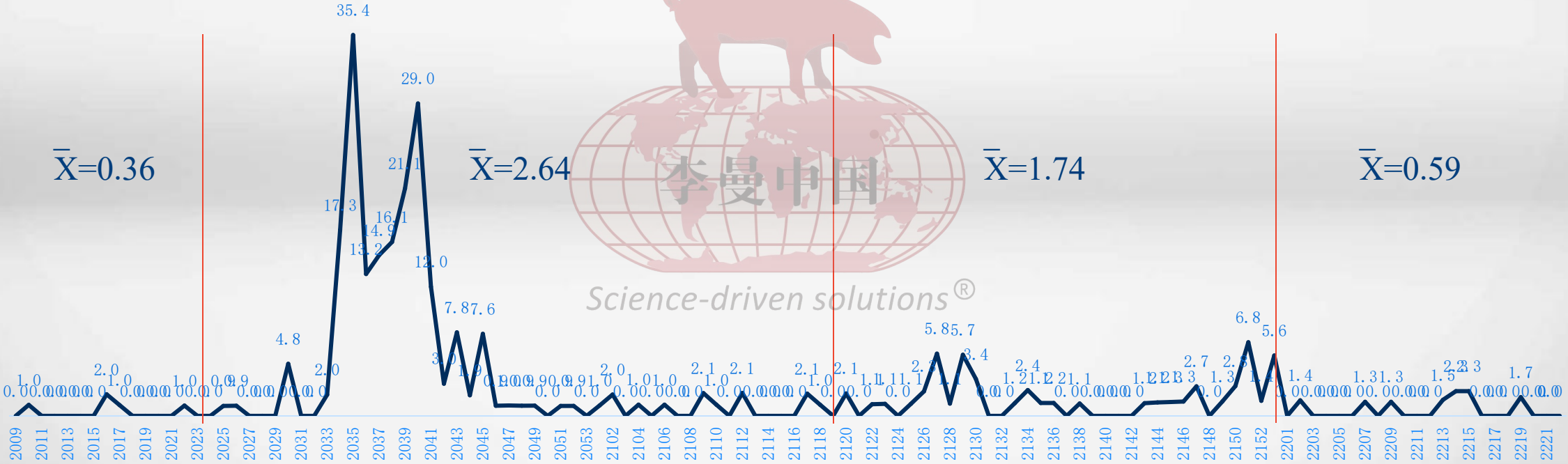
negative (IV)

	PCR	ELISA
Breeding pigs	-	-
piglets	-	-

The number of miscarriages per thousand sows in different PRRSV status

Negative(IV) | Positive Unstable（I） | Positive Stable（II） | Provisional Negative (lll)

$\bar{X}$=0.36 | $\bar{X}$=2.64 | $\bar{X}$=1.74 | $\bar{X}$=0.59



Negative farms：+1 weaner/litter

negative（IV）		positive
（%）birth rate：96.2 ± 5.7	4.9%	（%）birth rate：91.3±11.5
（%）stillbirth rate：2.6 ± 4.7	4.9%	（%）stillbirth rate：7.5 ± 11.5
（kg）ADG：0.24 ±0.06	0.03	（kg）ADG：0.21 ±0.05
（%）pre-weaning mortality： 10.9%	8.8%	（%）pre-weaning mortality： 19.7%
（kg）average weaning weight ： 6.21 ± 1.49	0.49	（kg）average weaning weight ： 5.72 ± 1.29

Schelkopf A, Nerem J, Cowles B, Amodie D, Swalla R, Dee S. Reproductive, productivity, and mortality outcomes in late-gestation gilts and their litters following simulation of inadvertent exposure to a modified-live vaccine strain of porcine reproductive and respiratory syndrome (PRRS) virus. Vaccine. 2014 Aug 6;32(36):4639-43. doi: 10.1016/j.vaccine.2014.06.073. Epub 2014 Jun 24. PMID: 24975816.

PRRS+ medication costs for nursery pigs enhanced 3 times

Rearing phase:	Weaning		Fattening	
Productive chain[1]:	PRRS–	PRRS+	PRRS–	PRRS+
Pig (thousand)	116.0	65.3	108.2	54.4
Starting live weight, kg	7.1	6.9	32.0	31.7
Final live weight, kg	32.2	31.3	170.9	168.5
Average age, days	55.9	59.8	199.0	203.4
Daily live weight gain, g	449	407	699	674
Feed to gain	1.71	1.83	3.43	3.62
Total losses[2]	2.55^{A}	14.49^{B}	4.64^{A}	9.04^{AB}
Cost index[2]	1.63^{aB}	1.91^{bB}	0.88^{A}	0.93^{A}
Medication costs[2], €/kg	0.04^{A}	0.12^{B}	0.03^{A}	0.04^{A}

Trevisi P, Amatucci L, Ruggeri R, Romanelli C, Sandri G, Luise D, Canali M, Bosi P. Pattern of Antibiotic Consumption in Two Italian Production Chains Differing by the Endemic Status for Porcine Reproductive and Respiratory Syndrome. Front Vet Sci. 2022 Mar 28;9:840716. doi: 10.3389/fvets.2022.840716. PMID: 35419448; PMCID: PMC8996257.

Values in other respects

1. State policy
2. Premiums of PRRSV naïve gilts
3. Brand value
4. Animal Welfare
5. Reduction of PRRSV recombination and mutation

…

Target and Introduction of the Eradication Program

Program scope and target

- Use **the PRRSV positive herd in the existing system** to establish a PRRSV negative herd of 1500 base sows in a new farm and to sell PRRSV naïve breeding pigs
- Requirements: shorten the cycle; a certain range of losses is acceptable.

Program introduction

- The eradication program **includes environmental assessment of the target farms, assessment and selection of breeding farms/breeding programs, implementation of the eradication program, monitoring of the program process, and eradication validation.**
- It takes 16 weeks from homogenous infection of herd to stable TTS and 37 weeks from homogenous infection of herd to . Thirteen plus months have passed since the start of the whole program.

Implementation of Eradication Strategy

1. Direct purchase of PRRSV naïve pigs to build herd - genetic loss of our own herd
2. Test - removal: suitable for stabilized small herds
3. Introduction – closure – homogenous infection
   - Build herds by introducing high-litter-production sows – farms do not have the required number of high-litter-production sows

- Gilts introduced to farms
  - Wild virus/vaccine?

Wild virus/vaccine : make decisions in light of eradication cycle and economic losses

Wild virus

Estimated economic losses： 95,784RMB

TTS[1]： 26.3 weeks

Cost1.Medicine=16.9 RMB/head*1500 head=25,350RMB

Cost2.Death： vaccine + feed cost before death=70,434RMB

- Cost of weaning: 420RMB
- Weight of dead weaners : 7KG
- Average weight of dead pigs: 21 KG
- Weight of weaners – new added weight before death: 14KG (21KG – 7KG)
- FCR（Weight of weaners – new added weight before death）: 1.96
- FCR before death: 27.44KG (14KG x 1.96 FCR)
- Unit price of nursery feed: 4.58RMB/KG（freight included）
- Feed consumption of dead pigs ： 126RMB/Head
- Losses caused by dead pigs: 546RMB/Head（126+420）
- No. of dead pigs=1500*8.6%=129 Head

Vaccine

Estimated input： 62,148RMB

TTS[1]： 32 weeks

Cost1.Medicine： 6.5RMB/Head*1500Head=9,750RMB

Cost2.Death： vaccine + feed cost before death=34,398元

- Losses caused by dead pigs: 546RMB/Head（126+420）
- No. of dead pigs=1500*4.2%=63Head

Cost3.Vaccine： 12RMB/Head*1500Head=18,000RMB

Specific parameters refer to the statistics of death and drug cost after the introduction of two batches pigs in Kui Qiang Farm
Refer to Luiz.2023 for the economic model

1. Linhares DC, Cano JP, Torremorell M, Morrison RB. Comparison of time to PRRSv-stability and production losses between two exposure programs to control PRRSv in sow herds. Prev Vet Med. 2014 Sep 1;116(1-2):111-9. doi: 10.1016/j.prevetmed.2014.05.010. Epub 2014 Jun 2. PMID: 24931129.

Eradication process of new farms： **4 critical juncture**+4 stages+7 topics

August 17, 2022
Juncture 1: First batch introduction

November 9, 2022
Juncture 2: PRRSV naïve pigs introduction + active infection of the whole herd

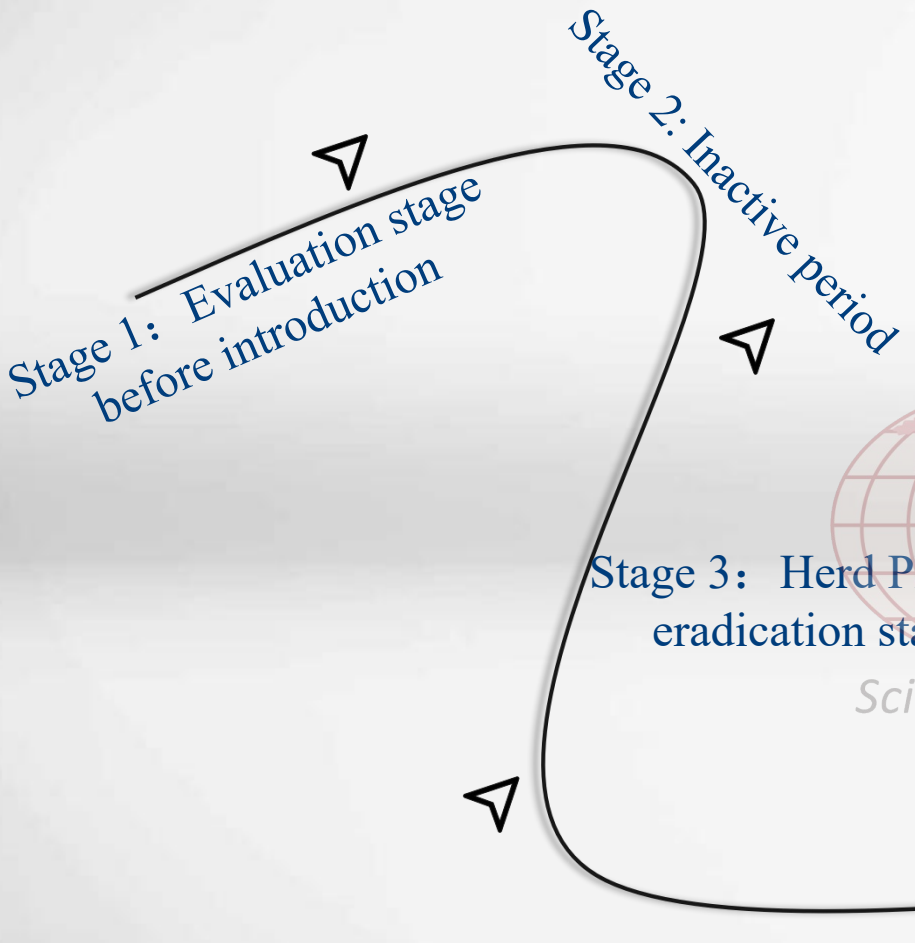
TTS=16th Week

March 8, 2023
Juncture 3： PRRSV status remained stable for the herd

21th week

August 15, 2023
Juncture 4: PRRSV naïve 6-week piglets

Eradication process of new farms： **4 stages+7 topics**



1. Evaluation of eradication capacity of target farm
2. Selection of farms where we introduce pigs and their batches
3. Reduce the death number of PRRSV positive piglets
4. Parameter setting for artificial active infection
5. Infection-eradication PRRSV infection dynamics & surveillance protocols
6. Biosafety optimization
7. Eradication evaluation

Stage 1：Evaluation stage before introduction

Objective：capabilities for eradication?

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Parameterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

PRRSV Risk Assessment

1. Environmental assessment
2. Climate assessment
3. Biosafety assessment

- Distribution of surrounding pig farms
- Vegetation coverage around pig farms
- Terrain of pig farms
- Proportion of 4-10℃ weather in the whole year in the area where the pig farm is located
- Proportion of smoggy days
- Frequency of recent pig movements on the public trunk road
- Whether there is an air filter device
- Frequency of pigs moving outside
- Frequency and method of gilt introduction
- …

Moeller J, Mount J, Geary E, Campler MR, Corzo CA, Morrison RB, Arruda AG. Investigation of the distance to slaughterhouses and weather parameters in the occurrence of porcine reproductive and respiratory syndrome outbreaks in U.S. swine breeding herds. Can Vet J. 2022 May;63(5):528-534. PMID: 35502250; PMCID: PMC9009730.
Andréia G Arruda, Nannong Pig Industry Conference, 2022
Sanchez F, Galvis J A, Cardenas N, et al. Spatiotemporal relative risk distribution of porcine reproductive and respiratory syndrome virus in the southeastern United States[J]. arXiv preprint arXiv:2301.05774, 2023.
Galvis JA, Corzo CA, Machado G. Modelling and assessing additional transmission routes for porcine reproductive and respiratory syndrome virus: Vehicle movements and feed ingredients. Transbound Emerg Dis. 2022 Sep;69(5):e1549-e1560. doi: 10.1111/tbed.14488. Epub 2022 Mar 3. PMID: 35188711; PMCID: PMC9790477.

- The distance of the target farm to the surrounding farms is 2.78, 4.39 and 7.44 km, respectively

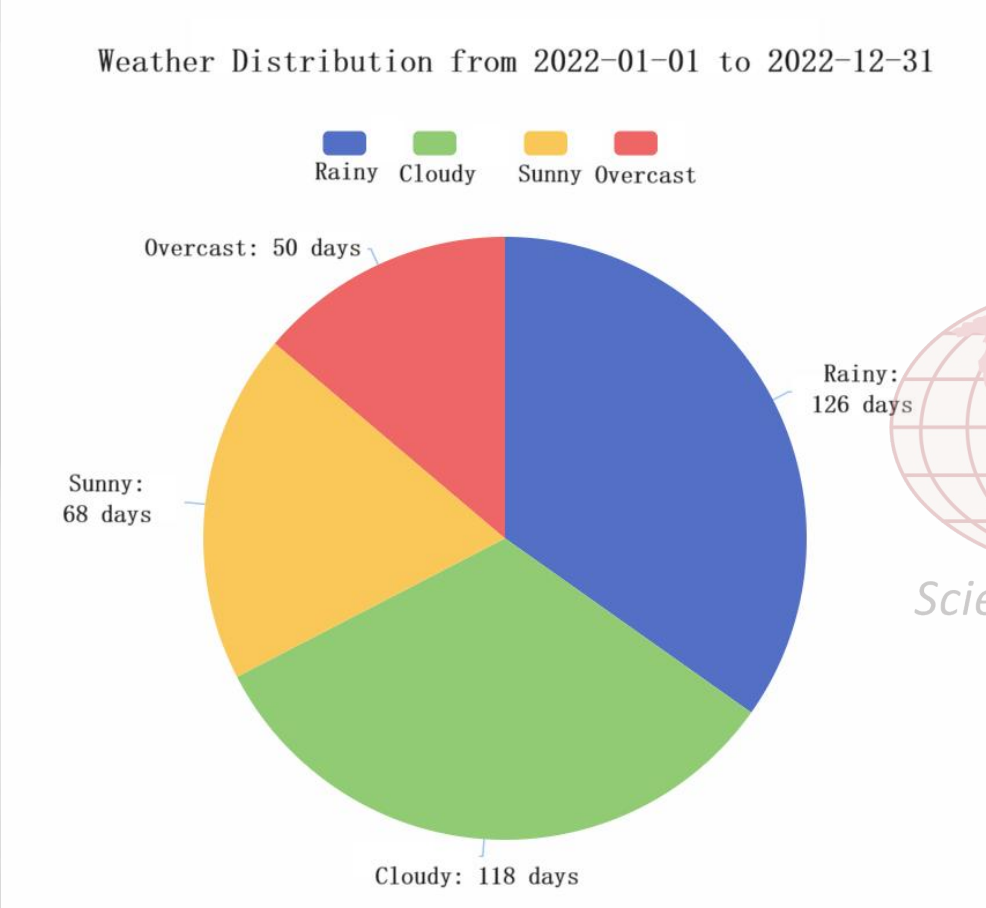
Vegetation coverage around pig farms

About 1 km from the nearest public trunk road, trees are straight in front of the farms;
The farm air outlet faces the trunk road

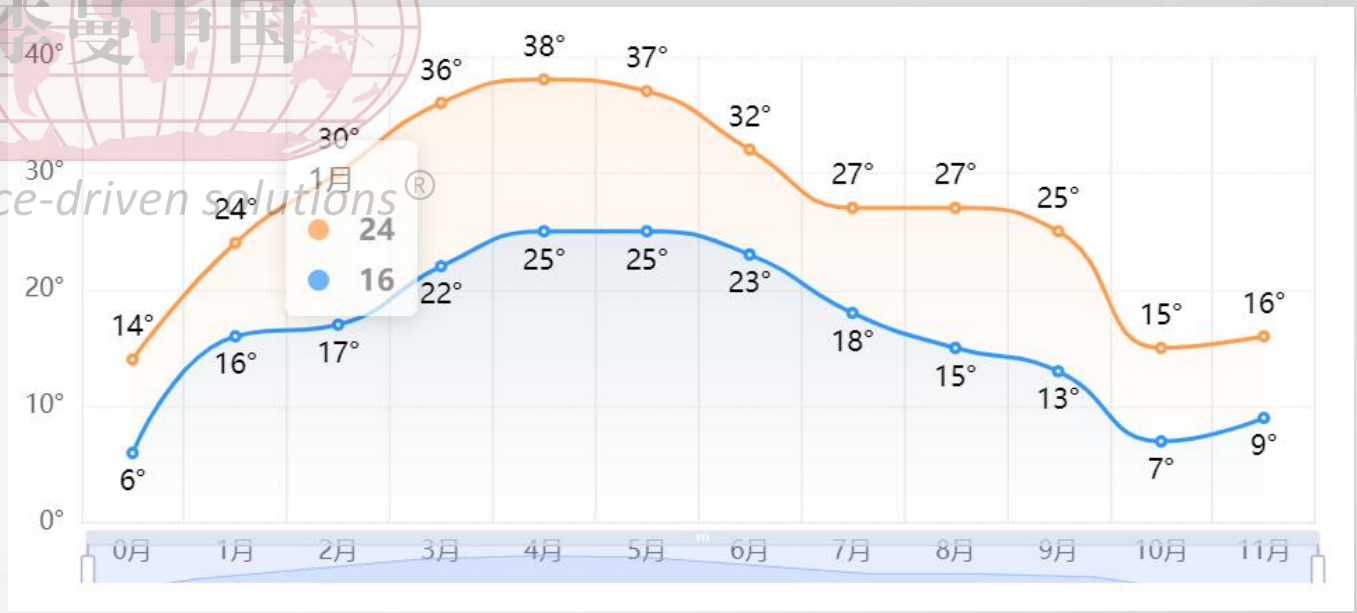
The target farm is on the hillside, level with the trunk road and the nearest pig farm

- Newly renovated farm
- One-site
- Farrowing to slaughtering
- 3 weeks/batch
- 2400 heads
- Independent boar stud inside

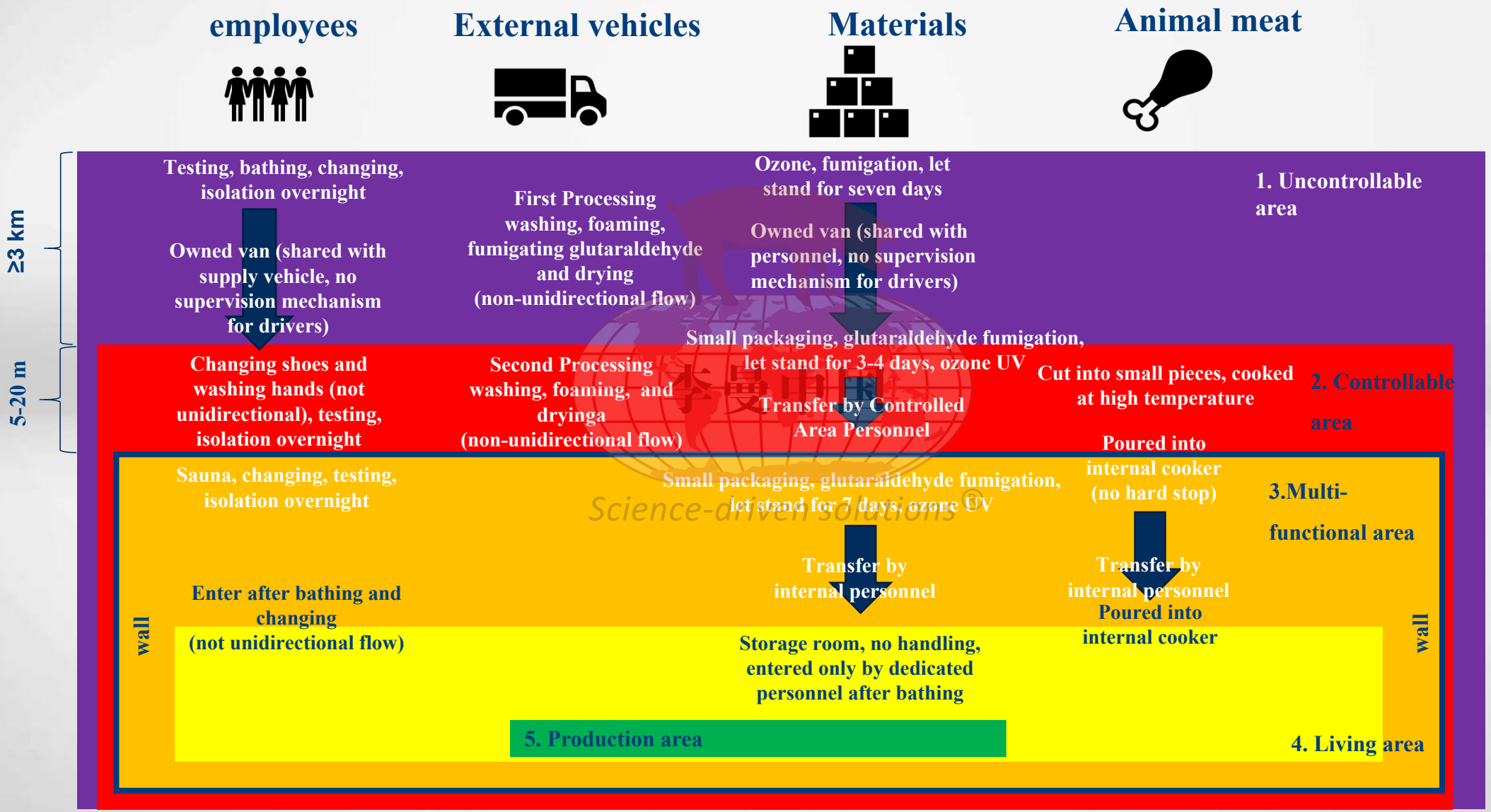
Almost no sandstorm or haze; Wind speed Class 1- light air; < 10℃ less than 3 months



Average high temperature	Average low temperature	Average wind speed	Average visibility
27℃	16℃	4.1(km/h)	21.9(km)
Extreme high temperature	**Extreme low temperature**	**Average daily air index**	**Total rainfall**
41℃ (07/13)	-2℃ (12/17)	31 Good	1620.7(mm)



There is still room to improve biosecurity level of outside the farm



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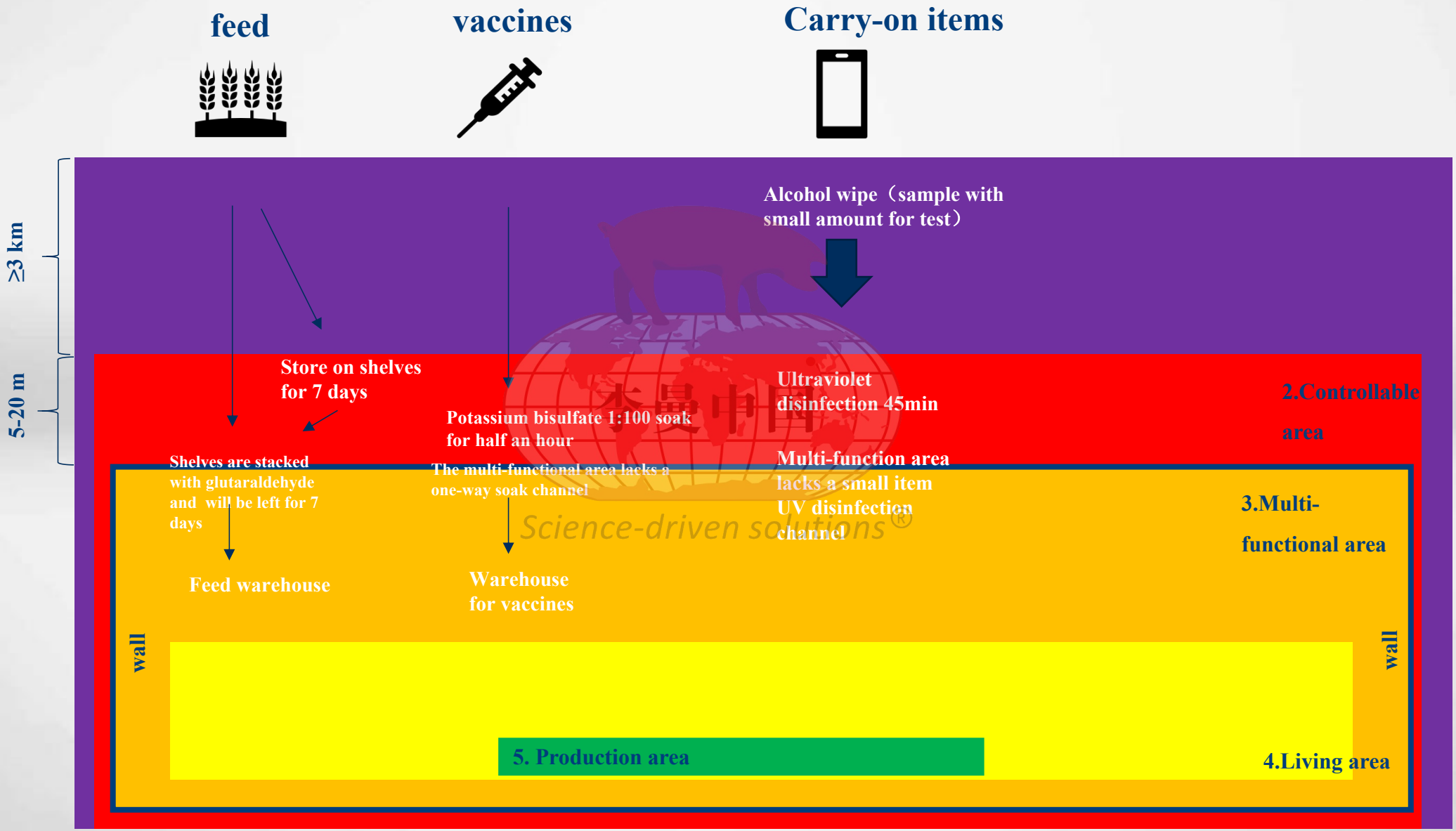


Quick review of evaluation of PRRSV eradication capacity

- The newly built introduced farm enjoys a relatively ideal location with few pig farms in the surrounding areas and natural barriers between pig farms
- The weather conditions in the area are good with breeze and properly high temperature. There is almost no physical carrier of virus such as haze. Therefore, PRRSV is relatively easy to inactivate.
- There is still room to improve biosecurity level of outside the introduced farm, but the plans are always implemented in a timely and forceful manner. So, we have more opportunities to unleash the potential for upgrading the system.

Juncture 1: Batch introduction

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Characterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

Principle of selecting farms where we introduce pigs and their batches:

reduce the diversity of introduced strains

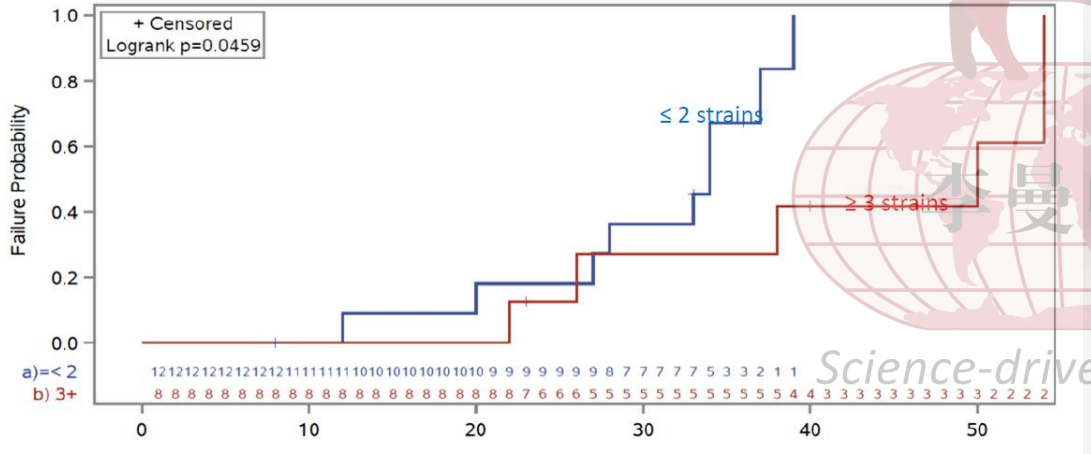
Objective: reduce the death rate of introduced herd and shorten the eradication period

- Negative farm＞positive stable farm＞positive unstable farm
- few types of strains＞many types of strains
  - Introduction to single farm＞Introduction to multi-farms
  - Single-batch introduction＞multi-batch introduction

- Pileri E, Mateu E. Review on the transmission porcine reproductive and respiratory syndrome virus between pigs and farms and impact on vaccination. Vet Res. 2016 Oct 28;47(1):108. doi: 10.1186/s13567-016-0391-4. PMID: 27793195; PMCID: PMC5086057.
- Moura CAA, Philips R, Silva GS, Holtkamp DJ, Linhares DCL. Comparison of virus detection, productivity, and economic performance between lots of growing pigs vaccinated with two doses or one dose of PRRS MLV vaccine, under field conditions. Prev Vet Med. 2022 Jul;204:105669. doi: 10.1016/j.prevetmed.2022.105669. Epub 2022 May 13. PMID: 35594607.
- Daniel Linhares.et.al. Managing PRRSV outbreak.2023

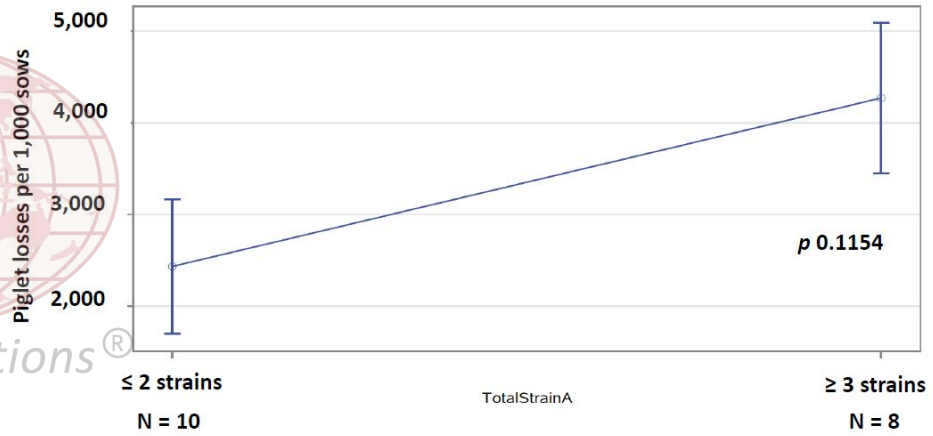
The more diverse the strain is, the more the losses and the longer the stable period are

Farms with ≥ 3 strains reached low prevalence 12 weeks after farms with ≤ 2 PRRSV strains



Differences in 12 weeks

Farms with ≤ 2 PRRSV strains detected had 1,837 fewer piglet losses/1,000 sows than farms with ≥ 3 PRRSV strains



1837 weaners /1000 sows

Trevisan, *et al*. 2022, Transboundary and Emerging Diseases

Breeding farm A	20220514
Testicular processing fluid	
Preimmune piglets	MLV
6 weeks of age	MLV
9 weeks of age	FALSE
12 weeks of age	HP-PRRS
Gilts	

Breeding farm B	20220514
Testicular processing fluid	
Preimmune piglets	MLV
6 weeks of age	MLV
9 weeks of age	HP-PRRS
12 weeks of age	HP-PRRS
Gilts	

Status of PRRSv and Diversity of Virus Strains at Breeding Farms

Test results in May 2022: Positive Stable With Vaccination (II-Vx) for all breeding farms

Query?

1. Sources of viruses in late nursery period?
2. Will MLV affect the sequencing results of field strains?
3. Can a single test determine the PRRSv class/status of a herd?

Improved testing plan:

1. Increase sample size of piglets in weaning period and increase the estimated prevalence from 5% to 2-3%
2. Delay MLV immunization: from 14 days of age to post-weaning
3. Transform random sampling into risk-based sampling
4. Sequencing for all whose CT≤32
5. Monitor 3 batches continuously

Three Batches of 1,500 Weaned Gilts Were Introduced Continuously From the Breeding Farm A with Low PRRSv Diversity

Breeding farm A	20220514	20220612	20220626	20220725	20220813
Testicular processing fluid		FALSE		IP-PRRS(25%）	
Preimmune piglets	MLV	HP-PRRS 25%	HP-PRRS(17%)	HP-PRRS(14%)	
6 weeks of age	MLV	MLV	LSE		
9 weeks of age	FALSE	HP-PRRS	HP-PRRS		
12 weeks of age	HP-PRRS	FALSE	FALSE		
Gilts					

Breeding farm B	20220514	20220612	20220626
Testicular processing fluid			
Preimmune piglets	MLV	MLV	HP-PRRS(46%)
6 weeks of age	MLV	QYYZ HP-RRS	MLV
9 weeks of age	HP-PRRS	SE	HP-PRRS
12 weeks of age	HP-PRRS	MLV	HP-PRRS
Gilts			

Breeding farm A

- Positive Unstable Low Prevalence（I-B）
- Single strain diversity：HP-PRRS

Breeding farm B

- Positive Unstable High Prevalence（I-A）
- High strain diversity
  - HP-PRRS
  - QYYZ

PRRSV等级划分参考AASV2020

Measures to Reduce Infection Pressure in Breeding Pig Supply Batches

Immunization/drug schedule adjustments: **Reduce the potential of PRRS MLV introduction into breeding sites**

- Piglets: adjust the immunization time of Ingelvac® PRRS MLV to post-weaning immunization, mixed with twistpak
- Sows: stop immunization of Ingelvac® PRRS MLV

Biosecurity optimization plan: **Reduce field strain diversity and infection pressure of pigs in farrowing house**

- Strictly prevent cross-fostering and no longer foster piglets after 48 hours (suckling piglets of target batches)
- Personnel management: Nursery house personnel are not allowed to enter farrowing house and mating and pregnancy house; Minimize the movement of personnel in farrowing house
- Strengthen material management: vaccine trolleys, pig stoppers, etc., and sterilize them well to reduce the risk of cross-contamination of tools
- Reduce pig stress before and after farrowing (medication and operation)
- Set up separate farrowing house for gilts
- Only introduce piglets born from multiparous sows
- Suspend entry of gilts into herds (especially after the target batch is in late gestation)

Thanks for the support of Mr. Xia Tian from PIC

Summary

Optimize testing plans to better understand PRRSv status/grade of breeding farms

- PRRSv Positive Stable (II) farm, or there is a certain probability of low prevalence, need to increase the sample size and change to a risk-based sampling method
- The success of wild-virus sequencing is affected in the short term after immunization with live PRRSv vaccines, and sampling for sequencing is recommended prior to immunization
- Increase sequencing volume to better understand the diversity of on-farm strains
- Choose an introduced site/batch with a single diversity of strains

Restrictive factors

- On-farm prevalence of HP-PRRS, not immune to MLV vaccine, rise in piglet mortality

Stage 2: Inactive period

- Slow the transmission of PRRSV in the herd
  - Reduce secondary bacterial infections

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Parameterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

Main objectives for each stage

PRRSV surveillance

Stage 1：before introduction	Stage 2： During introduction	Stage 3： after introduction-before artificial infection
Objectives	Objectives	Objectives
• Reduce the proportion of pigs infected with PRRSV • Reduce the diversity of PRRS strains	• Prevent/monitor potential infection of exogenous pig diseases during transport	• Slow the transmission of PRRSV • Reduce the death rate

Methods and Principles of Implementation

1. Control positive rate in lactating piglets of introduced batches
2. Increase feeding area
3. Environmental control & stress management
4. Reduce the flow of people & materials across units
5. Isolation of common pathogenic bacterium&drug susceptible test for sensitive drug screening
6. Inject sensitive antibiotics promptly

Phase I: Before Introduction

- Purpose:
- Mitigating infection rates in pre-starter through internal biosecurity
- Knowledge of prevalent strains and herd prevalence of transfer batches

1.1 Management of farrowing house

- Transfer piglets at 25 days of age
- Introduced batches should farrow within 3 days
- Abondon 6 piglets born from introduced gilts among the first batch

1.2 Drugs and vaccines (purpose: to prevent secondary infections between 4-10 weeks of age, mainly for common secondary bacteria in nursery period)

- Follow normal health care procedures at breeding farms
- No immunization of live PRRS vaccine to transfer piglets

1.4 Laboratory monitoring (purpose: to understand the prevalence and circulating virus strains the pig herds before transfer, and to find infectious disease materials)

- Testicular fluid from piglets of 3-5 days of age , full coverage, one sample per building
- Sampling at 21 days of age: Requirements: castrated boars, 60 samples/batch, one boar per litter, covering all farrowing houses in the batch, marking the corresponding farrowing house number and pen number, and marking the sampled piglets for the production of disease material.

Thanks for the support of Mr. Xia Tian from PIC

Phase II: The Introduction Process

Purpose:

- Preventing exogenous pathogens from entering the herd at the transfer stage

- Transfer pigs early in the morning and avoid transfer during the rainy season. Try to separate piglets produced by gilts and sows in transfer process from the farrowing houses at breeding farms to nursery houses at introduced farms
- After cleaning, disinfection and drying, third-party pig transfer trucks should be tested for ASF, PEDV, and PRRS antigens (This can be tested locally)
- On transfer trucks' arrival at breeding farms, use 1:50 Virkon to disinfect the trucks' tire tailgate with low-pressure spray, and wait for 5-10 minutes after disinfection.
- On transfer trucks' arrival at introduced farms, samples from tires are collected before disinfection and tested for PRRS , ASF and PEDV. (This can be tested locally)
- Piglets produced by gilts and sows should be transferred seperatly.

Thanks for the support of Mr. Xia Tian from PIC

Phase III: After Introduction - Before Artificial Infection

Purpose:

- Slow down PRRS infection in nursery pigs and reduce PRRSv-induced secondary infections in herds through strict internal biosecurity and production management.
- Track herd infection status and prevalent strains through regular surveillance

3.1 Production Management

- Piglets born from gilts and sows of the same batch should be raised in separate buildings
- Different batches cannot be mixed and housed together
- No sharing of personnel and materials between pig houses and batches if not necessary
- If necessary, operate sow houses before gilt houses

3.2 Pharmaceutical health care

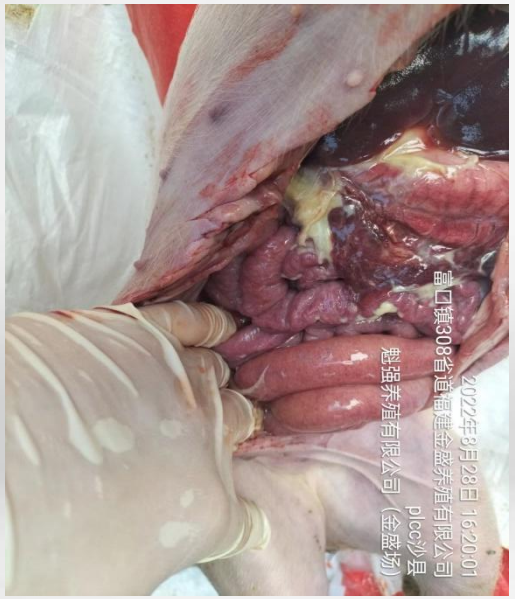
- Day 1 of arrival at new farms: offspring of gilts should be injected with 1 dose of macrolide antibiotics. Other pigs can be injected as needed
- Pulse feeding/water medication: florfenicol + tylvalosin + doxycycline. After stopping the medication for one week, add for another week.
- Adjust medication according to clinical symptoms

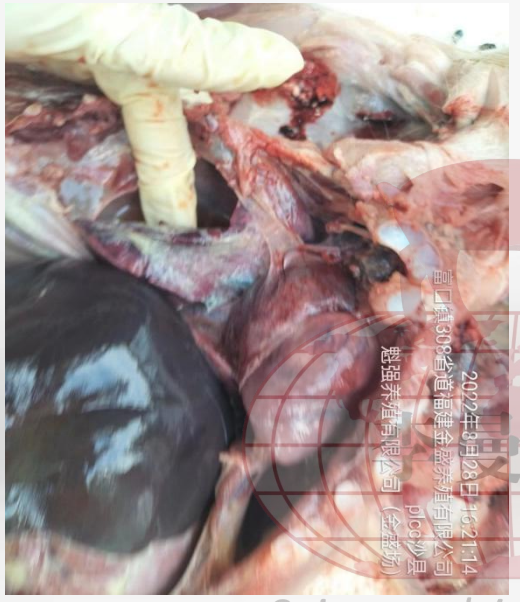
3.3 Monitoring

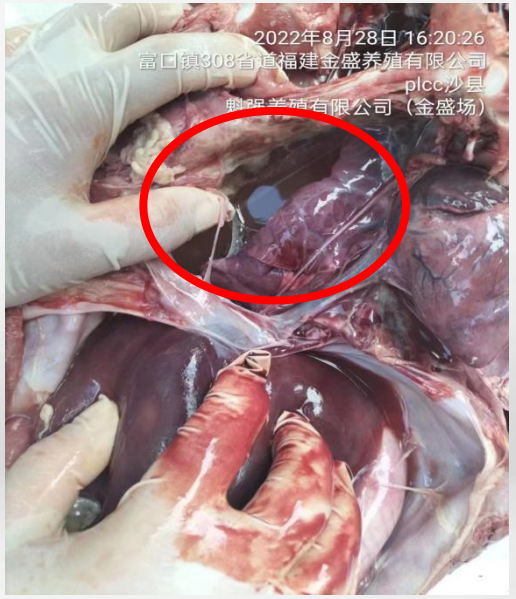
- Sampling type: saliva sampling, (≤ 15 pigs/cotton rope)
- Number of samples: collected from the whole herd
- Monitoring frequency: 2 weeks/times
- Sampling time: about 30 minutes/time

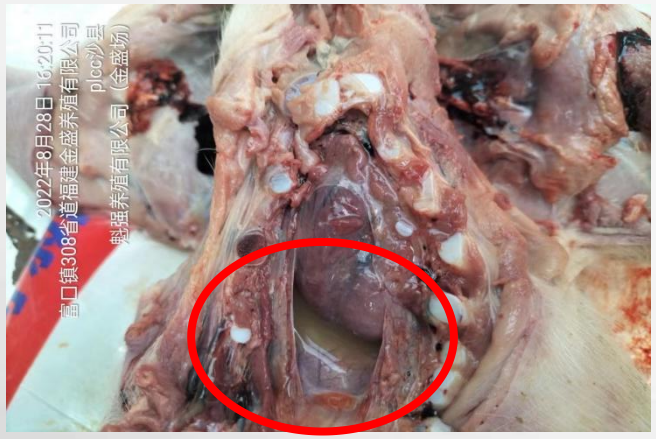
Thanks for the support of Mr. Xia Tian from PIC

Anatomical sampling of 1 batch of sick and weak pigs for identification









Bacterial isolation and antimicrobial susceptibility test

Sample Type	Sample No.	Result	Pathogenicity
pericardial effusion	A1	Lactobacillus rhamnosus	-
		Pseudomonas fluorescens	-
pericardial effusion	A2	Bacillus licheniformis	-
		Bacillus subtilis	-
		Rothia nasimurium	-
		Kurthia gibsonii	-
		Enterococcus faecalis	-
		Aerococcus viridans	-
		Corynebacterium stationis	-
		Staphylococcus epidermidis	-
		Kocuria	-
joint exudate	B1	Kocuria	-
joint exudate	B2	Streptococcus suis	pathogenic
plasma	C1	Streptococcus suis	pathogenic
plasma	C2	Rothia nasimurium	-
fibrous exudate	D1	NA	NA
fibrous exudate	D2	Aerococcus viridans	-
		Bacillus licheniformis	-
		Vagococcus fluvialis	-
		Corynebacterium stationis	-
tissue	E1	NA	NA

Three samples were collected:

- Pericardial effusion, joint exudate, blood
- Fibrous exudate swab
- lesion tissue

- Identification of bacterial isolates: Streptococcus suis & Haemophilus parasuis
- The antimicrobial susceptibility test showed that β-lactam antibiotics were more sensitive to the above two bacteria.

Acknowledgments: Support from Boehringer Ingelheim HMC

Batch 1: 0/21 detected at 3 weeks of age, 21/21 detected at 11 weeks of age

Layout of B8 house

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7
door	corridor						

3 weeks of age

Layout of B9 house

	Pen 1	Pen 3	Pen 5	Pen 7	Pen 9	Pen 11	Pen 13
door	corridor						
	Pen 26	Pen 24	Pen 22	Pen 20	Pen 18	Pen 16	Pen 14

Layout of B8 house

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7
door	corridor						

5 weeks of age

Layout of B9 house

	Pen 1	Pen 3	Pen 5	Pen 7	Pen 9	Pen 11	Pen 13
door	corridor						
	Pen 26 (29)	Pen 24	Pen 22	Pen 20	Pen 18	Pen 16	Pen 14

Layout of B8 house

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7
door	corridor						

7 weeks of age

Layout of B9 house

	Pen 1	Pen 3	Pen 5	Pen 7	Pen 9	Pen 11	Pen 13
door	corridor						
	Pen 26 (29)	Pen 24	Pen 22	Pen 20	Pen 18	Pen 16	Pen 14

11 weeks of age

Layout of B8 house

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7
door	corridor						

Layout of B9 house

	Pen 1	Pen 3	Pen 5	Pen 7	Pen 9	Pen 11	Pen 13
door	corridor						
	Pen 26 (29)	Pen 24	Pen 22	Pen 20	Pen 18	Pen 16	Pen 14

9 weeks of age

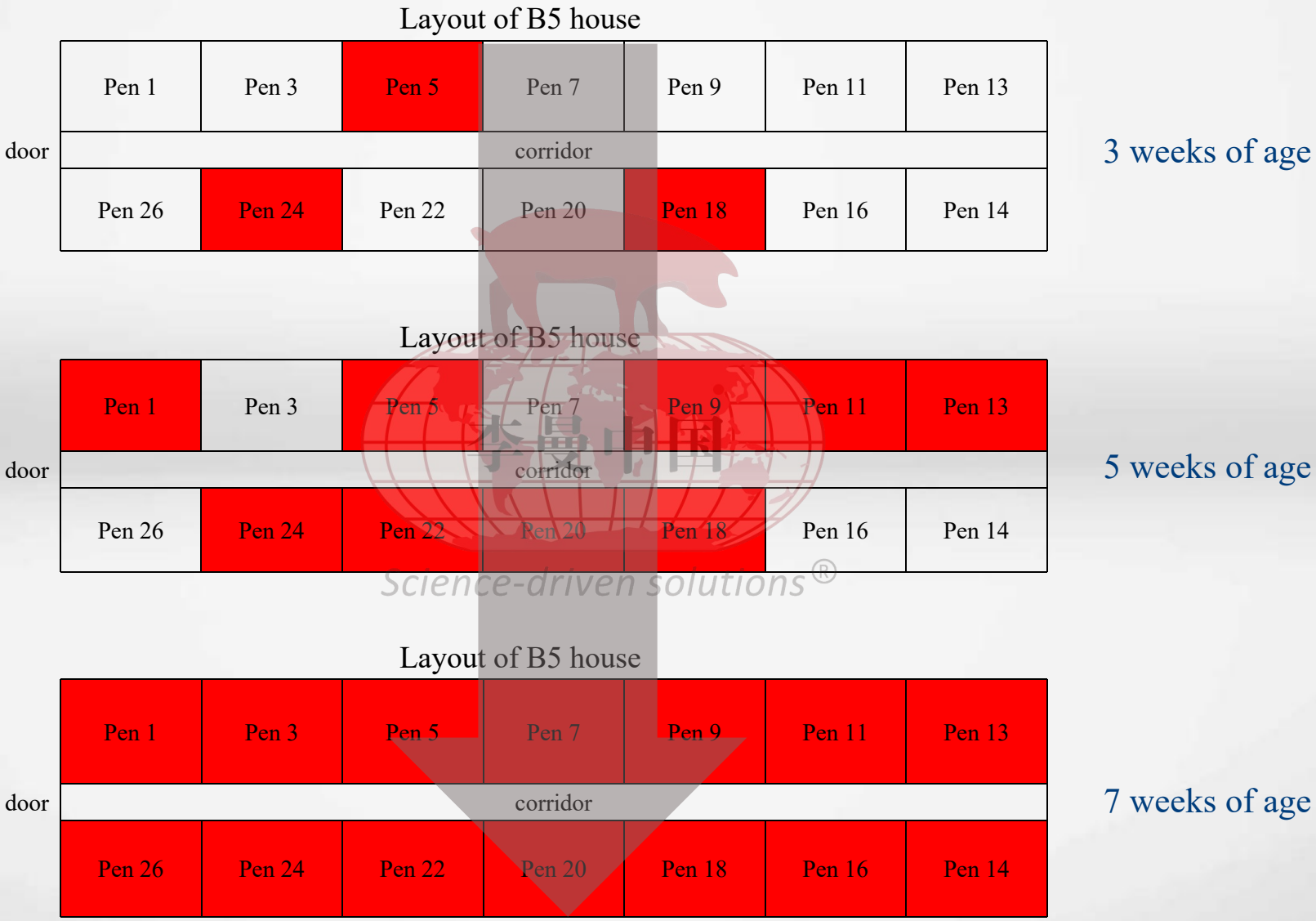
Layout of B8 house

	Pen 1	Pen 2	Pen 3	Pen 4	Pen 5	Pen 6	Pen 7
door	corridor						

Layout of B9 house

	Pen 1	Pen 3	Pen 5	Pen 7	Pen 9	Pen 11	Pen 13
door	corridor						
	Pen 26 (29)	Pen 24	Pen 22	Pen 20	Pen 18	Pen 16	Pen 14

Batche 2: 3/14 detected at 3 weeks of age, 14/14 detected at 7 weeks of age



Average space and medication cost

MLV immunized group: Death registration form for nursery batches at breeding farms

Batch	Weaning date	No. of weaned piglets(head)	Death and culling rate	Space(m²)	Medication cost per head	WOA
1	8.17	901	**6.1%**	**0.31**	**9.8 yuan**	**13**
2	9.7	884	**4.2%**	**0.27**	**6.5 yuan**	**10**
3	9.28	795	**2.8%**	**0.3**	**6.5 yuan**	**7**

MLV non-immunized group: Death registration form for nursery batches on introduced farms

Batch	Weaning date	No. of weaned piglets(head)	Death and culling rate	Space(m²)	Medication cost per head	WOA
1	8.17	569	**10.7%**	**0.4**	**20.7 yuan**	**13**
2	9.7	489	**8.6%**	**0.47**	**16.9 yuan**	**10**
3	9.28	405	**3.2%**	**0.38**	**8.0 yuan**	**7**

Notes:

Introduced farms are new farms, stocking density is much smaller than the breeding farms, treatment cost vary by 2-3 times

Selected pigs from the first batch of introduced breeding pigs to make pathogenic material

Summary

- Targeted control of the proportion of piglets introduced into the batch that are infected early can delay the infection time of whole herd.

- Positive piglets in non-immunized group have higher death and culling rate and medication costs than immunized group.

Juncture 2: Whole herd artificial infection

Purpose: to unify infection state, reduce susceptible animals, and make the eradication schedule clearer

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Parameterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

Current situation: Due to genetic improvement, **a number of naïve boars and sows need to be introduced urgently.**

Objective: minimize the number of susceptible animals, unify herd immunity, reduce time from infection to eradication

Issues to be addressed

1. Sources of acclimation material
2. Selection of acclimation material?
   - Serum? (Dilution times? Add antibiotics or not? What antibiotics and what is the dose?)
   - Tissue? (Preparation process?)
   - Contact transmission/high frequency mixing? (Pre-assessment of whether infected, 3:10 ratio of infections, post-assessment of infections)
3. Selection of strains (4-5% nucleotide variation in strains from the same farm)
4. Choice of infection mode
5. Selection of infectious dose

Finding the Right Pathogenic Material

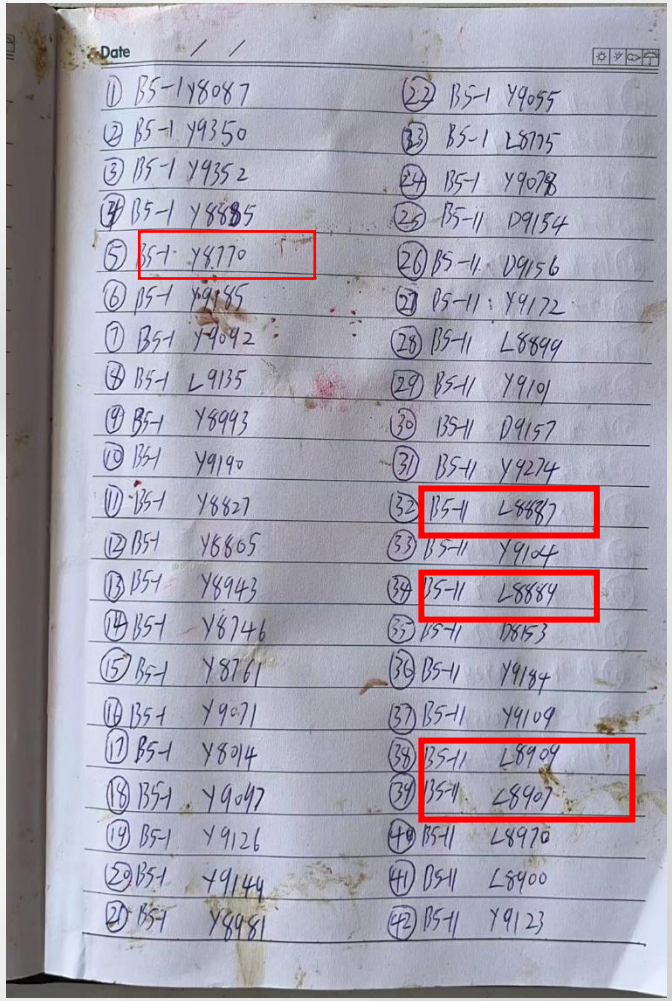
Step 1:
Primary screening of saliva fluid to screen pens with low CT values

No.	Sample No.	PRRSV-Ag (CTvalue)	Type
1	B1-1	36.88	2 batches of saliva
2	B1-3	31.92	2 batches of saliva
3	B1-24	-	3 batches of saliva
4	B1-26	36.45	3 batches of saliva
5	B5-1	32.68	saliva
6	B5-3	-	saliva
7	B5-5	37.12	saliva
8	B5-7	-	saliva
9	B5-9	37.18	saliva
10	B5-11	33.91	saliva
11	B5-13	35.91	saliva
12	B5-14	-	saliva
13	B5-16	-	saliva
14	B5-18	34.06	saliva
15	B5-20	37.84	saliva
16	B5-22	40.82	saliva
17	B5-24	37.68	saliva

Step 2:
Detecting viremia in pigs in the pen on a case-by-case basis

金盛1	-	FAM	金盛31	22.95	FAM
金盛2	33.98	FAM	金盛32	19.7	FAM
金盛3	27.44	FAM	金盛33	-	FAM
金盛4	-	FAM	金盛34	18.94	FAM
金盛5	19.93	FAM	金盛35	23.94	FAM
金盛6	25.66	FAM	金盛36	24.53	FAM
金盛7	23.29	FAM	金盛37	25.29	FAM
金盛8	-	FAM	金盛38	19.2	FAM
金盛9	27.15	FAM	金盛39	18.36	FAM
金盛10	29.66	FAM	金盛40	-	FAM
金盛11	34.28	FAM	金盛41	31.61	FAM
金盛12	29.48	FAM	金盛42	22.51	FAM
金盛13	28.44	FAM	金盛43	22.84	FAM
金盛14	-	FAM	金盛44	24.01	FAM
金盛15	24.4	FAM	金盛45	20.28	FAM
金盛16	-	FAM	金盛46	-	FAM
金盛17	24.4	FAM	金盛47	23.15	FAM
金盛18	29.72	FAM	金盛48	-	FAM
金盛19	23.81	FAM	金盛49	31.87	FAM
金盛20	-	FAM	金盛50	-	FAM
金盛21	-	FAM	金盛51	21.71	FAM
金盛22	27.16	FAM	金盛52	27.23	FAM
金盛23	26.57	FAM	金盛53	21.32	FAM
金盛24	-	FAM	金盛54	23.25	FAM
金盛25	26.88	FAM	金盛55	23.29	FAM
金盛26	33.59	FAM	金盛56	27.03	FAM
金盛27	-	FAM	金盛57	33.71	FAM
金盛28	23.57	FAM	金盛58	-	FAM
金盛29	27.18	FAM	金盛59	30.91	FAM
金盛30	30.39	FAM	金盛60	37.6	FAM

Step 3:
Screening of culled pigs



Selection of acclimation strains: the two most distantly related strains were selected as acclimation strains.

ORF5-based sequencing

13		
98.0	1	41-ORF5
97.0	2	9-15SanguB6-2-ORF5
98.0	3	9-15SanguB6-3-ORF5
98.0	4	9-15SanguB6-4-ORF5
97.0	5	9-15SanguB7-13-ORF5
96.0	6	9-15SanguB7-14-ORF5
97.0	7	9-15SanguB7-16-ORF5
95.0	8	9-15SanguB7-26-ORF5
97.0	9	9-15SanguY8312-ORF5
96.0	10	9-15SanguY8640-ORF5
96.0	11	9-2SanguB31-35-ORF5
97.0	12	9-15SanguY8655-ORF5
[redacted]	13	B50-ORF5
13		

ORF7-based sequencing

8		
93.3	1	0902SanguB31-35-ORF7
97.5	2	0922A2-ORF7
97.5	3	0922B26-30-ORF7
96.7	4	0922C5-ORF7
95.8	5	0929JinshengB6-1-ORF7
95.8	6	0929JinshengB7-14-ORF7
95.8	7	9-12SanguB9-18-ORF7
[redacted]	8	0902SanguB50-ORF7
8		

Lot 1: 2 ORF5s

Lot 2: 5 ORF5s

Lot 3: 1 ORF7

Theoretical Basis of Infection Route and Dose

Neutralizing antibodies were higher for nasal immunization than for intramuscular injection in naïve pigs infected for the first time

Immunization dose 4.8 Log^{10} $TCID_{50}$

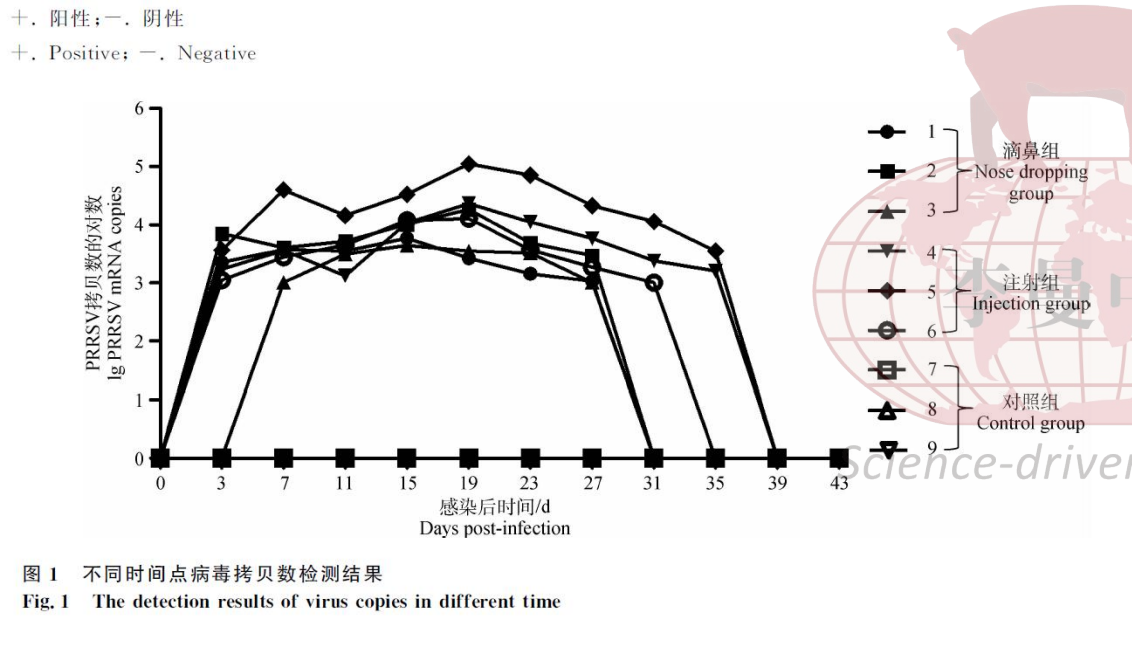


图 1　不同时间点病毒拷贝数检测结果

Fig. 1　The detection results of virus copies in different time

viremia titer

- Regardless of the route of infection, viremia was detected 3 days after infection in 5/6 pigs
- However, viremia in intramuscular injection group are 10-100 times higher than in nose dropping group

Tip: Newly introduced naïve pigs are better infected by nose dropping from the point of view of effectiveness

1. Opriessnig T, Rawal G, McKeen L, Filippsen Favaro P, Halbur PG, Gauger PC. Evaluation of the intranasal route for porcine reproductive and respiratory disease modified-live virus vaccination. Vaccine. 2021 Nov 16;39(47):6852-6859. doi: 10.1016/j.vaccine.2021.10.033. Epub 2021 Oct 24. PMID. 34706840.
2. LI Wen, MA Sixu, LI Xiangtong, SUN Yangyang, WEI Fengling, XU Ruiqin, YANG Guoyu, XIA Ping, ZHANG Gaiping, The Variance Analysis of Viremia and Antibody in Piglets Vaccinated against PRRSV VR2332 Attenuated Strain via Nasal Drip and Injection. 2019, Vol. 50 Issue (2): 373-381. DOI: 10.11843/j.issn.0366-6964.2019.02.015

Theoretical Basis of Infection Route and Dose

For infected pigs, the intramuscular dosage of the second immunization should follow the principle of small doses.

TABLE 3. Incidence of PRRSV genomic copies in serum on day of PRRSV challenge and days 7 and 14 postinoculation

Group	Incidence (no. of PRRSV-positive pigs/total no. of pigs in group [group mean of log-transformed data ± SE]) on postinoculation day[a]:		
	0	7	14
L-VAC	0/10 (0.0 ± 0.0)	2/10 (0.6 ± 0.5)*	0/10 (0.0 ± 0.0)*
H-VAC	0/10 (0.0 ± 0.0)	0/10 (0.0 ± 0.0)*	0/10 (0.0 ± 0.0)*
SDSU73-L-VAC	1/10 (0.2 ± 0.2)	1/10 (0.3 ± 0.3)*	0/10 (0.0 ± 0.0)*
SDSU73-H-VAC	0/10 (0.0 ± 0.0)	0/10 (0.00 ± 0.0)*	0/10 (0.0 ± 0.0)*
Control	0/10 (0.0 ± 0.0)	8/10 (3.9 ± 0.7)**	8/10 (2.0 ± 0.5)**

[a] Different symbols (* and **) within a column indicate significant ($P < 0.001$) differences in group mean values and incidences.

Tip:

1. In the case of uninfected herds, small doses of intramuscular injection is insufficient to elicit a strong immune response: that's why infected pigs have to be acclimated again.
2. In infected pigs, the protection against reinfection is not dose-dependent.

Compared to the control group and the low vaccine dose group, the high vaccine dose (IM) group, the low dose challenged group, and the high dose challenged group had mild clinical symptoms and significantly lower viremia (2.0 $TCID_{50}$ in the low dose group and 5.0 $TCID_{50}$ in the high dose group), all of which were administered intramuscularly.

Opriessnig T, Baker RB, Halbur PG. Use of an experimental model to test the efficacy of planned exposure to live porcine reproductive and respiratory syndrome virus. Clin Vaccine Immunol. 2007 Dec;14(12):1572-7. doi: 10.1128/CVI.00332-07. Epub 2007 Oct 10. PMID: 17928430; PMCID: PMC2168385.

Theoretical Basis of Infection Route and Dose

Oral route of exposure:
$10^{5.3}$ $TCID_{50}$ (95% CI, $10^{4.6}$ and $10^{5.9}$)

Intranasal route of exposure:
$10^{4.0}$ $TCID_{50}$ (95% CI, $10^{3.0}$ and $10^{5.0}$)

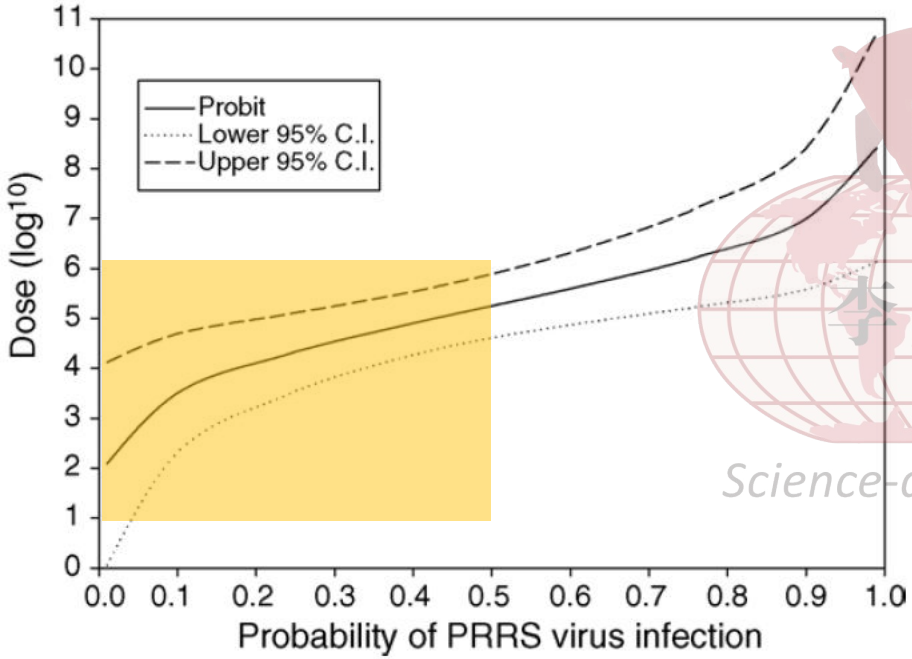


Fig. 1. Dose response curve for oral route of exposure.

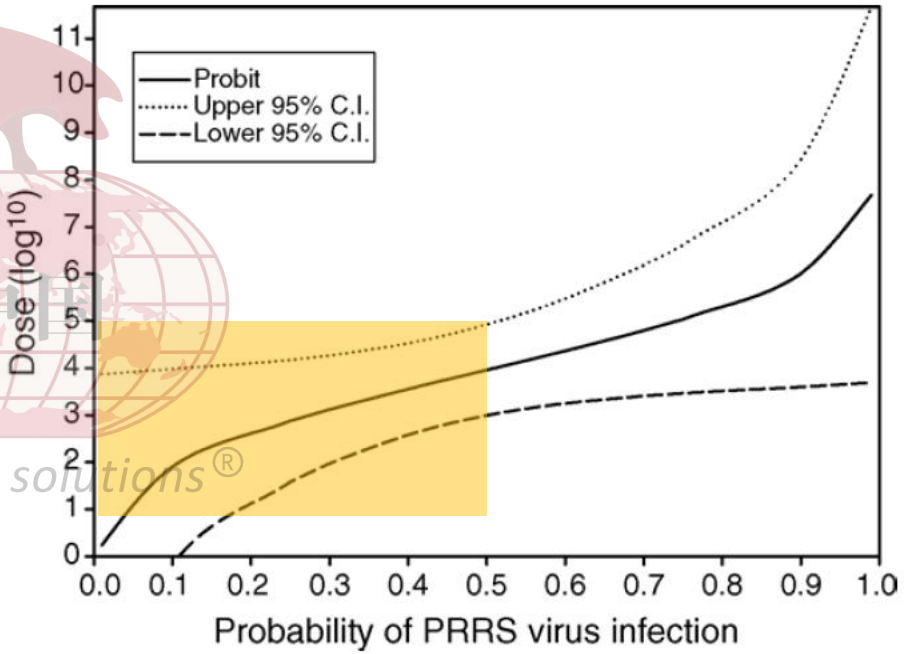


Fig. 2. Dose response curve for intranasal route of exposure.

Hermann JR, Muñoz-Zanzi CA, Roof MB, Burkhart K, Zimmerman JJ. Probability of porcine reproductive and respiratory syndrome (PRRS) virus infection as a function of exposure route and dose. Vet Microbiol. 2005 Sep 30;110(1-2):7-16. doi: 10.1016/j.vetmic.2005.06.012. PMID: 16098692.

Preparation of acclimation materials

- naïve herds: nasal spray, use lung tissue homogenate filtrate, infection dose 3.25-4.25 Log^{10} $TCID_{50}$
- Positive herds: intramuscular injection, using serum dilutions, infection dose approx. 2 Log^{10} $TCID_{50}$
- Naïve pig herds: pilot experiment using 10% small groups

Preparation of acclimation materials[1] (Preparation of diluent and acclimation materials)

1. Diluent preparation: 250 ml sterile saline + 1g ceftiofur as diluent; saline is kept refrigerated (4℃) in advance.
2. Lung milling fluid acclimation material preparation: the whole preparation process was carried out in 4 ℃.
3. B50 lungs were weighed, divided into small pieces and added to the 4 ℃ diluent at 2 ml/g; together they were placed in a tissue homogenizer, stirred well, and filtered through three layers of sterilized gauze, and then filtered through filter paper (solution cups) and placed on ice for later use.
4. Serum was diluted at a ratio of 1:100 with 4 ℃ diluent for later use.
5. Required items and tools: 5L of saline (1500 heads * 2ml serum = 3L of saline; 3000ml / 100-fold dilution = 30ml of viremia serum); 20g of ceftiofur powder; 100ml measuring cylinder; sterile scalpel and scissors, tissue homogenizer, weighing scale (for weighing lung tissues), sterile gauze, 100 centrifuge tubes of 50ml, equipment for sterilization (e.g., pot and stove for boiling and disinfection), ice box and ice
6. 2 acclimation materials, prepared in the morning, used in the morning, stored at 4 ℃ throughout the process

1. Opriessnig T, Baker RB, Halbur PG. Use of an experimental model to test the efficacy of planned exposure to live porcine reproductive and respiratory syndrome virus. Clin Vaccine Immunol. 2007 Dec;14(12):1572-7. doi: 10.1128/CVI.00332-07. Epub 2007 Oct 10. PMID: 17928430; PMCID: PMC2168385.

Pre-testing of pathogenic material treatment

Pathogenic material preparation for gilt acclimation

Health programs during the acclimation period

1. Infected herds (batch 1, 2 and 3 pigs)
   - Drinking water health program: amoxicillin + tilmicosin + multivitamins; amoxicillin dosed according to body weight (kg): 10mg effective content of compound amoxicillin/kg body weight + 30% of wasted drinking water.
   - Injectable therapy: antimicrobial susceptibility tests recommend using long-acting ceftiofur.
   - Have epinephrine on hand if pigs have stress reaction
2. Newly introduced naïve pigs were infected twice
   - Injection of macrolide antibiotics on the same day after acclimation with wild virus
   - Health care for the whole herd on the day of infection using amoxicillin, multivitamins, sulfa and antipyretics in drinking water for 5-7 days
   - Symptomatic treatment to pigs with obvious clinical symptoms, early detection and early treatment, antimicrobial susceptibility tests recommend the use of long-acting four-generation ceftiofur
   - Have epinephrine on hand if pigs have stress reaction

Death and culling rate within 4 weeks of acclimation: (13+4)/1533=1.1%

Batch	Week of age of acclimation	Number of dead + culled pigs/pigs in stock within 4 weeks of infection
batch 1	16	(6+0)/569
batch 2	13	(6+2)/489
batch 3	10	(1+0)/405
Introduction of naïve pigs	24	(0+2)/70

Stage 3: Herd eradication period

Purpose: To track the progress of eradication and intervene and control in a timely manner

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Parameterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

Monitoring program:

Purpose: 1. to determine whether infection was successful; 2. infection rate; 3. whether shedding was stopped

Reinfection of the herd

1. Determine if infection is successful: do nnucleic acid test 1 week post-infection by collecting whole herd saliva
2. Determination of infection rate: do antibody test 3 to 8 weeks post-infection by selecting 4 pens of pigs per batch weekly
3. Determine if the herd is no longer infectious: do nucleic acid test every two weeks after infection by collecting whole herd saliva

Naïve pig herd

1. Determine if infection is successful: do nucleic acid test within 7 days of infection by collecting blood from the whole herd
2. Determination of infection rate: do antibody test 3 weeks post-infection by collecting blood from the whole herd
3. Determine if the herd is no longer shedding: do nucleic acid test every two weeks after infection by collecting whole herd saliva

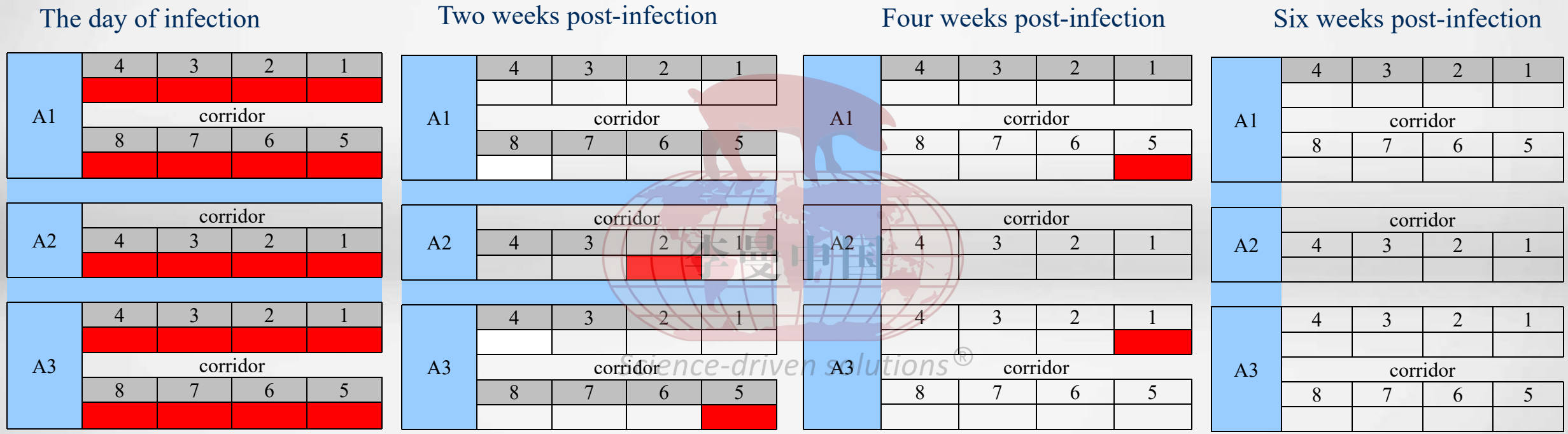
Confirmation of infection success: PRRSv nucleic acid turns positive on day 4 of naïve pig after nasal infection

孔号	样品名	靶名	类型	染料	Ct	结果
A1	1.Y146707	PRRS北美型	UNK	FAM	18.53	阳性
A1	1.Y146707	PRRS欧洲型	UNK	VIC	0.00	阳性
A2	2.Y143503	PRRS北美型	UNK	FAM	17.74	阳性
A2	2.Y143503	PRRS欧洲型	UNK	VIC	0.00	阳性
A3	3.L141202	PRRS北美型	UNK	FAM	18.93	阳性
A3	3.L141202	PRRS欧洲型	UNK	VIC	0.00	阳性
A4	4.Y147106	PRRS北美型	UNK	FAM	16.78	阳性
A4	4.Y147106	PRRS欧洲型	UNK	VIC	0.00	阳性
A5	5.L142306	PRRS北美型	UNK	FAM	19.23	阳性
A5	5.L142306	PRRS欧洲型	UNK	VIC	0.00	阳性
A6	6.L1535047	PRRS北美型	UNK	FAM	16.72	阳性
A6	6.L1535047	PRRS欧洲型	UNK	VIC	0.00	阳性
A7	7.D149506	PRRS北美型	UNK	FAM	16.95	阳性
A7	7.D149506	PRRS欧洲型	UNK	VIC	0.00	阳性

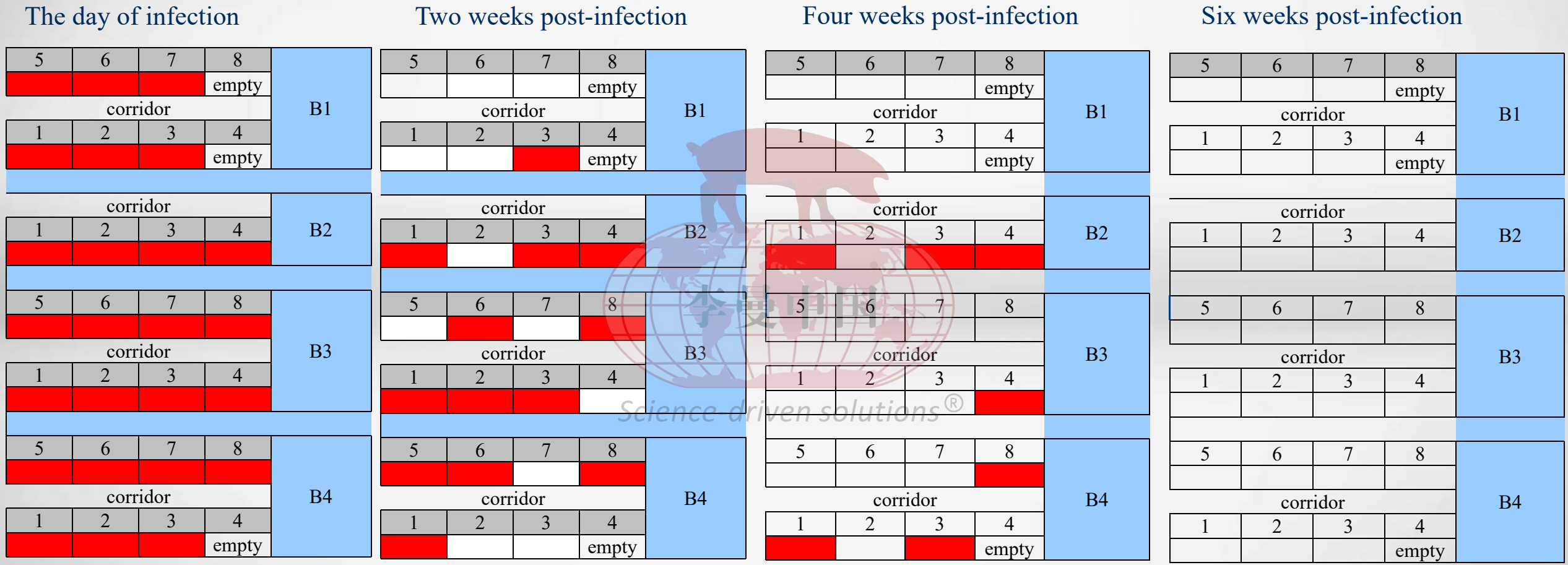
Determination of infection rate: 64/64 positive conversion rate of antibody three weeks after infection in pigs

No.	S/P value	European genotype	American genotype	No.	S/P value	European genotype	American genotype	No.	S/P value	European genotype	American genotype
								B43	1.337	-	39.02
B1	1.635	-	-	B22*	1.42	-	29.96	B44	1.446	-	34.91
B2	1.309	-	31.13	B23	1.147	-	34.55	B45	1.64	-	-
B3	1.74	-	-	B24	1.729	-	-	B46	1.48	-	-
B4	1.507	-	27.12	B25	1.669	-	-	B47	1.993	-	30.57
B5	1.656	-	30.27	B26	2.088	-	32.97	B48	1.537	-	32.66
B6*	1.293	-	32.55	B27	0.874	-	37.41	B49*	1.488	-	31.59
B7	1.492	-	-	B28	0.671	-	36.17	B50*	0.865	-	25.84
B8	1.622	-	36.03	B29	1.105	-	31.38	B51*	0.437	-	35.2
B9	0.635	-	29.95	B30	1.367	-	-	B52*	0.658	-	28.73
B10	1.949	-	32.12	B31	1.397	-	26.48	B53*	0.778	-	29.57
B11	1.554	-	36.22	B32	1.572	-	32.84	B54*	1.574	-	30.06
B12	1.701	-	29.14	B33	1.377	-	35.06	B55	1.378	-	30.09
B13*	1.334	-	31.18	B34	0.841	-	28.93	B56	1.673	-	33.66
B14	1.724	-	26.79	B35	0.775	-	-	B57	1.245	-	36.31
B15	1.692	-	30	B36	1.544	-	-	B58	1.646	-	31.94
B16	1.873	-	30.27	B37	1.579	-	37.33	B59	1.168	-	35.84
B17	1.417	-	30.81	B38	1.515	-	-	B60	1.431	-	31.63
B18	1.831	-	32.16	B39	1.166	-	30.05	B61	1.443	-	28.9
B19	1.587	-	32.14	B40	1.859	-	34.92	B62	1.815	-	29.08
B20*	1.401	-	31.51	B41*	1.656	-	35.04	B63	0.635	-	29.7
B21	1.669	-	33.85	B42*	1.191	-	31.98	B64	1.833	-	33.06

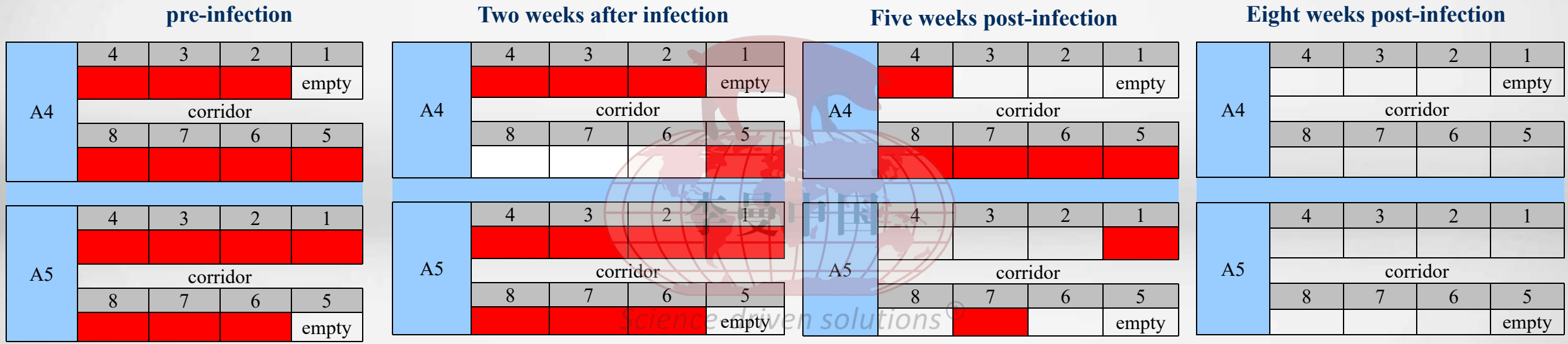
Whether or not the herd stops shedding: batch 1
Six weeks after the second infection, the whole herd is almost no longer shedding



Whether or not the herd stops shedding: batch 2
Six weeks after the second infection, the whole herd is almost no longer shedding



Whether or not the herd stops shedding: batch 3
Eight weeks after the second infection, the whole herd is almost no longer shedding



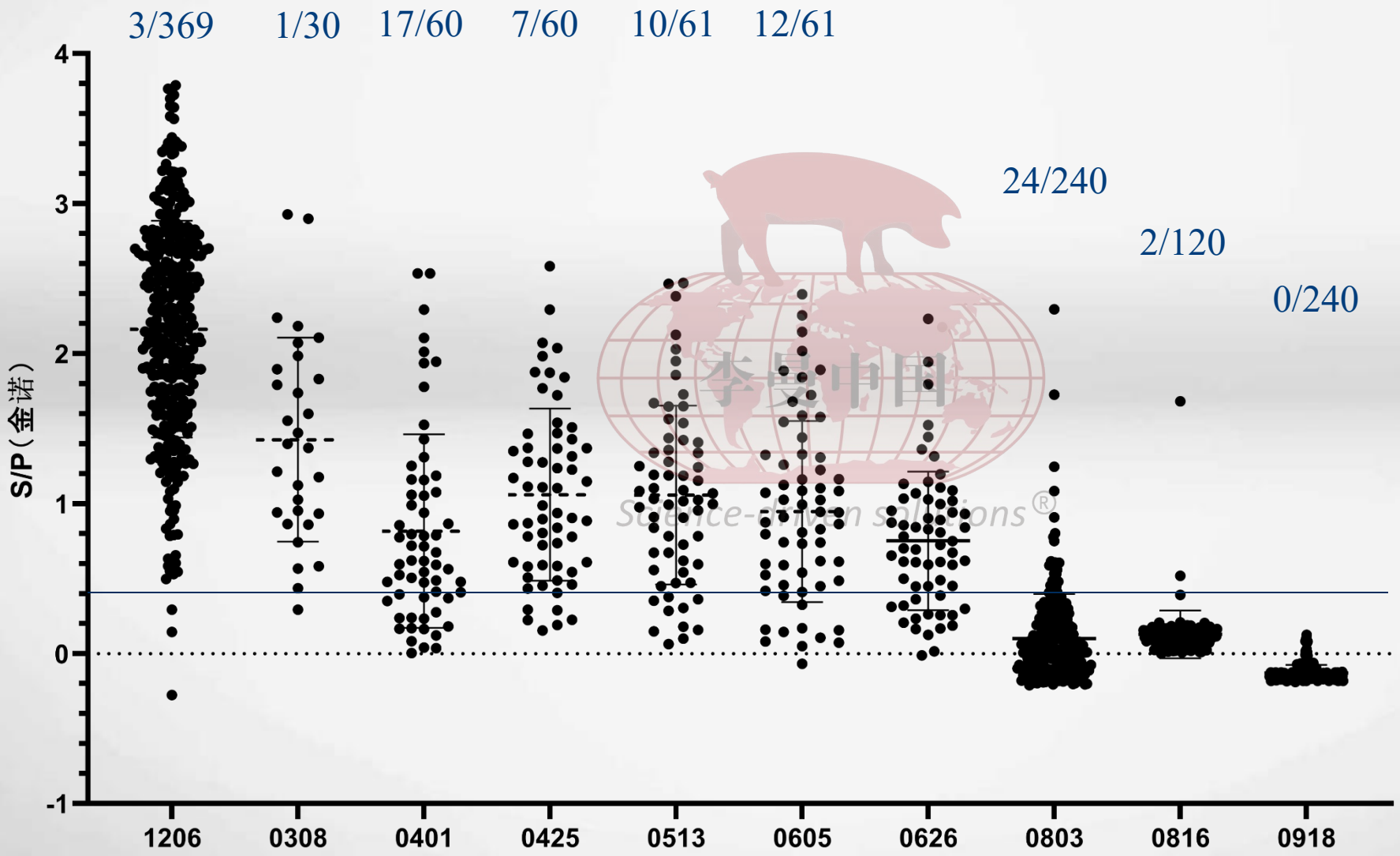
Juncture 3: Stabilization of herds

1. Assessment of the eradication capacity of introduced farms
2. Selection of breeding pig supply farms and batches
3. Reducing death and culling rate in PRRSv positive piglets
4. Parameterization of human-initiated infections
5. Infection-eradication PRRSv Infection Kinetics & Surveillance Programs
6. Biosafety optimization
7. Evaluation of eradication

PRRSv nucleic acids became continuously negative on March 8: PRRSv stable state reached

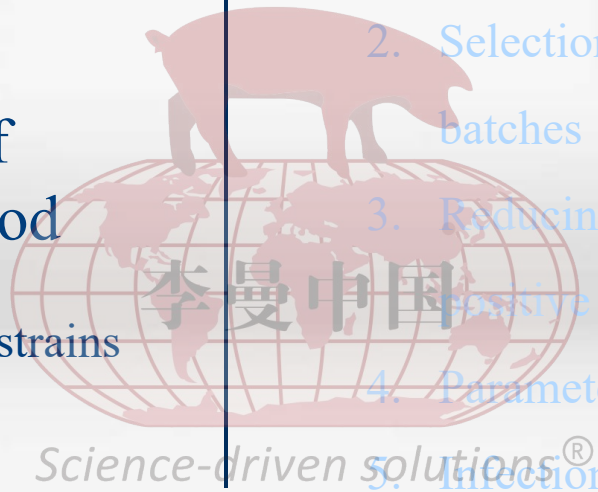
Date/Sample Type	Semen PCR negative ratio	Sow serum PCR negative ratio	Sow serum ELISA negative ratio
March 8 (batch 1)	0/19	0/31	1/31
April 1 (batch 2)	0/29	0/60	17/60
April 25 (batch 3)	0/39	0/60	7/60
May 13 (batch 4)	0/30	0/61	10/61
June 5 (batch 5)	0/34	0/61	12/61
June 26 (batch 6)	0/30	0/62	15/62
July 17 (batch 7)	0/9	0/62	-
August 7	0/12	-	-
August 9	0/8	-	-
August 16	0/9	-	-
September 6	0/14	-	-
September 18	0/8	-	-

Almost all antibodies turned negative on September 18, with less than 1% positive rate



Stage 4: Maintenance of stability/evolutionary period

Preventing the introduction of new strains



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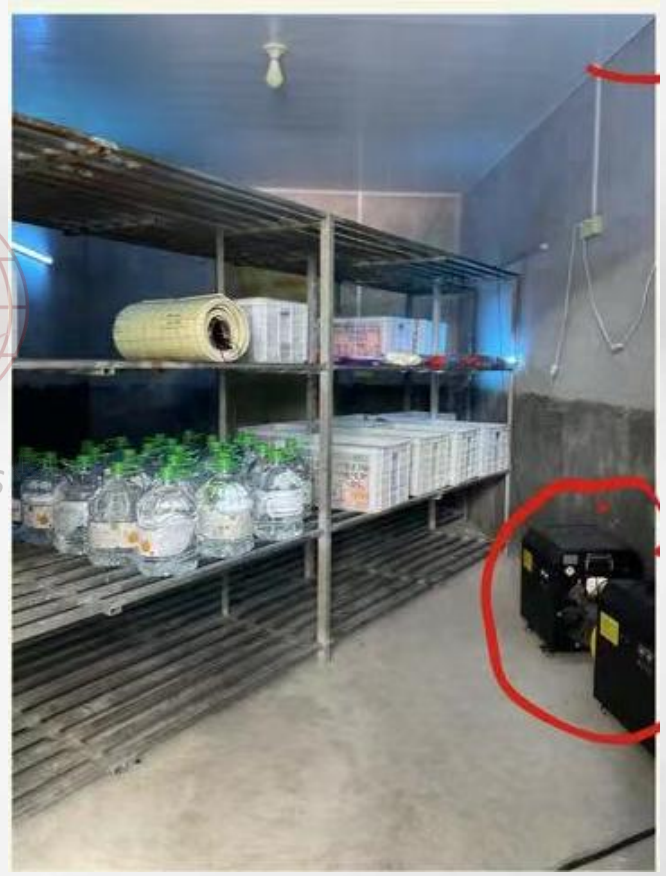
Continuous optimization of biosafety

1. Heating temperature monitoring of the material handling room in the front multifunctional area
2. Preventing sewage backflow on pig exit platforms for culled pigs
3. Reasonable placement and use of the soaking box & carry-on items disposal box
4. Optimization of the meal delivery windows from living area to the front multifunctional area
5. Optimization of the pickup process in warehouses in the company's controllable area
6. Optimizing the shoe changing process in the front multifunctional area
7. Employee baggage management optimization
8. Marking roads in farm
9. Incorporating biosafety reminder labels & standardized and papered biosafety procedures for new employee training

Continuous optimization of external biosecurity

The shelves in the material disinfection channel in the front multifunctional area have been rectified, and the treatment method has been changed from ozone and UV disinfection to heating and soaking.



Continuous optimization of external biosecurity

Anti Backflow Sinks

Increase in the number of soaking buckets and carry-on item sterilizers

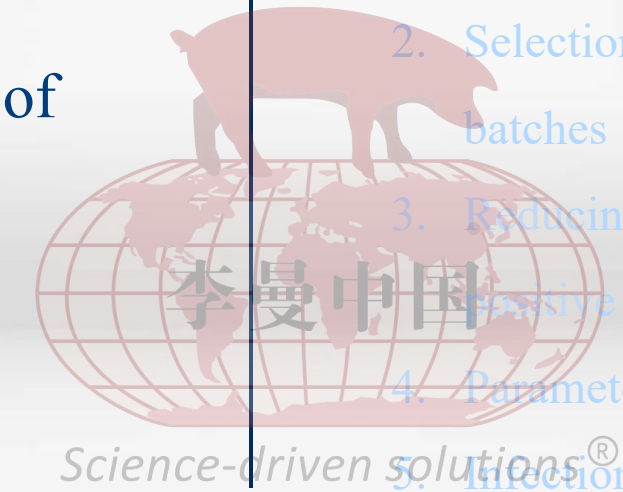
Reasonable placement and use of the soaking box & carry-on item disposal box

Optimization of meal delivery windows from living area to the front multifunctional area

Optimize the shoe changing process in the front multifunctional area

Juncture 4: Negative conversion of antibody in 6-week-old piglets

Positioning the eradication process



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Eradication process and evaluation method

positive instable		
	PCR	ELISA
breeding pig	+	+
piglet	/	/

March 8, 2023

positive stable		
	PCR	ELISA
breeding pig	-	+
piglet	/	/

August 15, 2023

naïve piglet		
	PCR	ELISA
breeding pig	-	+
piglet	-	-

Projected November 2023

almost eliminated (III)		
	PCR	ELISA
breeding pig	-	+
piglet	-	-
sentinel Pig	-	-

eliminated (IV)		
	PCR	ELISA
breeding herd	-	-
piglet herd	-	-

Eradication evaluation: monitoring program

Objective:

1. Samples from delivery rooms test negative for PRRSV nucleic acid (<1%)

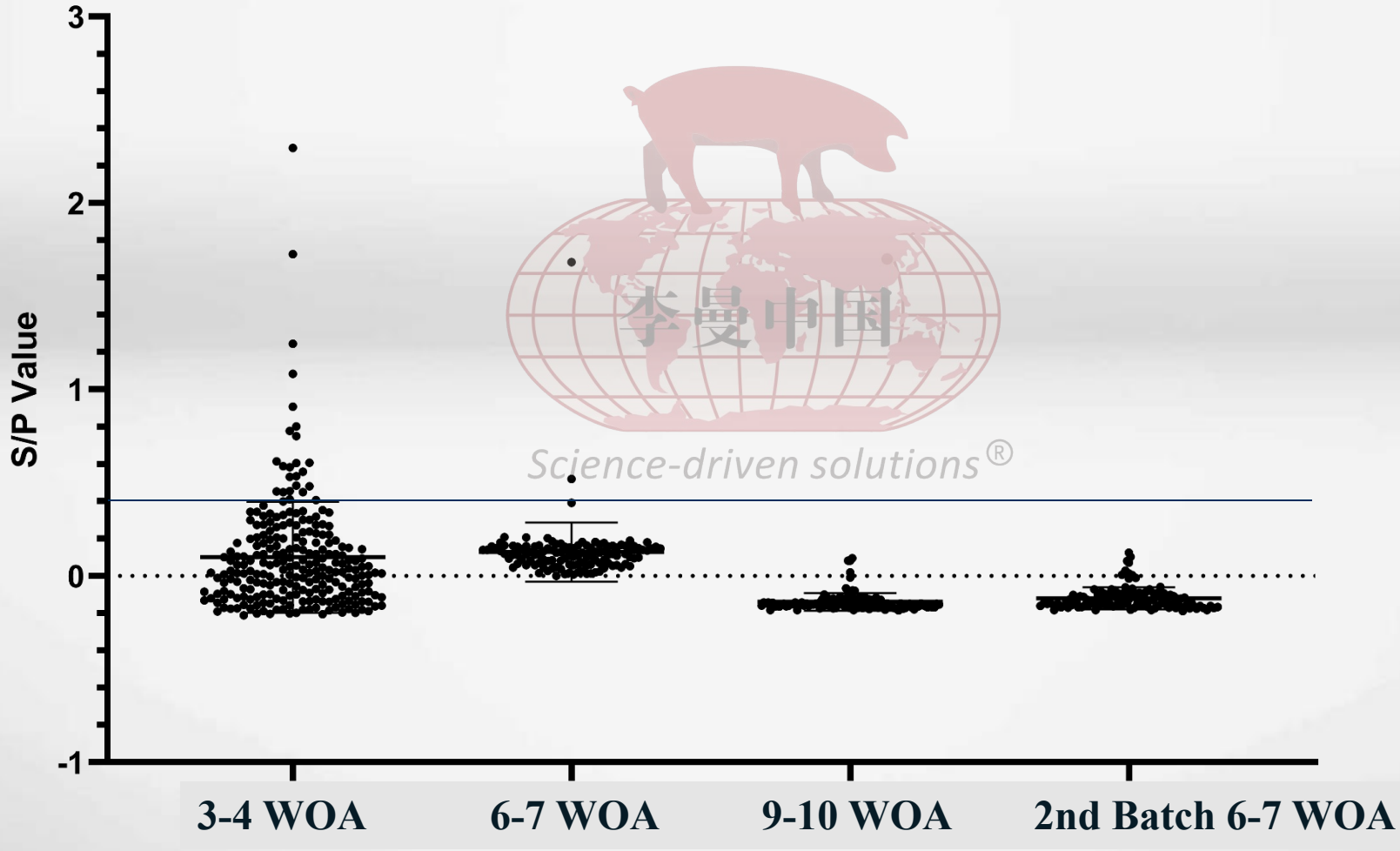
2. Maternal antibodies in 6-7 weeks of nursery herd turn negative (<1%)

Each batch	Sample size	Detection method
Ineffective tongue tip exudate of piglet	20-100 tongues tip/sample, full coverage	PRRSv PCR
Processing fluid of piglet	20-30 litters/sample, whole herd sampling	PRRSv PCR
Blood of weaned piglets at 3-4 weeks of age	240 weak piglet/batch	PRRSv PCR&ELISA
Blood of 6-week-old piglets	240 weak piglet/batch	PRRSv PCR&ELISA
Blood of 9-week-old piglets	240 weak piglet/batch	PRRSv PCR&ELISA

All farrowing piglets tested negative for PRRSv nucleic acid

	piglets of batch 1	piglets of batch 2	piglets of batch 3	piglets of batch 4	piglets of batch 5
tongue tip exudate	0/2	0/2	0/2	0/2	0/3
Testicular processing fluid	0/7	0/7	0/7	0/7	-
3-4 weeks of age	0/240	-	-	-	-
6-7 weeks of age	0/120	0/120	0/120	-	-
9-10 weeks of age	0/120	-	-	-	-

- piglets of batch 1 at 10 weeks of age were all negative for antibodies; piglets of batch 2 at 7 weeks of age were all negative for antibodies



Next Steps

Naïve sentinel pigs were placed in the sow herd, and antibody levels were tested twice consecutively at 30-day intervals. The herd remained negative and the virus tended to be eliminated.

(Reference to US AASV 2020 standards)

date of birth	Sentinel pig entry time	heads	Antigen-antibody monitoring status for the first time (0 day)	Second time (30-day-interval)	Third time (60-day interval)	Fourth time (90-day interval)	Fifth time (120-day interval)	Sixth time (150-day interval)
6.1	6.22	65	100% negative for both antigen and antibody	100% negative	100% negative	100% negative	100% negative	100% negative
6.11	7.1	40	100% negative for both antigen and antibody	100% negative	100% negative	100% negative	100% negative	100% negative
7.1	7.22	21	100% negative for both antigen and antibody	100% negative	100% negative	100% negative	100% negative	100% negative
7.12	8.1	61	100% negative for antigen, 86.7% negative for antibody (s/p 0.489, 0.559)	100% negative	100% negative	100% negative	100% negative	100% negative
7.25	8.14	40	100% negative for both antigen and antibody	100% negative	100% negative	100% negative	100% negative	100% negative
8.10	9.2	35	100% negative for antigen, 94.1% negative for antibody (s/p0.425)	100% negative	100% negative	100% negative	100% negative	100% negative
8.26	9.18	61	100% negative for both antigen and antibody	100% negative	100% negative	100% negative	100% negative	100% negative

Conclusion & Discussion

1. PRRSv naïve herds are productive, but not all pig farms are suitable for eradication
2. Prior to eradication, an assessment of the eradication capacity of the project site should be carried out, including topography, climate and biosafety.
3. Should unified infections using vaccines/wild viruses be based on demand, time or economics?
4. If it is not possible to introduce directly from naïve farms to repopulate herds, try to choose breeding farms with low diversity of PRRSv strains, and preferably with low PRRSv infection rate in multiparous sows/weaned piglets.
5. Rash acclimation with wild virus may cause abortion in sows and steep increase in piglet mortality. Before acclimation, it is necessary to consider the virulence of the wild virus, the age of the herd at the time of infection, the route of infection and the dose of infection, to ensure the establishment of a solid protection while reducing losses.
6. Continuous herd monitoring allows the entire acclimation process to become traceable, visualized and intervened. For example, is there an actual infection? Percentage of infection? Whether they are no longer shedding, etc.
7. Establishing a virus eliminated herd is easier to achieve than maintaining eradication.

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- Virus identification support from Shandong Agricultural University
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Ranking in no particular order.