Control Challenges of African Swine Fever Low-Virulence Strains: Identification, Detection, and Eradication

非洲猪瘟弱毒株的防控挑战—识别、检测与清除

李曼中国
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I. Epidemiological Characteristics of ASFV

非洲猪瘟的流行病学特点

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1. ASFV的病原学特点 The etiological characteristics of ASFV

- 五层结构：基因组、核心壳层、双层内膜、衣壳和外囊膜组成；
  Five-layer structure: consisting of the genome, core shell layer, double-layer inner membrane, capsid and outer envelope.
- 病毒粒子直径200-260nm，含有43811个微粒组成，呈正二十面体；
  The diameter of the virus particles is 200 - 260 nm, and they consist of 43,811 particles, forming a regular icosahedron.
- 基因组为线性双链DNA分子，约为**170-193kb**，不同毒株的基因组大小存在差异；
  A linear double-stranded DNA molecule, approximately 170-193 kb in size.
- 基因组编码**151-167种蛋白**，成熟的病毒粒子**包含54（现68）种结构蛋白和100多种非结构蛋白**（病毒粒子包含3万余个蛋白亚基），感染宿主细胞时病毒可**包裹25种宿主蛋白**形成免疫逃避;
  The genome encodes 151-167 types of proteins. The mature virus particle contains 54 (currently 68) structural proteins and over 100 non-structural proteins (the virus particle consists of more than 30,000 protein subunits). When infecting host cells, the virus can encapsulate 25 host proteins to form immune evasion.
- 目前流行毒株主要有I型、II型和I/II型重组毒株。
  The currently prevalent strains mainly include type I, type II and type I/II recombinant strains.

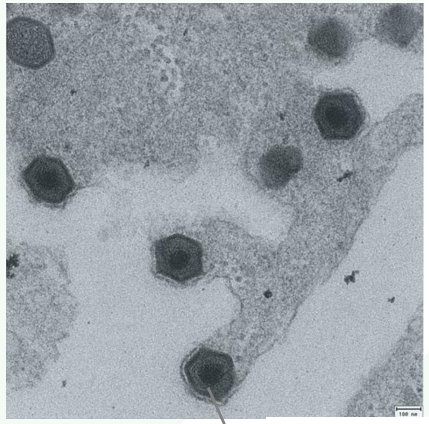
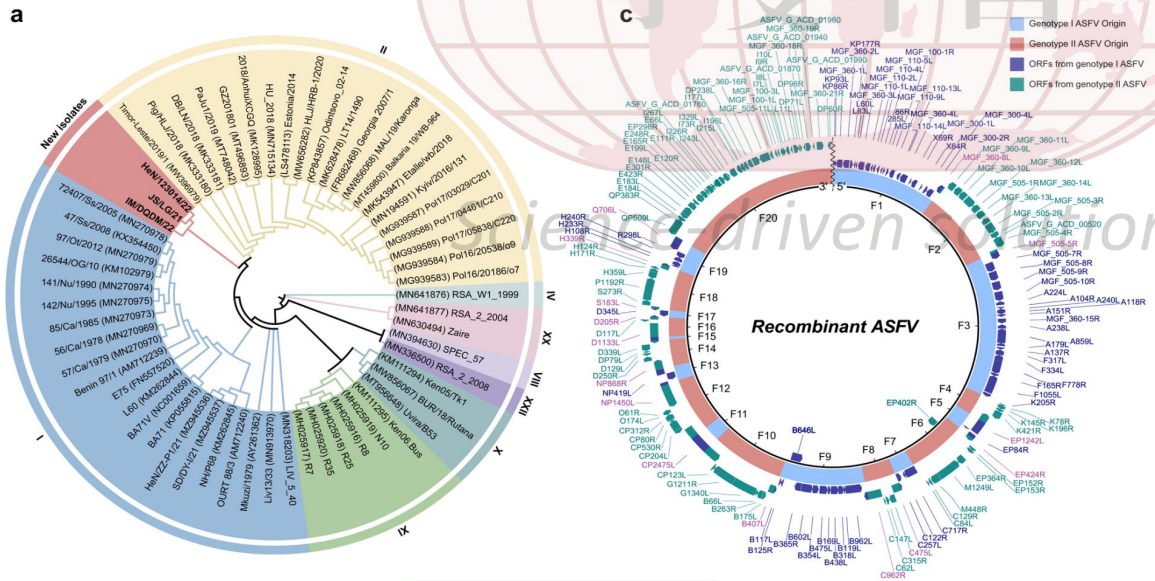


图1. ASFV的电子显微镜
引自《Swine disease》，



200-260nm

Zhao Dongming *et. al* Nat Commun. *2023*

2．非瘟的流行传播模型
The epidemic and transmission model of ASFV

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➢ **定殖循环**

Colonization cycle

✓ 野猪-野猪

✓ Wild boar within

✓ 家猪-家猪

✓ Domestic pig within

✓ 野猪-家猪

✓ Wild boar to domestic pig

✓ 野猪-蜱

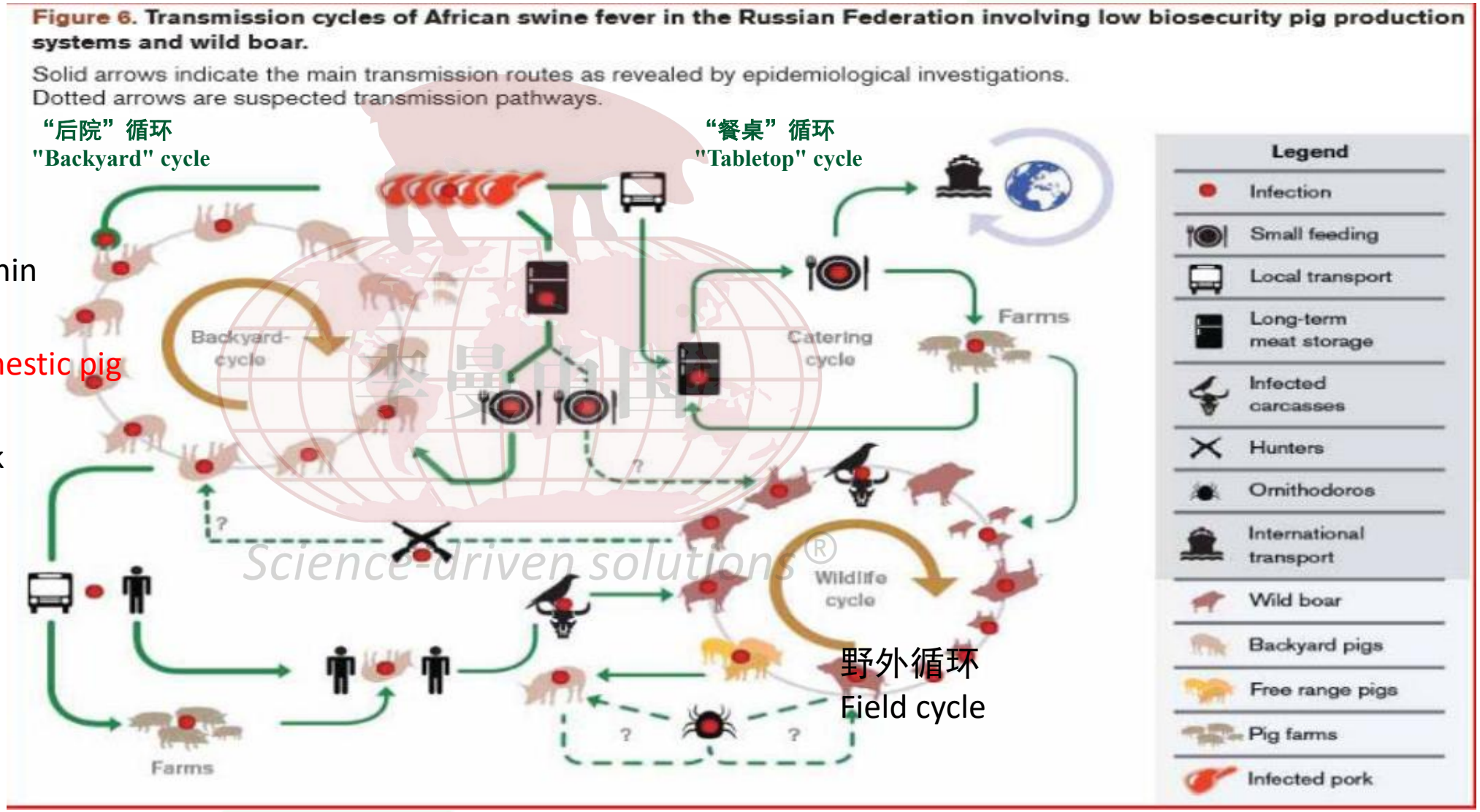
✓ Wild boars to tick

✓ 猪-蜱

Domestic pig to tick

Figure 6. Transmission cycles of African swine fever in the Russian Federation involving low biosecurity pig production systems and wild boar.

Solid arrows indicate the main transmission routes as revealed by epidemiological investigations.
Dotted arrows are suspected transmission pathways.



野外循环
Field cycle

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3. ASFV的变异趋势
The trend of ASFV mutations

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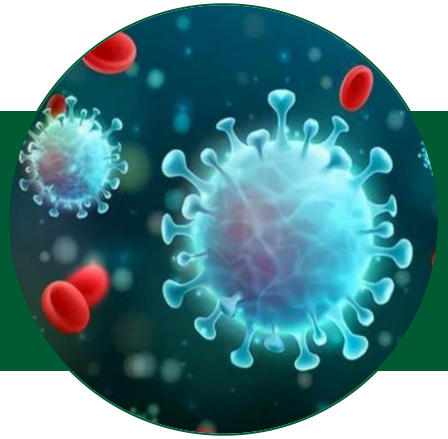
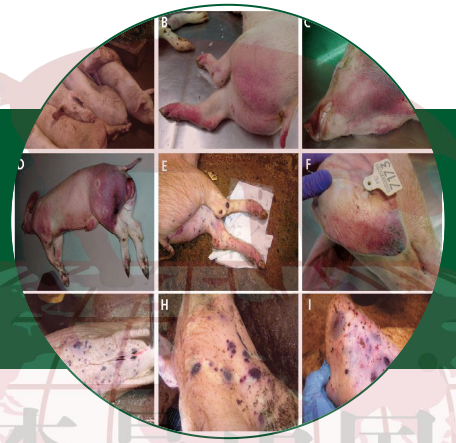


病毒变异多样性
Viral diversity in mutation

流行病学调查结果显示，
病毒出现多种变异和缺失

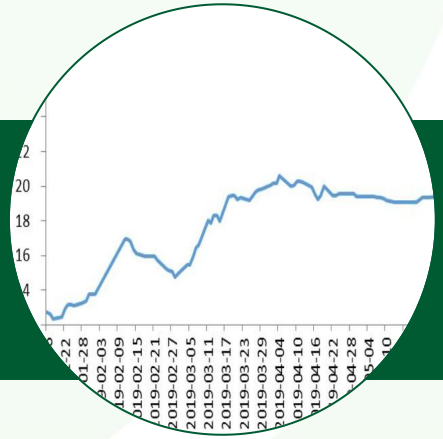
The results of the epidemiological investigation show that the virus has undergone various mutations and deletions.



毒力差异多样性
Diversity of virulence variations

野毒毒力较强，缺失株毒力相对较弱！

The wild strain has a stronger toxicity, while the missing strain has relatively weaker toxicity.



流行态势多样性
Diversity of popular trends

以直接接触传播为主，间接接触、
粉尘等气溶胶传播为辅

The main mode of transmission is through direct contact, while indirect contact and aerosol transmission (such as from dust) are secondary.

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4. ASFV防控形势分析
Analysis of the Prevention and Control Situation of ASFV

受季节、雨水等因素影响，表现为局部散发流行或地方流行！

Affected by factors such as seasons and rainfall, it is manifested as sporadic or local epidemics!

传播方式未改变
The mode of transmission has not changed.

直接或间接接触传播，经口、呼吸道、伤口、注射或产道等感染

Direct or indirect contact transmission, infection through the mouth, respiratory tract, wounds, injections or the birth canal, etc.

理化特性未改变
The physical and chemical properties have not changed.

仍然对高温、干燥、强酸、强碱敏感

Remains sensitive to high temperatures, dryness, strong acids, and strong bases.

常用消毒剂仍然有效

Common disinfectants remain effective.

主要防控策略未变
The main prevention and control strategies remain unchanged.

无有效疫苗和特效药，仍需要依赖有效的生物安全措施

There is no effective vaccine or specific drug, so we still need to rely on effective biosecurity measures.

I型/II型野毒、MGF/CD2V等功能基因、非功能基因缺失交叉重组毒株

Type I/II wild-type viruses, functional genes such as MGF/CD2V, and non-functional gene-deficient cross-recombined strains

病毒不断突变，毒力差异大The virus is constantly mutating, with significant differences in its virulence.

变异毒株血液间歇性排毒、临床症状不明显

The variant strain intermittently excretes toxins in the blood and shows no obvious clinical symptoms.

难发现、难检测、难剔除、难溯源

Difficult to detect, difficult to eliminate, difficult to trace.

精准剔除技术不太好用
The precise elimination technique is not very effective.

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II. Identification and detection of ASFV attenuated strains

非洲猪瘟弱毒株的识别与检测

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1. 野毒感染的临床症状 Clinical symptoms of wild virus infection

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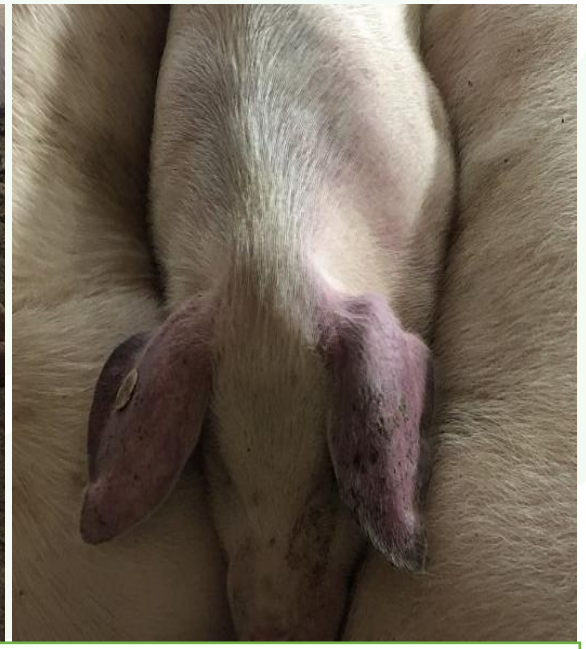
- 主要表现为发烧、减料、通体发红等症状，发病急，病程短，死亡率高！The main symptoms include fever, weight loss, and overall redness of the body. The onset is sudden, the disease course is short, and the mortality rate is high!
- 可通过分餐饲喂观察发现明显不食的病猪！By observing the situation of divided feeding, it is possible to identify the sick pigs that refuse to eat!

2.变异毒感染临床症状（I型缺失）
Clinical symptoms of variant virus infection (Type I is absent)

- 主要表现为采食量逐渐下降，皮肤渐进性发红，耳朵发绀！The main manifestations are a gradual decrease in feed intake, progressive redness of the skin, and cyanosis of the ears!
- 相较于野毒感染发病缓和，死亡率下降！Compared to wild-type infections, the onset of the disease is milder and the mortality rate has decreased!

感染初期，猪群出现食欲下降，精神沉郁现象，无其他明显症状
In the early stage of infection, the pig herd exhibited decreased appetite and lethargy, with no other obvious symptoms.

感染4-5天后，猪只皮肤出现微微泛红现象（不明显）
After 4-5 days of infection, the skin of the pigs shows a slight reddening (not very obvious)



感染7天后，猪只耳朵变红，皮肤变红（十分明显），食欲废绝，卧地不起，严重者身体出现抽搐现象
Seven days after infection, the pig's ears turn red, the skin also turns red (very noticeably), its appetite disappears, it lies down and cannot get up, and in severe cases, it experiences convulsions.

3．变异毒感染临床症状（II型缺失）
Clinical symptoms of variant virus infection (Type II deficiency)

- 最初食欲下降不明显，精神稍显沉郁；关节肿胀、脚痛、跛行、跪卧；At first, there was no significant decrease in appetite, but the spirit became a little listless; the joints were swollen, the feet hurt, the gait was abnormal, and the animal would kneel or lie down.
- 皮肤可出现大小不一的坏死斑，且可在感染后3-5d逐渐增多；The skin may develop necrotic patches of varying sizes, and these patches will gradually increase over a period of 3 to 5 days after the infection.
- 腹股沟淋巴结肿胀、突起！Swollen and protruding inguinal lymph nodes!

4. 其他变异缺失株临床症状
Clinical symptoms of other variant deletion strains

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An African swine fever vaccine-like variant with multiple gene deletions caused reproductive failure in a Vietnamese breeding herd

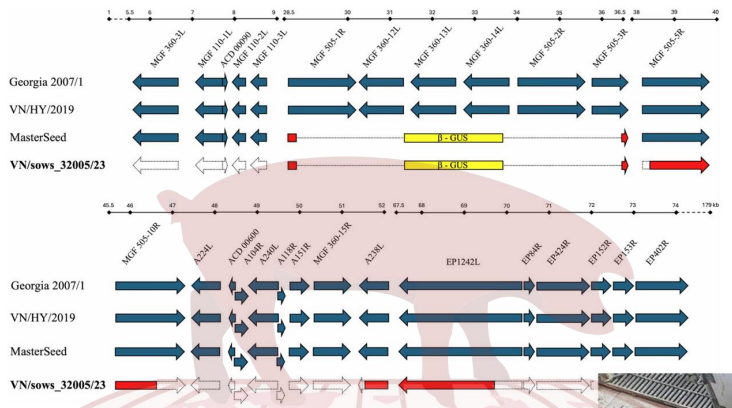


Fig. 3. Schematic diagram of indel mutations among ASF strains: vaccine-like (VN/sov Eurasian isolate (Georgia 2007/1), the first Vietnamese outbreak isolates (VN/HY/2019 (MasterSeed). Reference strains are downloaded from the NCBI GenBank database. Ge and partial deletions were visualized as dotted lines, yellow boxes, and red arrows, respe were depicted as dark blue arrows with open reading frame annotation above.

Ear tag no.	Heart	Liver	Kidney	Spleen	Lung	LN	Udder	Uterus	Ovary	Oviduct
Reproductive failure										
33700	–	–	–	+	–	+	–	–	–	–
30256	–	–	–	+	–	+	+	–	–	–
03300	n/a	–	–	+	+	+	+	–	+	–
Ulcerative dermatitis										
32242	–	n/a	–	+	+	+	+	–	+	–
32005	–	n/a	–	+	+	+	+	–	–	–
34550	–	+	–	+	+	+	+	–	–	–

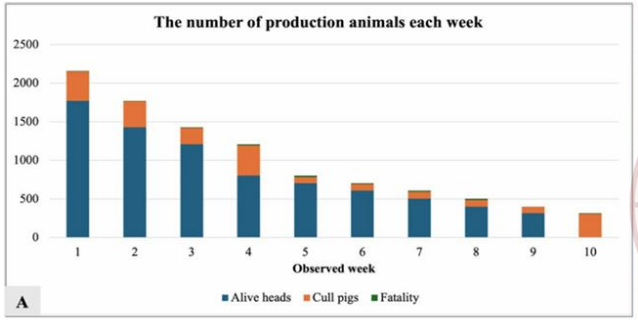
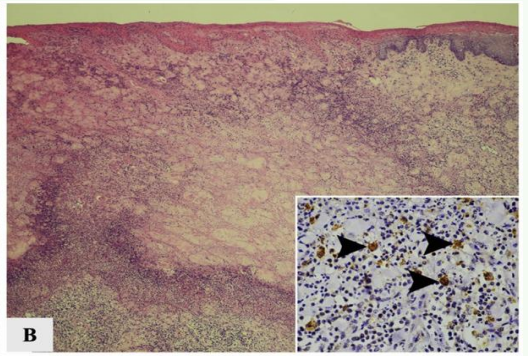
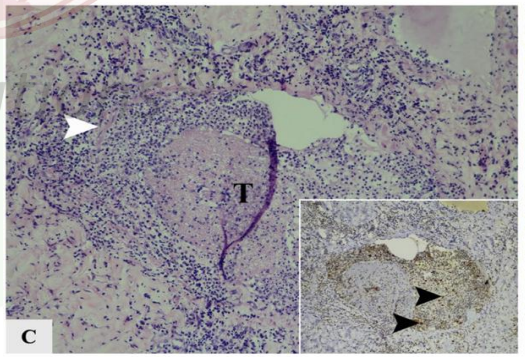


Fig. 1. The swine population in the herd and significant observed clinical signs in ASFV-infected sows. (**A**) A total of production animals was recorded each week during the study period. (**B**) Reproductive failure in pregnant sows in the third stage of gestation with aborted mummifies. (**C**) Moderate-to-severe multiple dermatologic ulcerations in lactating sows (arrowhead).





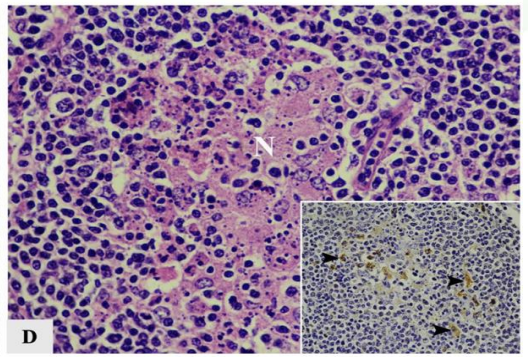


Fig. 4. Pathological and immunohistochemical findings in ASF-infected sows. (**A**) A variable-sized ulceration on the udder of lactating sows at 1–2 weeks postpartum. (**B**) Massive necrosuppurative inflammation of the skin that extended from the epidermis to the reticular dermis. (**C**) Vasculitis (white arrowhead) with thrombus (T) at the interstitium of the mammary gland. (**D**) The lymphoid necrosis (N) in the germinal center of the lymph node. ASFV-antigen localization was detected in the macrophage-like cells in infected lesions (arrowhead, insets).

4. 其他变异缺失临床症状
Clinical symptoms of other variant deletion strains

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Published in partnership with the Sealy Institute for Vaccine Sciences

https://doi.org/10.1038/s41541-025-01099-9

African swine fever virus vaccine strain Asfv-G-ΔI177l reverts to virulence and negatively affects reproductive performance

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Erwin van den Born[1], Ferenc Olasz[2,3], István Mészáros[2], Eszter Göltl[2,3], Barbara Oláh[2], Jui Joshi[1], Emma van Kilsdonk[1], Ruud Segers[1] & Zoltán Zádori[2,3]

ASFV-G-ΔI177L is a modified-live African swine fever virus (ASFV) strain that has been incorporated into a commercially available vaccine. Its safety in pregnant sows and genetic stability in an in vivo passaging experiment were investigated. Upon inoculation of two pregnant sows with ASFV-G-ΔI177L, one developed moderate ASF-related clinical signs. In terms of reproductive performance, 43% of the offspring was born dead and the live-born piglets developed ASF-specific clinical signs, became viremic, and only 17% survived until the end of study. During passaging in pigs, ASFV-G-ΔI177L reverted to virulence with severe ASF-specific clinical signs at passages 3 and 4, associated with increased viremia. Whole genome sequencing identified C257L mutations as a potential driver of increased replication fitness and virulence. The data show that ASFV-G-ΔI177L is not genetically stable and, therefore not safe for use in ASF vaccines and suggest that ASF vaccine candidates should be tested for safety in pregnant animals.

https://doi.org/10.1038/s41541-025-01099-9

Article

pregnancy were inoculated with 1.5×10^2 $TCID_{50}$ of ASFV-G-ΔI177L. This inoculation dose corresponded to the lowest effective and safe dose described for this vaccine strain[2]. Both inoculated sows (group I) were viremic on day 4 post-inoculation and remained viremic until the end of the study (Fig. 1A). They also showed blue discolouration around the nipples at 9 dpi. Inoculated sow 1 did not show any other clinical signs, whereas the other inoculated sow (sow 2) had elevated temperatures, was largely inactive, and did not drink and eat on days 7 and 8 post-inoculation (Fig. 1B and C). By day 11, she was still eating less and moving slowly, but by day 14, she was showing signs of recovery with rectal temperatures back to normal, and by day 17, she fully recovered. The two control sows (group II) did not display any ASF-specific clinical signs before and after farrowing, but between 1 and 4 days post-farrowing both control sows were eating less, resulting in a low level of clinical scores (Fig. 1C).

All sows were farrowed at the expected farrowing date at approximately day 115 in gestation and about 15 days post-inoculation. However, significant differences were observed in terms of reproductive performance between inoculated and control sows (Table 1). In the case of inoculated sows, 43% of the piglets were stillborn as compared to 17% of the controls. While all live-born piglets of the control sows survived and were healthy until the end of the study, only 4 of the 23 live-born piglets of the ASFV-G-ΔI177L-inoculated sows survived. Only 8 piglets from inoculated sow 1 and 6 piglets from inoculated sow 2 managed to stay in the study beyond the day of birth, of which all showed ASF-specific clinical signs, and many were euthanized on reaching HEP (Table S5). The average daily rectal temperature of these group I piglets was above the normal temperature range for pigs (38.7–40.0 °C) between 7 and 11 days post-farrowing, whereas the temperatures of piglets born to the control sows remained normal (Fig. 1D). The 4 piglets in the inoculated group that survived until the end of the study

Table 1 | Reproductive performance of the ASFV-G-ΔI177L-inoculated and control sows

	ASFV-G-ΔI177L-inoculated sows		Control sows	
	1	2	1	2
Total piglets born	23	17	27	19
Normal piglets	8	6	21	15
Weak piglets	9	0	2	0
Dead piglets	6	10	4	4
Mummified piglets	0	1	0	0
Alive at end of experiment	4	0	23	15

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接种的母猪在接种后第4天出现病毒血症，并持续到研究结束。它们在9天后乳头周围出现蓝色变色。接种的母猪1没有表现出其他临床症状，而另一只接种的母猪（母猪2）在接种后第7和第8天体温升高，活动减少，不饮水和进食。到第11天，她仍然吃得少，行动缓慢，但到第14天，她开始恢复，直肠温度恢复正常，到第17天，她完全康复。

5.ASFV的再生感染系数R0变化
The variation of the re-infection coefficient R0 of ASFV

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$$R_0 = \sum_{s=1}^{t} I(t-s)\,ws \longrightarrow \lambda t = \sum_{s=1}^{t-1} ysw(t-s)$$

Transbound Emerg Dis. 2017 Dec;64(6):1858-1866. doi: 10.1111/tbed.12583. Epub 2016 Sep 25.

Estimating the Basic Reproductive Number for African Swine Fever Using the Ukrainian Historical Epidemic of 1977

F I Korennoy [1], V M Gulenkin [1], A E Gogin [2], T Vergne [3], A K Karaulov [1]

Affiliations + expand

PMID: 27667658 DOI: 10.1111/tbed.12583

Abstract

In 1977, Ukraine experienced a local epidemic of African swine fever (ASF) in the Odessa region. A total of 20 settlements were affected during the course of the epidemic, including both large farms and backyard households. Thanks to timely interventions, the virus circulation was successfully eradicated within 6 months, leading to no additional outbreaks. Detailed report of the outbreak's investigation has been publically available from 2014. The report contains some quantitative data that allow studying the ASF-spread dynamics in the course of the epidemic. In our study, we used this historical epidemic to estimate the basic reproductive number of the ASF virus both within and between farms. The basic reproductive number (R_0) represents the average number of secondary infections caused by one infectious unit during its infectious period in a susceptible population. Calculations were made under assumption of an exponential initial growth by fitting the approximating curve to the initial segments of the epidemic curves. The R_0 both within farm and between farms was estimated at 7.46 (95% confidence interval: 5.68-9.21) and 1.65 (1.42-1.88), respectively. Corresponding daily transmission rates were estimated at 1.07 (0.81-1.32) and 0.09 (0.07-0.10). These estimations based on historical data are consistent with those using data generated by the recent epidemic currently affecting eastern Europe. Such results contribute to the published knowledge on the ASF transmission dynamics under natural conditions and could be used to model and predict the spread of ASF in affected and non-affected regions and to evaluate the effectiveness of different control measures.

乌克兰，1977年流行毒株

群内传播，R0值7.46（5.68-9.21）

场间传播，R0值1.65（1.42-1.88）

ELSEVIER

Veterinary Microbiology

Volume 165, Issues 3–4, 30 August 2013, Pages 296-304

Transmission rate of African swine fever virus under experimental conditions

H.C. de Carvalho Ferreira [a, c], J.A. Backer [b], E. Weesendorp [a], D. Klinkenberg [c], J.A. Stegeman [c], W.L.A. Loeffen [a]

Abstract

African swine fever (ASF) is a highly lethal, viral disease of swine. No vaccine is available, so controlling an ASF outbreak is highly dependent on zoosanitary measures, such as stamping out infected herds and quarantining of affected areas. Information on ASF transmission parameters could allow for more efficient application of outbreak control measures. Three transmission experiments were carried out to estimate the transmission parameters of two ASF virus isolates: Malta'78 (in two doses) and Netherlands'86. Different criteria were used for onset of infectiousness of infected pigs and moment of infection of contact pigs. The transmission rate (β), estimated by a Generalized Linear Model, ranged from 0.45 to 3.63 per day. For the infectious period, a minimum as well as a maximum infectious period was determined, to account for uncertainties regarding infectiousness of persistently infected pigs. While the minimum infectious period ranged from 6 to 7 days, the average maximum infectious period ranged from approximately 20 to nearly 40 days. Estimates of the reproduction ratio (R) for the first generation of transmission ranged from 4.9 to 24.2 for the minimum infectious period and from 9.8 to 66.3 for the maximum infectious period, depending on the isolate. A first approximation of the basic reproduction ratio (R_0) resulted in an estimate of 18.0 (6.90–46.9) for the Malta'78 isolate. This is the first R_0 estimate of an ASFV isolate under experimental conditions. The estimates of the transmission parameters provide a quantitative insight into ASFV epidemiology and can be used for the design and evaluation of more efficient control measures.

马耳他毒株，1978年，每天的传播感染速率为0.48-3.63，

R0值18（6.9-46.9）

荷兰毒株，1986年，每天的传播感染速率为0.45-0.92，

frontiers | Frontiers in Veterinary Science

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Estimation of basic reproduction number (R_0) of African swine fever (ASF) in mid-size commercial pig farms in Vietnam

Basic reproduction number (R0) in sow and fattening pig farms

Table 1. Mean, standard deviation of infected cases per day and R_0 values

Farm	Type of pig	Actual pig population	Mean	Standard Deviation	Basic reproduction number (R0) (95% C.I)
HY1	Sow	384	4.5	2.78	1.78 (1.35 – 2.35)
	Fattening	1682	13.94	15.98	4.76 (4.18 – 5.38)
HY2	Sow	192	3.3	2.54	1.55 (1.08 – 2.18)
	Fattening	981	14.28	10.25	3.80 (3.33 – 4.28)

Note: C.I: Confident interval,

Front Vet Sci. 2022; 9: 918438.
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PMCID: PMC9556723
PMID: 36246317

Estimation of basic reproduction number (R_0) of African swine fever (ASF) in mid-size commercial pig farms in Vietnam

Nguyen Tuan Anh Mai, [1,†] Thi Bich Ngoc Trinh, [1,†] Van Tam Nguyen, [1] Thi Ngoc Ha Lai, [1] Nam Phuong Le, [1] Thi Thu Huyen Nguyen, [1,2] Thi Lan Nguyen, [1] Aruna Ambagala, [3] Duc Luc Do, [4,*] and Van Phan Le [1,*]

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animals

MDPI

Article

Estimation of a Within-Herd Transmission Rate for African Swine Fever in Vietnam

Van Phan Le [1], Nguyen Thi Lan [1], Jose Tobias Canevari [2], Juan Pablo Villanueva-Cabezas [3,4,*], Pawin Padungtod [5], Thi Bich Ngoc Trinh [6], Van Tam Nguyen [6], Caitlin N. Pfeiffer [2], Madalene V. Oberin [2], Simon M. Firestone [2] and Mark A. Stevenson [2]

越南毒株，2019年，

母猪群，R0值1.78（1.35-2.35），1.55（1.08-2.18），

肥猪群，R0值4.76（4.18-5.35），3.80（3.33-4.28）。

6. 如何做实验室的检测和鉴别诊断
How to conduct laboratory tests and make differential diagnoses

YANGXIANG 扬翔股份

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Veterinary Research

REVIEW Open Access

Strategic nucleic acid detection approaches for diagnosing African swine fever (ASF): navigating disease dynamics

Yuanshou Zhu[1†], Meng Zhang[3†], Zhijun Jie[3,4], Shujuan Guo[2], Zhigang Zhu[1*] and Sheng-ce Tao[2*]

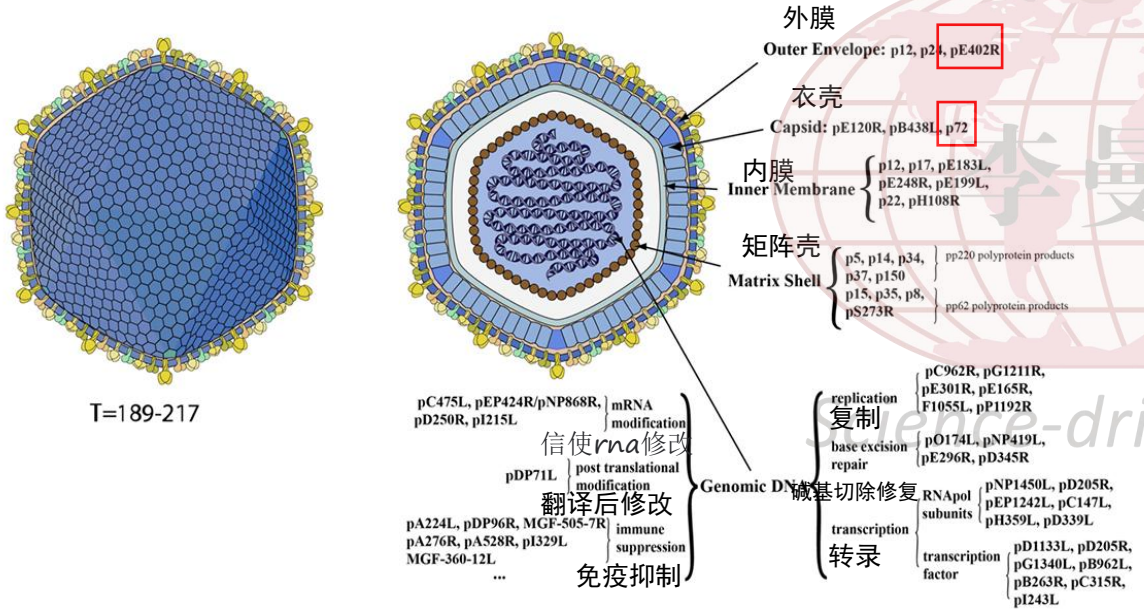

Figure 1 Illustration of the ASFV structure and genome. ASFV belongs to the family *Asfarviridae* and has an icosahedral enveloped virion. From

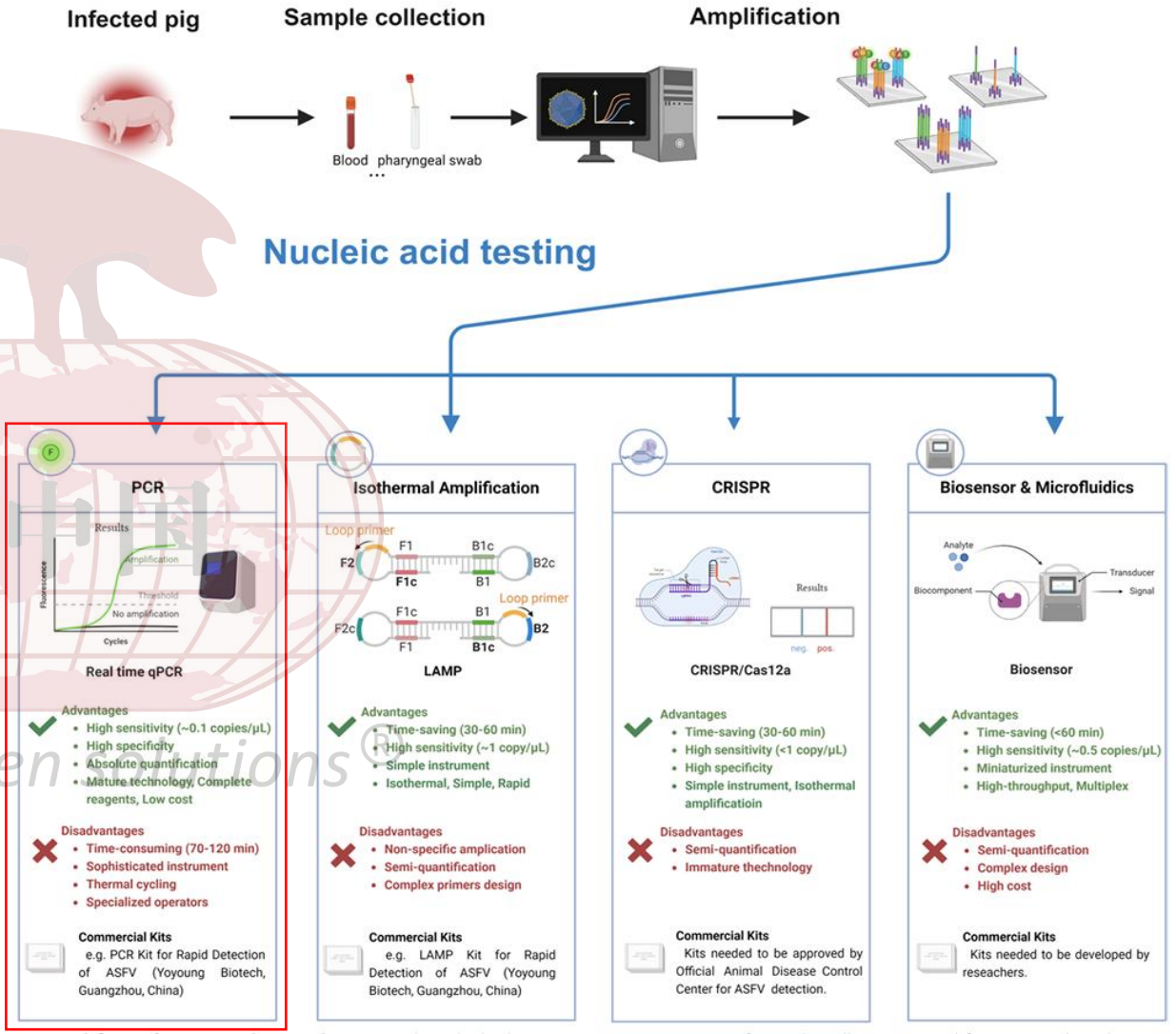

Figure 2 Workflow of ASFV nucleic acid testing. The whole detection process consists of sample collection, amplification, and nucleic acid testing. The samples were collected from the blood or pharyngeal swabs of infected pigs. Sample treatment includes DNA extraction from deactivated viruses or simple lysis by specialized reagents. Various nucleic acid testing methods, including PCR, isothermal amplification, CRISPR, and biosensors and microfluidics, can be used for ASFV detection. The advantages and disadvantages of different methods are also listed.

Zhu et al. Veterinary Research (2024)

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6. 如何做实验室的检测和鉴别诊断
How to conduct laboratory tests and make differential diagnoses

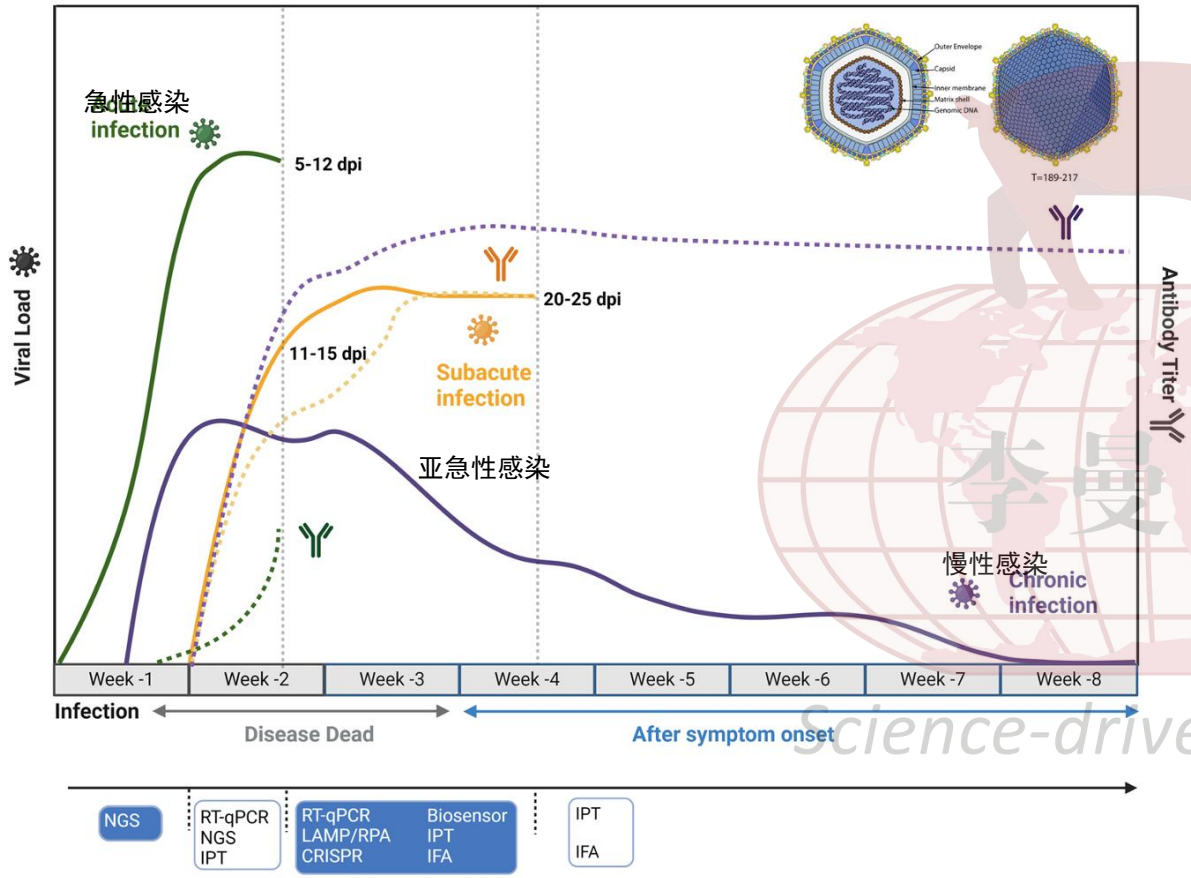

Figure 4 Disease dynamics and recommended detection methods for ASFV.

非洲猪瘟的动态和推荐的检测方法

Zhu et al. Veterinary Research (2024)

Table 1 Detection methods targeting the B646L gene of ASFV

Method	Classifications	Strain	Ref.
Based on PCR	A duplex fluorescent quantitative PCR assay	Genotype I and II strains	[132]
	A fast quantitative real-time PCR	ASFV	[46]
	A novel quantitative real-time PCR	ASFV-China	[45]
	Combination of $Fe(OH)_3$ modified diatomaceous earth and qPCR	ASFV in water	[47]
	Nanofluidic chip digital PCR	ASFV	[105]
Based on isothermal amplification	Recombinase-based isothermal amplification assay (RPA/RAA)	Different genotypes ASFV	[70]
	Exonuclease III-assisted recombinase-aided amplification colorimetric assay	ASFV	[72]

Table 2 Detection methods targeting genes of ASFV other than B646L

Mutation	Detection method	Strain	Ref.
p54	A novel real-time PCR assay	ASFV	[134]
E248R	A quantitative PCR method	ASFV	[135]
B646L and B438L	A probe-based duplex real-time PCR assay	ASFV	[136]
p72, CD2v and MGF	CRISPR-Cas12a	ASFV	[108]
A137R	Real-time PCR	ASFV	[137]
MGF-360-12L, I177L	Real-time PCR Assays	Highly virulent Georgia strain of ASFV	[138]
E296R	A duplex real-time PCR assay	Genotypes I and II ASFV	[139]
I73R and I329L	The tandem repeat sequences	p72 genotype II and intergenic region (IGR) II variant	[140]
I73R, I329L, Q174L, K145R, I329L, I215L	Sequencing and PCR	24 different European II-ASFV genotypes	[111]

Table 3 Mutations in candidate live attenuated vaccines

Mutation	Characteristic	Function	Ref.
F317L	An inhibitor of the NF-kB pathway and production of proinflammatory cytokines	Immune evasion; promote viral replication	[146]
EP364R, C129R	Target cyclic GMPAMP to inhibit the cGAS-STING signalling Pathway	Immune evasion; promote viral replication	[112]
MGF360-9L, MGF505-7R	Decrease ASFV-specific IFN-g response and increase macrophage infiltration	Promote viral replication; enhance virulence	[147]
H108R	/	Promote viral replication; enhance virulence	[148]
A137R	/	Promote viral replication; enhance virulence	[35]
E165R	Encode dUTPase	Promote viral replication	[149]
E184L	A transcribed gene in the infectious cycle	Enhance virulence	[150]
DP148R, DP71L, DP96R	/	Immune evasion; enhance virulence	[151]
L7L-L11L	Clustered gene on open reading frames and located at the right variable region	Enhance virulence	[152]
I177L	Belong to ASFV-G genome	Enhance virulence	[153]
EP402R	Hemagglutinin of ASFV	Immune evasion; enhance virulence	[154]

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7. ASF新型检测方法的应用与评价
Application and Evaluation of the New ASF Detection Method

表2 ASF新型检测方法应用

检测方法 Detection method	快速检测 Rapid detection	可在猪场使用 Field usability	可视化 Visualization	提取核酸 Nucleic acid extraction	经济性 Economy
生物传感器	√	√	×	√	/
基于耳标的生物传感器	√	√	×	×	/
人工智能(AI)	√	√	√	×	×
磁珠	√	√	×	×	√
微流体技术	√	√	×	√	/
蜂巢芯片(Hive-chip)技术结合 LAMP 技术	√	×	√	×	/
纳米流控芯片数字 PCR(cdPCR)	×	×	/	√	×
磁流体装置	√	√	×	√	√

ASF新型检测方法的开发聚焦点：

——便捷、高效、省时、省力！

The focus of the development of the new ASF detection method: Convenient, efficient, time-saving and labor-saving!

检测方法 Detection method	灵敏度 Sensitivity	特异性 Specificity	检测时间 Detection time	样品类型 Sample type	评价 Comment
间接 ELISA	*	*	2～3 h	血清	灵敏度和特异性高，但易发生交叉反应
双夹心 ELISA	*	*	2～3 h	血清	灵敏度高，但操作复杂
双重 ELISA	*	*	2～3 h	血清	可区分 ASFV 野毒株和 CD2v 缺失株
阻断 ELISA	*	*	2～3 h	血清	操作复杂且繁琐
免疫印迹检测试验	*	*	2～3 h	免疫器官、血清	灵敏度高，但无法实现高通量检测
免疫荧光素酶沉淀试验(MB-LIPS)	*	*	30 min	口腔液	检测时间短
荧光免疫层析试验(QDs-FICA)	*	*	20 min	血清	检测时间短
化学发光免疫分析法(CLIA)	*	*	20 min	血清	辅助检测手段
荧光抗体试验(FAT)	***	***	75 min	血清	用于急性 ASF 检测，一般作为辅助检测手段
时间分辨荧光免疫分析法(TRFIA)	**	**	45 min	口鼻分泌物	检测时间短，灵敏度比 ELISA 高
聚合酶链式反应(PCR)	**	**	2～3 h	血清、厨余垃圾	常用的检测方法
实时荧光定量 PCR(qPCR)	***	***	5～6 h	血液、组织样品、饲料、粉尘	灵敏度比 PCR 高

检测方法 Detection method	灵敏度 Sensitivity	特异性 Specificity	检测时间 Detection time	样品类型 Sample type	评价 Comment
双重 qPCR	***	***	5～6 h	血液、组织样品、鼻拭子、环境拭子	可区分 ASFV 基因Ⅰ型和Ⅱ型，以及野毒株和基因缺失型毒株
多重 qPCR	***	***	5～6 h	冷冻猪肉制品、组织样品	可鉴别 ASFV 野毒株和基因缺失型毒株，区分 ASFV 和 CSFV
微滴式数字 PCR(ddPCR)	***	***	5～6 h	粪便、血清、组织样品	检测灵敏度最高，但仪器昂贵
双重 ddPCR	***	***	5～6 h	血清	可区分 ASFV 野毒株和基因缺失型毒株
环介导等温扩增技术(LAMP)	***	***	20～45 min	全血、血清、熟猪肉、口腔拭子、直肠拭子	检测时间短，结果可视化，但显色效果可能受到血清中其他成分的影响
重组酶介导的等温扩增技术(RAA)	***	***	15～25 min	全血、猪肉、组织样品	检测时间短、便捷，结果可视化
重组酶聚合酶扩增技术(RPA)	***	***	10 min	全血、厨余垃圾	检测时间短、便捷，结果可视化
CRISPR/Cas12a	***	***	40 min～2 h	病毒 DNA、全血、组织样品	灵敏度高、便捷，结果可视化
CRISPR/Cas13a	***	***	1 h	病毒 DNA、全血、组织样品	灵敏度高、便捷，结果可视化
胶体金免疫层析试纸条	*	*	10～20 min	血清	可用于 ASFV 早期诊断，作为辅助检测手段
荧光免疫层析试纸条(FICTS)	*	*	10 min	血清	辅助检测手段
QDM 免疫层析试纸条(QDM-ICS)	*	*	25 min	血清	辅助检测手段

陈画菡等，中国兽医杂志，2024

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III、The precise elimination of ASFV

非洲猪瘟弱毒株的精准剔除

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精准剔除的主要原则
The main principle of precise elimination

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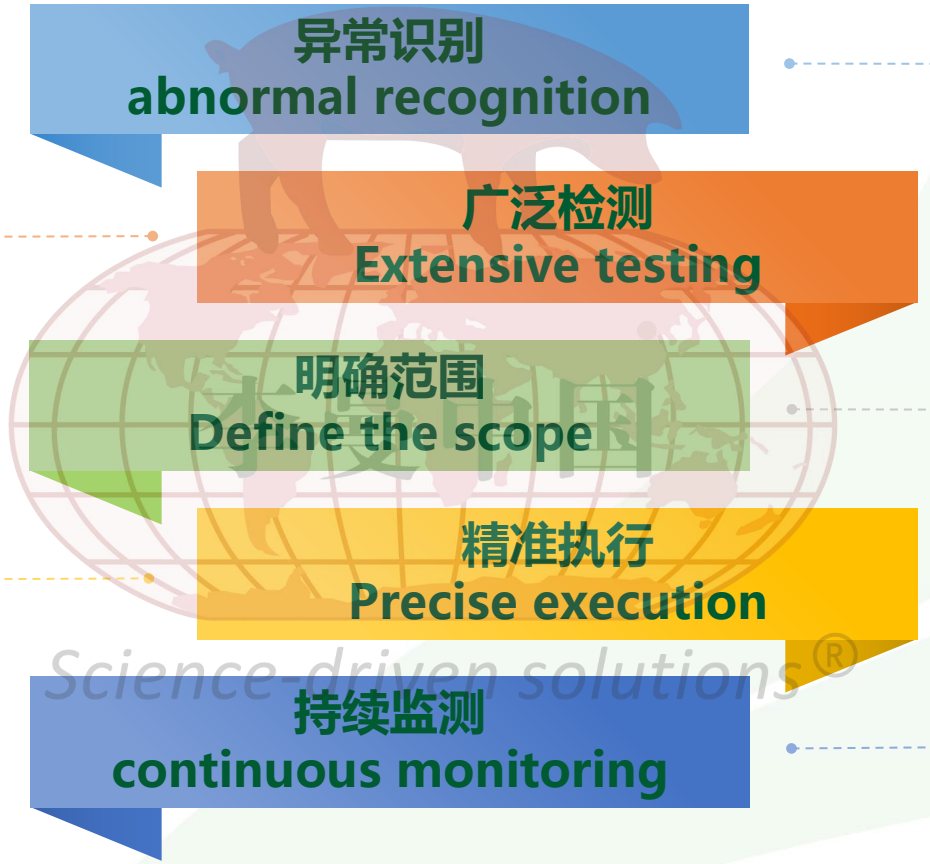
异常猪只样品（血样、口鼻拭子、组织样），其他样品（环境、车辆、物资、人员等）
Abnormal pig samples (blood samples, nasal and oral swabs, tissue samples), as well as other samples (environment, vehicles, supplies, personnel, etc.)

人员熟悉精准剔除流程
Personnel are familiar with the precise elimination proces
物资准备充足
The supplies are well-prepared.
消毒切断彻底
Disinfection and sterilization are thorough.



精准识别猪群在采食行为、精神状态、体表、肢蹄等方面的异常 Accurately identify the abnormalities in the feeding behavior, mental state, body surface, and limbs and hooves of the pig herd.

明确流行病学调查方法和逻辑，通过案例复盘和检测来确定感染面；
Clarify the epidemiological investigation methods and logic, and determine the extent of infection through case reviews and tests.

条件性静默生产，异常猪100%监测，配合范围性普查（1、7、14、21d） Conditional silent production, 100% monitoring of abnormal pigs, combined with comprehensive screening (at 1, 7, 14, and 21 days)

采样方式：少污染，易操作（尾根血拭子搭配口鼻拭子）
Sampling method: Low contamination, easy to operate (tail-root blood swab combined with oral and nasal swabs)

01/02. 异常猪的识别与检测
Identification and Detection of Abnormal Pigs

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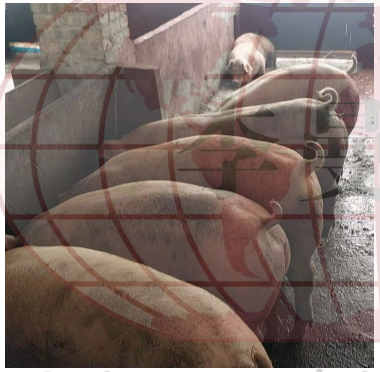
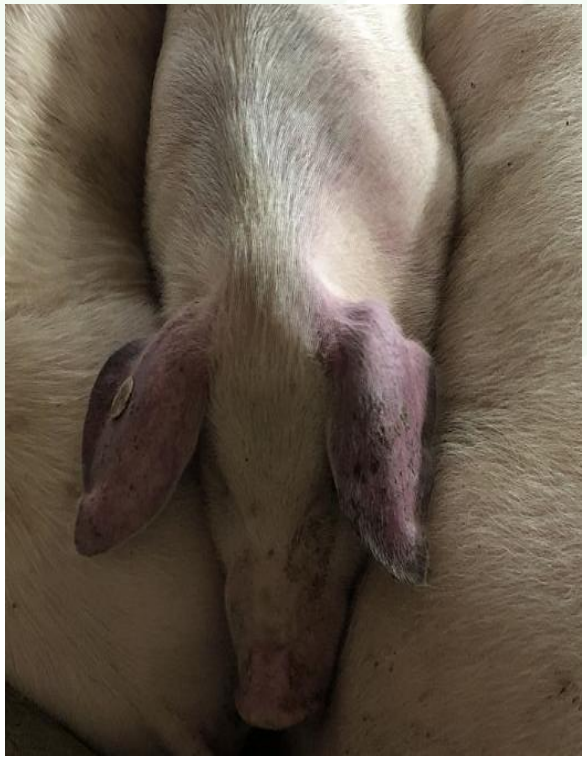
- ✓ 异常信号：发烧、减料、精神沉郁、脚肿、皮肤破溃、流产等……
- ✓ Abnormal symptoms: fever, reduced milk production, listlessness, swollen feet, skin ulceration, miscarriage, etc...
- ✓ 样品采集：口鼻拭子、尾根血拭子、淋巴液
- ✓ Sample collection: Nasopharyngeal swabs, tail root blood swabs, lymph fluid
- ✓ 实验室检测：RT-qPCR（鉴别诊断-I/II型，CD2V、MGF……）
- ✓ Laboratory test: RT-qPCR (diagnostic differentiation - type I/II, CD2V, MGF...)







03.明确范围—感染范围
Define the scope - Infection scope

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复盘分析 Review and analysis	从传播途径角度From the perspective of transmission routes					场内复盘关联动作Related action				应对方案 Solutions	消毒要求 Disinfection requirements
	感染途径 Route of infection	风险因素 Risk factor	业务频次 Business frequency	生物安全风险等级 Biological safety risk level	直接因素 Direct factor	第一级 Level 1	第二级 Level 2	第三极 Level 3	关联强度 Strength of association		
	注射感染 Injection of	疫苗免疫	低频	低	疫苗				弱		
		药物注射	低频	低	药物				无		

复盘分析 Review and analysis	从传播途径角度From the perspective of transmission routes					场内复盘关联动作Related action				应对方案 Solutions	消毒要求 Disinfection requirements
	感染途径 Route of infection	风险因素 Risk factor	业务频次 Business frequency	生物安全风险等级 Biological safety risk level	直接因素 Direct factor	第一级 Level 1	第二级 Level 2	第三极 Level 3	关联强度 Strength of association		
从病原入侵角度分析From the perspective of pathogen invasion	入口 Entrance	人员洗澡间	高频	高	人员				弱		
		物资消毒间	低频	高	物资				弱		
		食材消毒间	中频	高	食材				中		
	进出猪台 Pig delivery platform	引种	低频	高	过程感染				强		
		卖断奶猪	低频	中	交叉污染				强		
		卖淘汰猪	低频	中	交叉污染				强		
	无害化处理口 Harmless Disposal Port	胎衣、仔猪	高频	中	交叉污染				弱		
		处理死猪	低频	高	交叉污染				弱		
	进出风口 Inlet and outlet vents	水帘	高频	低	气溶胶				弱		
		风机	高频	低	气溶胶				弱		
	管线端口 Pipeline port	料塔	低频	低	飞鸟				无		
		水塔	中频	低	水源				无		
		粪沟	低频	低	反流				无		

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03.明确范围—剔除范围
Define the scope - Eliminate the scope

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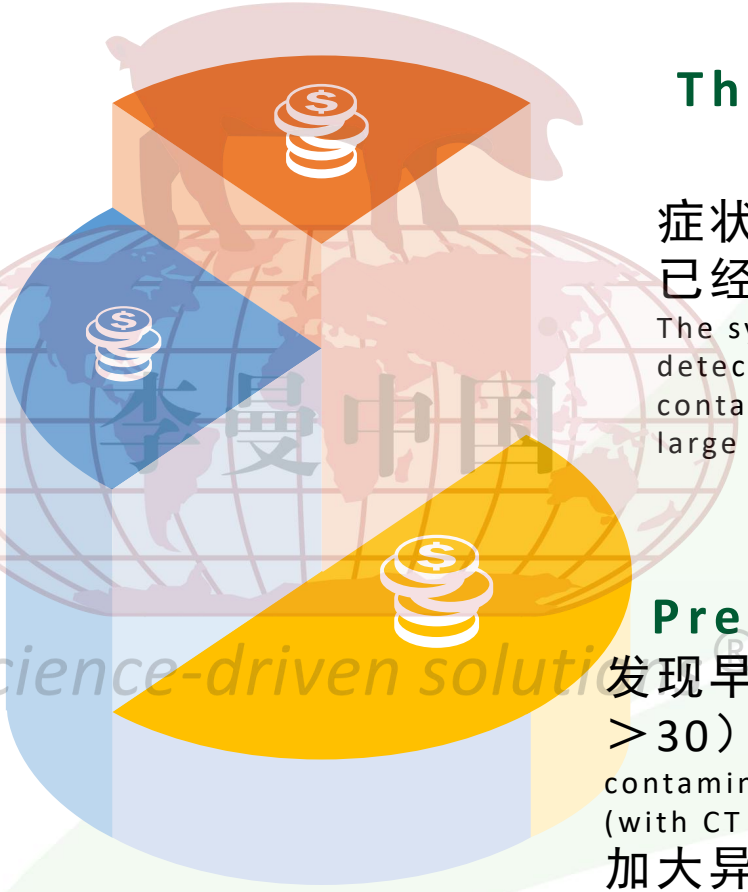
栋清
The entire building is cleared.

单栋多点感染，控制传染源，限制传播范围！

Single building with multiple infections. Control the source of infection and limit the spread!



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场清
The entire clearance operation

症状不典型，难于发现，发现时已经大面积污染扩散！

The symptoms are not typical and difficult to detect. By the time they are discovered, the contamination has already spread over a large area!

精准剔除-点拔
Precise elimination-single

发现早，污染面小，临床症状轻（CT值＞30）！ Early detection leads to a smaller contamination area and milder clinical symptoms (with CT values above 30)!

加大异常猪监测，稍有差异立刻淘汰！

Increase the monitoring of abnormal pigs and immediately eliminate those showing any slight differences!

4.精准执行Precise execution

YANGXIANG 扬翔股份

01 明确目的 Clear purpose

如何切断外部病原继续侵入；
如何切断内部病原继续扩散；
How to prevent external pathogens from continuing to invade;
How to prevent internal pathogens from continuing to spread;

02 确定方案 Determine solution

铁桶检查与堵漏；
异常猪只的剔除方式与路径；
其他猪群的监控策略；
Inspection and plugging of iron tanks;
Methods and procedures for eliminating abnormal pigs;
Monitoring strategies for other pig groups;

03 准备物资 Prepare supplies

人员防护物资、消毒类物资以及病死猪处理物资；
Personal protective equipment, disinfection supplies, and materials for handling dead pigs;

04 强化培训 Intensive training

培训内容包括人员分工、人员防护、传播切断与异常猪只的识别等；
The training content includes personnel division, personnel protection, transmission interruption and identification of abnormal pigs, etc.

05 演练实操 Practical drills

以病死猪、淘汰猪为例，定期开展应急演练；
通过视频和照片等形式记录演练过程；
Take dead pigs and culled pigs as examples.
Regular emergency drills should be conducted;
The drill process should be recorded through video and photo formats.

06 演练总结 Exercise Summary

对标生物安全管理措施是否执行到位；
判定演练行动是否符合要求！
Check whether the biological safety management measures have been implemented properly;
Determine whether the drill actions meet the requirements!

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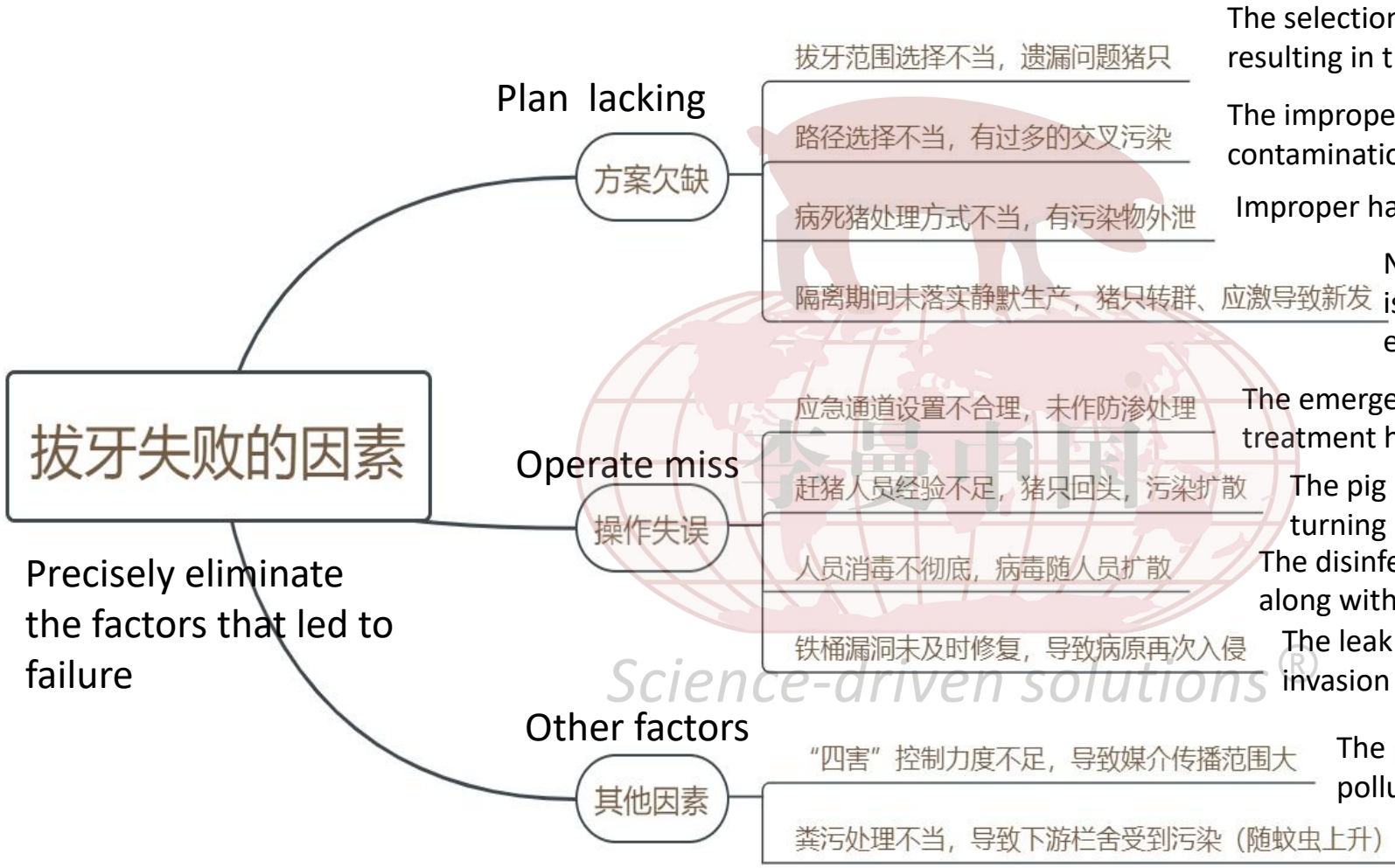
■ 清除失败的因素有哪些？
What are the factors that lead to failure in the process?



Precisely eliminate the factors that led to failure

The selection of the tooth extraction range was inappropriate, resulting in the omission of positive pigs.

The improper selection of the path resulted in cross-contamination.

Improper handling of dead pigs led to the leakage of pollutants.

No silent production was carried out during the isolation period. The transfer of pigs led to the emergence of new infected pigs.

The emergency exit is improperly set up and no anti-seepage treatment has been carried out.

The pig herding workers lacked experience, and the pigs' turning back led to cross-contamination.

The disinfection was not thorough, and the virus spread along with the movement of people.

The leak in the pig farm has not been fixed, resulting in the invasion of new pathogens.

The presence of vector organisms leads to secondary pollution.

Improper handling of manure has caused contamination of the pigs in the surrounding pens.

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05. 持续监测 Continuous monitoring

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✓ 异常猪采样监测（口鼻拭子+尾根血拭子）

Abnormal pig sampling monitoring (nasal and oral swabs + tail root blood swabs)

时间	线别	栋舍	异常猪位置	异常猪耳号	异常猪临床表现	是否采样	第1次采样	第2次采样	第3次采样	处理方式	备注

✓ 全场/线普查监测（尾根血拭子）

Full-scale/line census monitoring (tail root blood swab)

XX场猪群普检记录表-按栋舍顺序

序号	栋舍	第一轮普检（1d）				第二轮普检（5d）				第三轮普检（10d）				第四轮普检（20d）			
		采样日期	采样人员	头数	检测结果	采样日期	采样人员	头数	检测结果	采样日期	采样人员	头数	检测结果	采样日期	采样人员	头数	检测结果
1	产房1栋	2.18		53	0/53	2.23		53	0/53	2.28		53	0/53	3.10		53	0/53
2	产房2栋	2.18-19		53	0/53	2.23		53	0/53	2.28		53	0/53	3.10		53	0/53
3	产房3栋	2.18		53	0/53	2.23		53	0/53	2.28		53	0/53	3.10		53	0/53
4	产房4栋	2.18-19		53	0/53	2.23		53	0/53	2.28		53	0/53	3.10		53	0/53
5	产房5栋	2.18		53	0/53	2.23		53	0/53	2.28		53	0/53	3.10		53	0/53
6	产房6栋	2.18-19		59	0/59	2.23		59	0/59	2.28		59	0/59	3.10		59	0/59
7	产房7栋	2.19		57	0/57	2.23		57	0/57	2.28		57	0/57	3.10		57	0/57
8	产房8栋	2.18-19		58	0/58	2.23		58	0/58	2.28		58	0/58	3.10		58	0/58
9	产房9栋	2.18		57	0/57	2.23		57	0/57	2.28		57	0/57	3.10		57	0/57
10	产房10栋	2.19		56	0/56	2.23		56	0/56	2.28		56	0/56	3.10		56	0/56
11	定位1栋	2.19		146	0/146	2.23		146	0/146	2.28		146	0/146	3.10		146	0/146
12	定位2栋	2.18		141	0/141	2.21		141	0/141	2.26		141	0/141	3.8		141	0/141
13	定位3栋	2.19		130	0/130	2.23		130	0/130	2.28		130	0/130	3.10		130	0/130
14	定位4栋	2.19-2.20		120	0/120	2.23		120	0/120	2.28		120	0/120	3.10		120	0/120
15	定位5栋	2.18		73	0/73	2.23		73	0/73	2.28		73	0/73	3.10		73	0/73
16	定位6栋	2.20		127	0/127	2.24		127	0/127	3.1		127	0/127	3.11		127	0/127
17	定位7栋	2.19-2.20		136	0/136	2.24		136	0/136	3.1		136	0/136	3.11		136	0/136
18	定位8栋	2.19-2.20		136	0/136	2.24		136	0/136			136	0/136			136	0/136
19	定位9栋			空栏				空栏				空栏				空栏	
20	定位10栋			空栏				空栏				空栏				空栏	
21	定位11栋	2.20		120	0/120	2.24		120	0/120	3.1		120	0/120	3.11		120	0/120
22	定位12栋			空栏				空栏				空栏				空栏	
23	定位13栋			空栏				空栏				空栏				空栏	
24	定位14栋	2.20		138	0/138	2.24		138	0/138	3.1		138	0/138	3.11		138	0/138
25	定位15栋	2.17		121	0/121	2.21		121	0/121	2.26		121	0/121	3.8		121	0/121
26	定位16栋	2.18		111	0/111	2.23		111	0/111	2.28		111	0/111	3.10		111	0/111
27	定位17栋	2.19		137	0/137	2.23		137	0/137	2.28		137	0/137	3.10		137	0/137
28	定位18栋	2.18-19		134	0/134	2.23		134	0/134	2.28		134	0/134	3.10		134	0/134
29	后备1栋	2.19		28栏	0/18	清空		28栏		清空				清空			
30	后备2栋	2.19		28栏	2/25	清空		28栏		清空				清空			

持续汇报 | 全场平面图 | 全场布局图 | 普检记录-按栋舍 | 普检记录-按日期 | +

◘ 普查需要结合猪只的临床发病状态与CT值，并参考R0值的预估范围！

The census needs to take into account the clinical symptoms of the pigs and their CT values, and also refer to the estimated range of the R0 value!

✓ 急性型普查时间点：1d、5d、10d、20d

Acute type survey time points: 1 day, 5 days, 10 days, 20 days

✓ 亚急性/慢性型普查时间点：1d、7d、14d、21d

Preliminary survey time points for subacute/chronic type: 1 day, 7 days, 14 days, 21 days.

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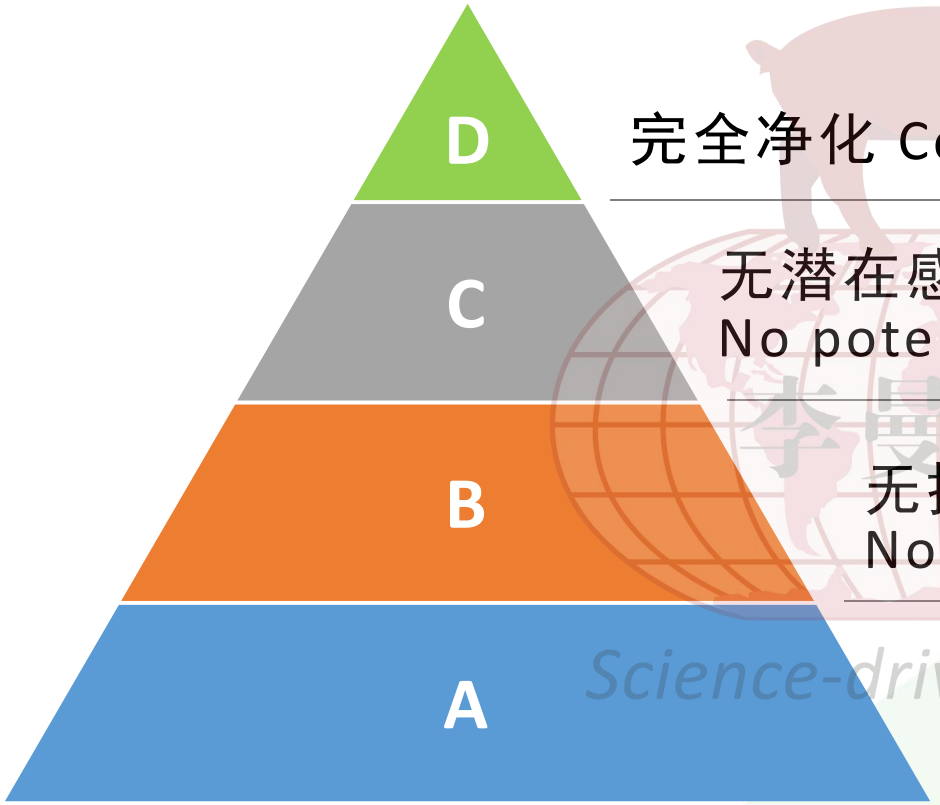
精准剔除的判定
Precise elimination determination



完全净化 Complete purification

无潜在感染风险因素
No potential risk factors for infection

无携带病原个体
No carrying of pathogenic individuals

无新增病例
No new cases were reported.

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6. ASFV阳性场的复产

Recovery of ASFV-positive farms

- 阻断病毒传入——打造“铁桶”猪场
- Preventing the spread of the virus - Building "impenetrable" pig farms

- 清除场内病毒——栏舍彻底的洗消烘
- Remove viruses from the site - thoroughly disinfect and dry the barns

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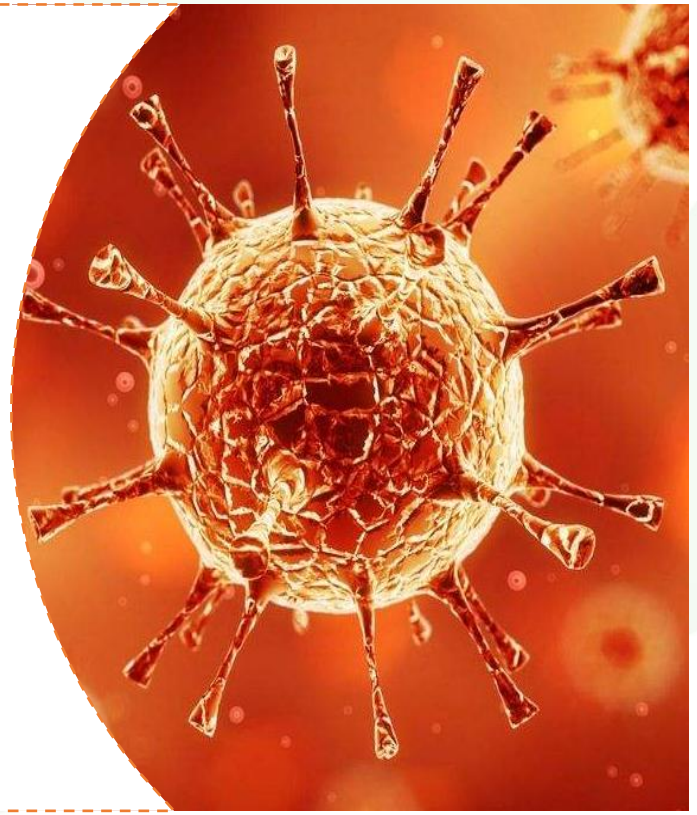
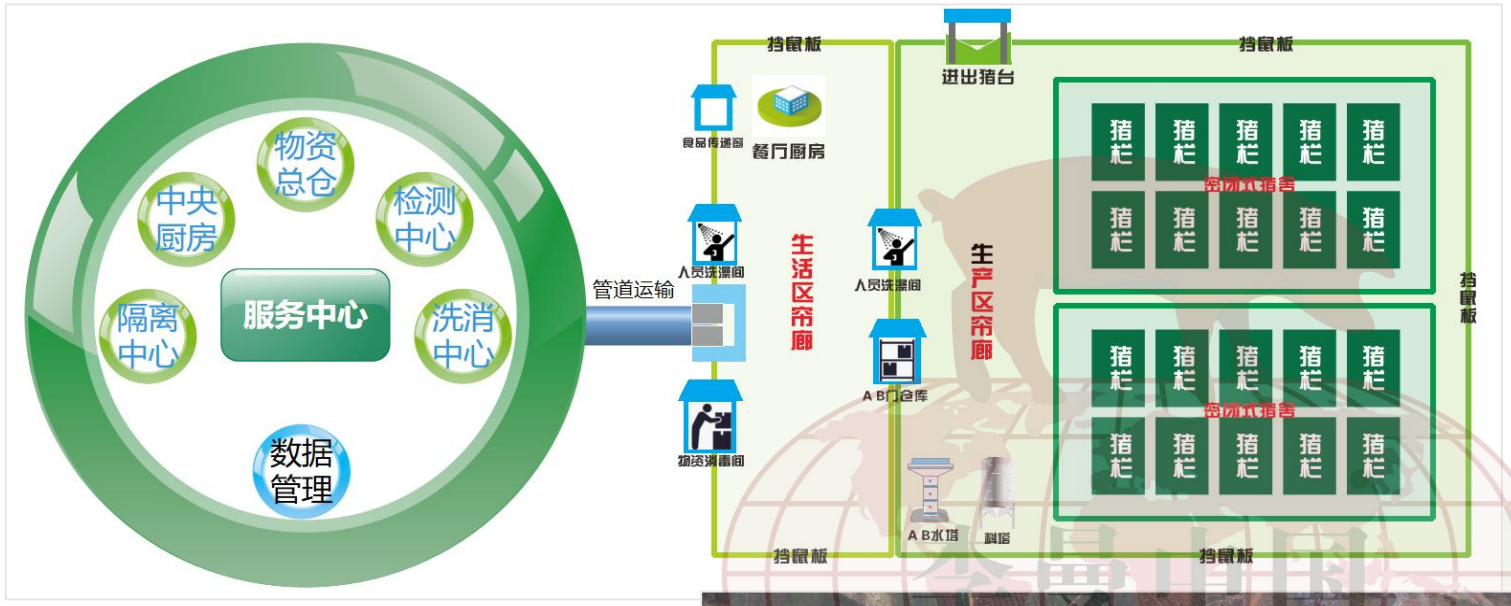


6.1打造“铁桶”猪场 Building "impenetrable" pig farms

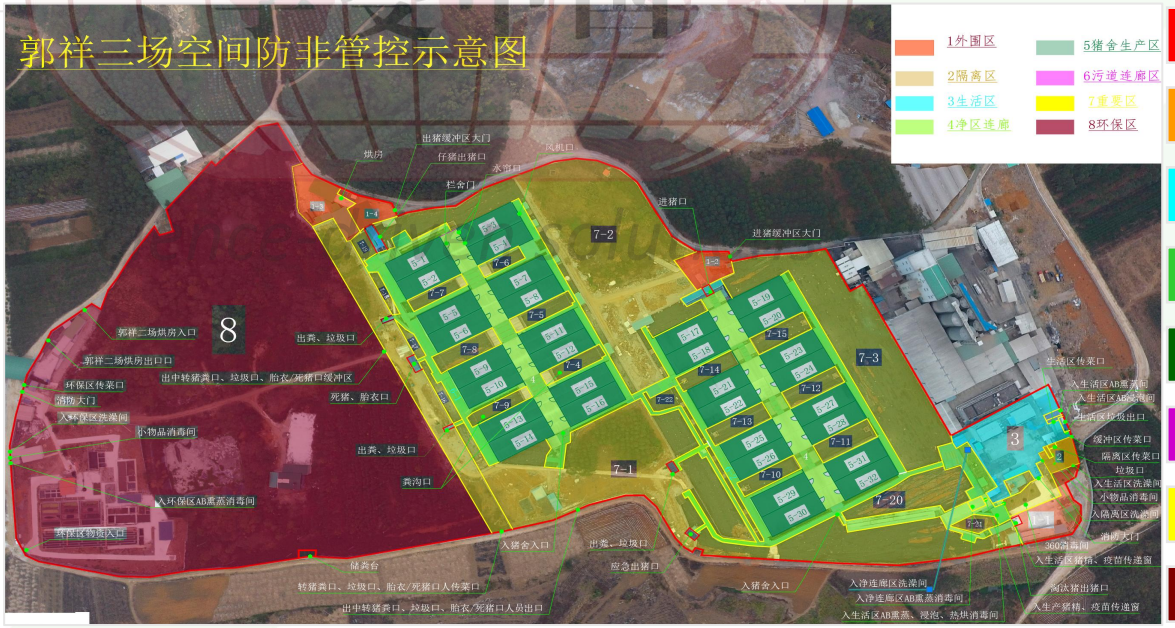


外部服务中心+“铁桶”猪场

External service center + "Iron Bucket" pig farm

生物安全空间结构划分

Biological safety spatial structure division



1外围区：红色

2隔离区：橙色

3生活区：蓝色

4净道连廊区：绿色

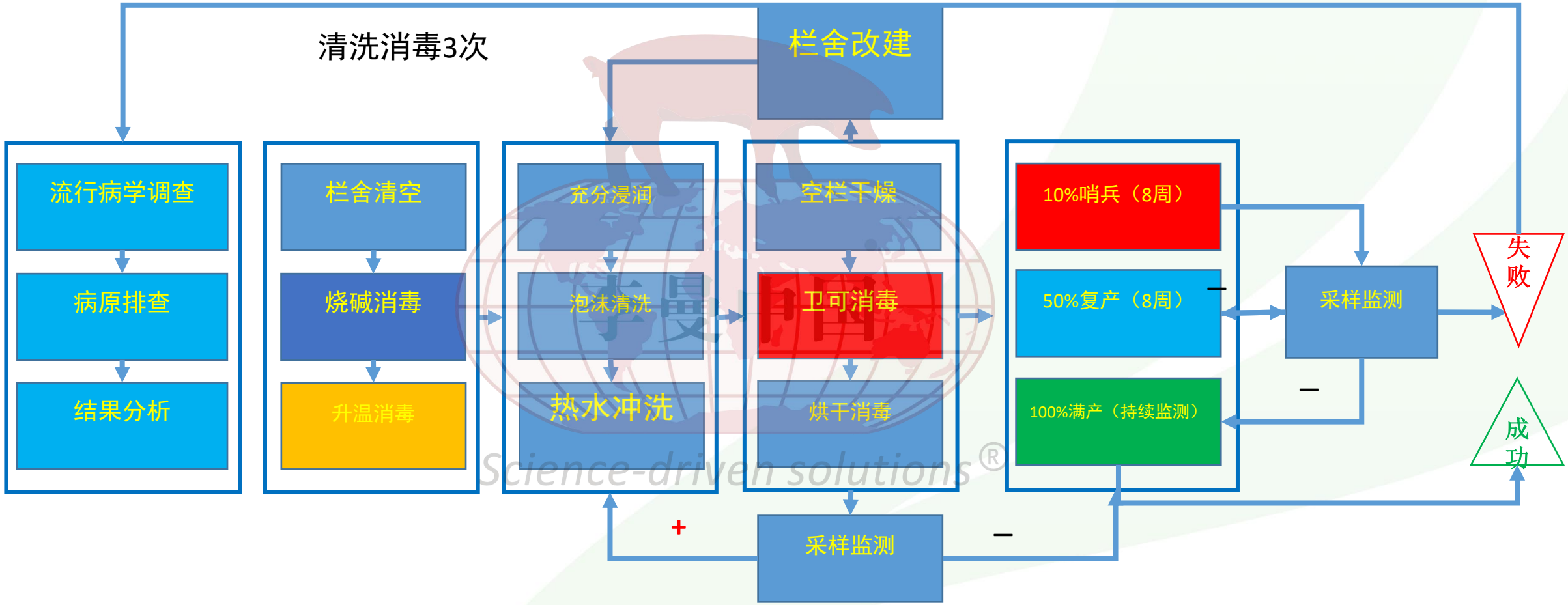
5猪舍生产区：深绿

6污道连廊区：洋红色

7重要区：黄色

8环保区：黑红色

6.2 阳性栏舍洗消烘的操作流程
The operation procedure for disinfection, cleaning and drying of the positive isolation sheds



6.3 ASFV的耐受条件
The tolerance conditions of ASFV

Product	Survival time
Meat with/without bone and ground meat 带/不带骨头的肉和绞肉	105 days
Salted meat 咸肉	182 days
Cooked meat (min. 30 mins @ 70 °C) 熟肉	0
Dried meat 干肉	300 days
Smoked and deboned meat 烟熏去骨肉	30 days
Frozen meat 冻肉	1000 days
Chilled meat 冷鲜肉	110 days
Offal 牛杂	105 days
Skin/Fat (also dried) 皮肤/脂肪（干燥）	300 days
Blood stored at 4 °C 储存在4°C血液	18 months
Faeces at room temperature 室温粪便	11 days
Putrefied blood 腐烂的血液	15 weeks
Contaminated pig pens 受污染猪圈	1 month

Beltrán-Alcrudo et al (2018). FAO

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- **TEM：**
  60℃，20min
  56℃，70min
  25-37 ℃ few weeks
  4 ℃ >1 year
  Frozen meat, several years to ten years

- **pH：**
  4~11.5 无血清 without serum
  4~13.4 有血清 with serum
  未熟肉品、腌肉、泔水中可长时间存活It can survive for a long time in undercooked meat, cured meat and food residues.

- 乙醚、氯仿等脂溶剂可破坏囊膜使其失活
- Lipid solvents such as ether and chloroform can damage the membrane and render it inactive.

6.4 空栏洗消的目的
The purpose of empty tank decontamination

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1）清除有机物
Remove organic matter

清除杂物

Clearing debris

清扫粪污

Remove fecal waste

栏舍初洗

The pen is freshly washed.

泡沫清洗

Foam cleaning

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2）栏舍消毒
Stable disinfection

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高温消毒/
High-temperature disinfection
热风烘烤
Hot air drying

烧碱：1-2%烧碱，清洗前，可疑场地、污染物的消毒；Caustic soda: 1-2% caustic soda, for disinfection of suspected areas and contaminants before cleaning;
复方戊二醛/卫可：1：200稀释，清洗后，所有待消毒区域；
Compound glutaraldehyde/Wacool: Dilute at a ratio of 1:200. After cleaning, all areas to be disinfected.

气体消毒：
甲醛、过氧乙酸、或CID20等
Gas sterilization:
Formaldehyde, peracetic acid, or CID20, etc.

火焰烘烤：异常死亡猪只的栏位地面、料槽、栏片、漏粪板等所有可耐受高温的地方；
热风炉烘烤：60-65℃，2h，栏舍必须密封！
Flame baking: The floor, feed troughs, cage panels, manure discharge plates and all other areas that can withstand high temperatures in the stalls of the deceased pigs;
Hot air furnace baking: 60-65℃, 2 hours. The barn must be sealed!

浸润消毒
烧碱、复方戊二醛、卫可
Dilution disinfection
Caustic soda, compound glutaraldehyde, Voco

气体熏蒸消毒：使用甲醛：高锰酸钾：水=2：1：1的比例进行熏蒸消毒，甲醛使用量为15-30ml/m³；过氧乙酸：3ml/m³熏蒸。
Gas fumigation disinfection: Use a ratio of formaldehyde : potassium permanganate : water = 2 : 1 : 1 for fumigation disinfection. The amount of formaldehyde used is 15-30ml/m³; peracetic acid: 3ml/m³ for fumigation.

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3）抽样检测 Sampling inspection

- **抽样地点**：病猪料槽、漏粪板、粪沟、料塔/房、洗澡通道、生活区宿舍和餐厅（多点取样，混合检测）；
- Sampling locations: pig feed troughs, manure discharge boards, manure ditches, feed towers/rooms, bathing passages, living area dormitories and dining halls (multiple samples taken and combined for testing);
- **抽样频率**：清洗后0、3、5天
- Sampling frequency: 0, 3, and 5 days after cleaning
- **混样检测**：环境样品每处混合一个样品进行检测！
- Sample mixture testing: For each location of the environmental samples, one sample is mixed together for testing!
- **结果处理**：若问题栏舍检测结果为阳性或可疑，请再次清洗并采样检测，直到连续至少1周监测为阴性。
- Result processing: If the test result in the problem section is positive or suspicious, please repeat the cleaning process and take samples for testing again. Continue this process until the test result remains negative for at least 1 consecutive week.

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4）猪群观察
Observation of the Pig Herd

限制饲喂
Limited feeding

限制采食量，定时饲喂，使猪对饲喂有较强的喂食反射！
Limit the amount of feed intake and provide regular feeding to enable pigs to develop a strong feeding reflex!

注意观察
close observation

观察是否有猪只对采食反应迟钝、厌食（剩料过多）、发烧、体表发红等！
Observe whether any pigs show signs of sluggish response to feeding, anorexia (excessive leftover feed), fever, or redness on the body surface!

异常送检
Abnormal sample submission

所有观察有异常的猪只、突然死亡的猪只采样送检！
All abnormal pigs and pigs that die suddenly need to be sampled and sent for testing!

5）加强异常猪只的监测
Strengthen monitoring of abnormal pigs

■ 异常猪只识别 Abnormal Pig Identification

发烧、减料、体表发红、坏死、突死、关节肿胀等逐头采样检测

Taking samples for testing one by one, including fever, reduced food intake, redness on the body surface, necrosis, sudden death, and joint swelling.

■ 临床样本采集 Clinical sample collection

口鼻拭子、咽拭子、血拭子、全血、抗凝血、腹股沟淋巴结

Nasopharyngeal swabs, throat swabs, blood swabs, whole blood, anticoagulated blood, inguinal lymph nodes

1d、7d、14d、28d大范围的采样监测筛查，排查隐性感染；

1d, 7d, 14d, 28d extensive sampling monitoring for screening, to detect latent infections;

■ 监测项目 Monitoring program

抗原Antigen （3-5dpi/7-9dpi）、抗体Antibody （7-10dpi）

6）引种时抗原、抗体监测的意义
The significance of antigen and antibody monitoring during introduction

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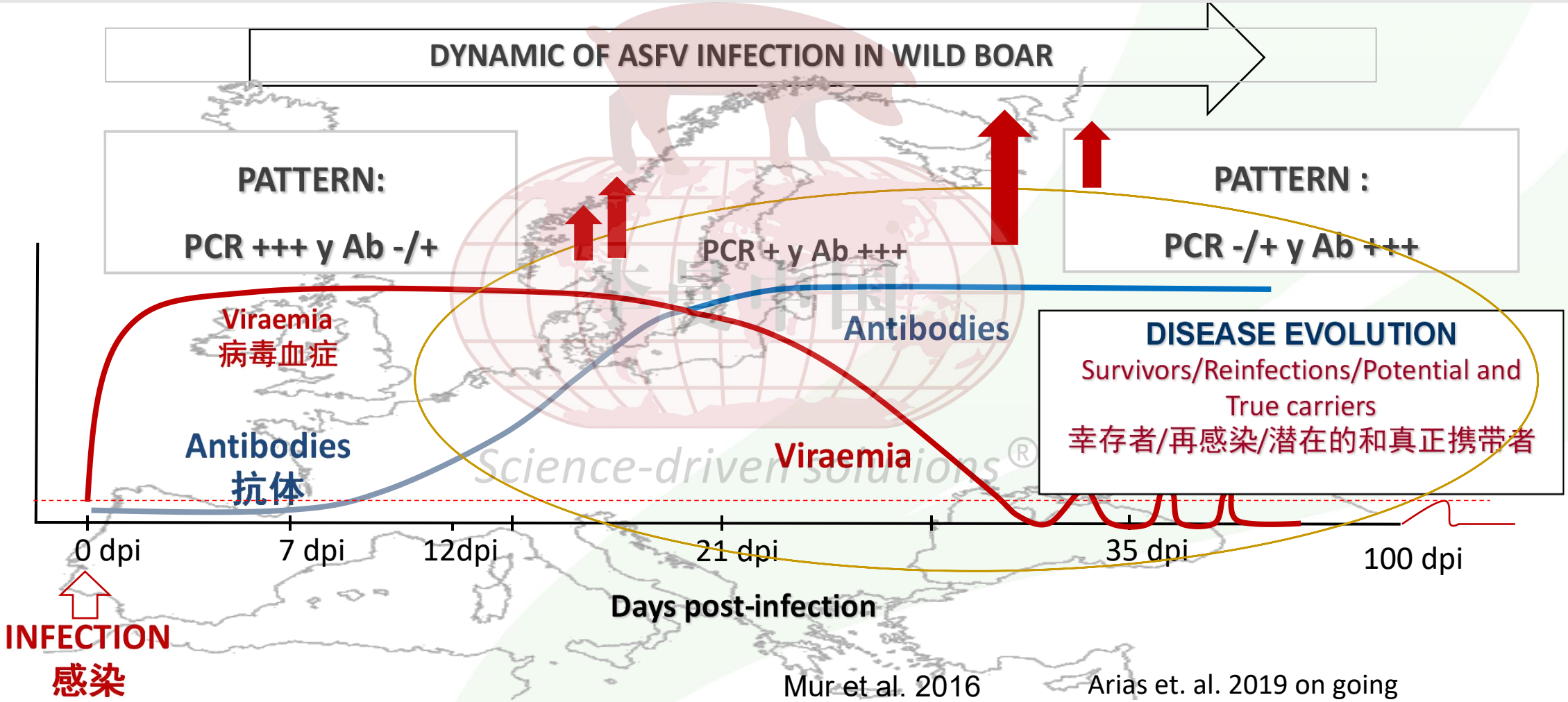
非洲猪瘟感染野猪后抗原抗体的变化趋势
The trend of antigen and antibody changes in wild boars infected with African swine fever



Mur et al. 2016

Arias et. al. 2019 on going

III、总结 Summary

- **统一标准，以防非保猪为首要目标；**

Establish a unified standard to prevent non-pig farmers from targeting this as their primary goal.

- **明确主次，优先解决最致命的问题；**

Clarify the priorities and give priority to addressing the most critical issues.

- **破釜沉舟，永远做好应对最坏局面的打算；**

Break through all barriers and make a commitment to always be prepared for the worst-case scenario.

- **答案永远在现场，不分职级高低，全员参与！**

The answer is always right here, regardless of rank or position. Everyone is involved!

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THANKS

谢 谢

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