饲料禁抗和非瘟下猪场生物安全措施

----饲料和饮水安全

Feed and drinking water biosecurity under AGP-free & ASFV risk

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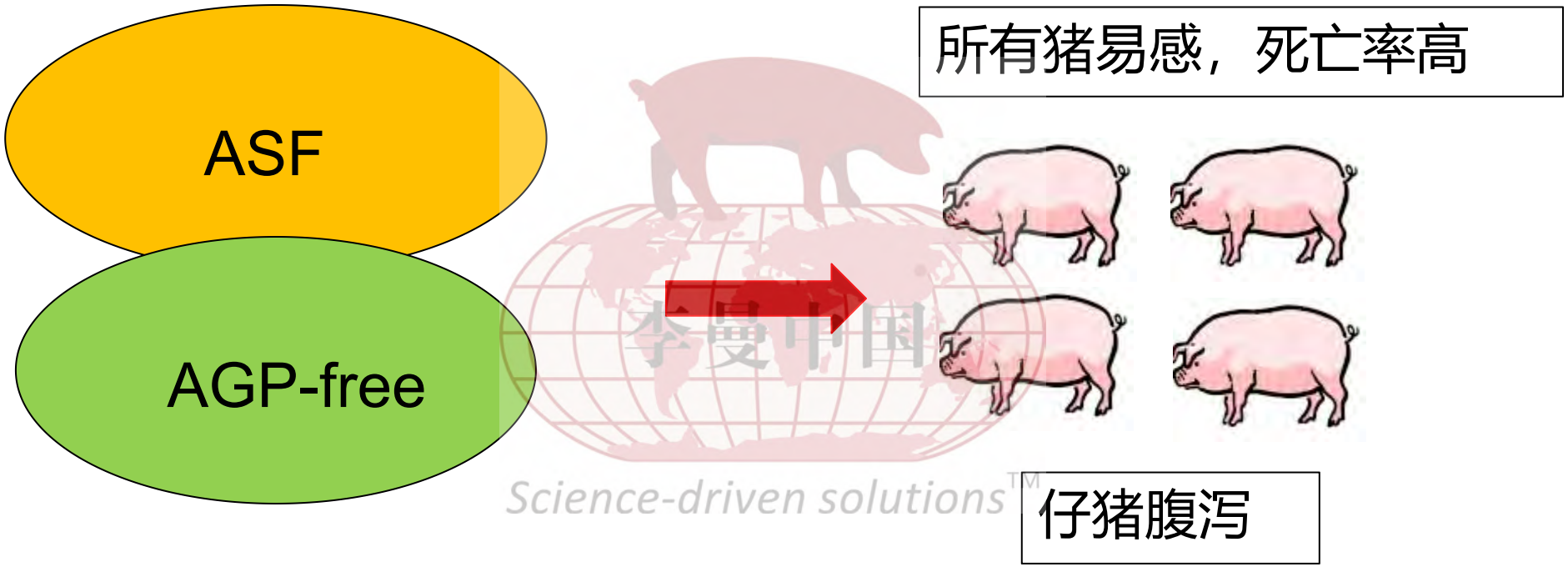
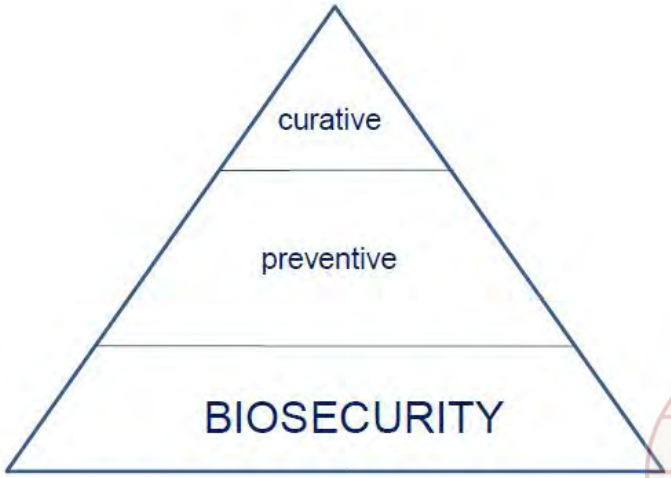
诺伟司国际 Novus International

2020.10.14

当前养猪面临的挑战



生物安全的再认识



- 生物安全是一切健康生产的基础；
- 生物安全是动态调整的；
- 环境载毒量
  - **外围：**病死猪的处理；猪场数量；地下水；交通等；
  - **内部：**密度；阳性个体；营养；洗消压力

你是否关注了饲料和饮水的生物安全?
Do you focusing on the Feed & Drinking water biosecurity?

NOVUS
SOLUTIONS SERVICE SUSTAINABILITY

生物安全防控体系
Biosecurity System

综合防控
Integrated prevention

饲料和饮水生物安全
Feed & water biosecurity

传染源
Infection source

传播途径
Transmission routs

易感动物
susceptible animals

饲料生物安全（Feed biosecurity）

- 饲料生物安全（feed biosecurity）是指饲料产品中不含对畜禽健康造成危害同时也不会在畜禽产品中引起残留、蓄积和转移的有毒、有害物质或因素，同时饲料产品以及通过饲喂该产品而生产的畜禽产品，不会对人体健康或人类的生存环境产生负面影响。
- 影响饲料生物安全的因素主要包括：虫害、螨害和鼠害，饲料中的微生物污染、抗营养因子、有毒有害化学物质及非营养性添加剂带来的污染。饲料中的微生物污染通常指细菌、真菌、病毒和寄生虫等。

武书庚等，2007，中国畜牧杂志

饲料的风险 Risk in feed

成分Ingredient	SVA（FMDV）	ASFV	PSV（SVDV）	PEDV
常规豆粕 Soybean meal-conventional	（+）	（+）	（+）	（+）
有机豆粕 Soybean meal-Organic	（-）	（+）	（+）	（+）
豆油粕 Soy oil cake	（+）	（+）	（+）	（+）
DDGS	（+）	（-）	（-）	NS
赖氨酸 Lysine	（+）	（-）	（+）	（+）
胆碱 Choline	（+）	（+）	（-）	（+）
Vitamin D	（+）	（-）	（+）	（+）
全价料（阳性） Complete feed（+positive）	（+）	（+）	（+）	NS
全价料（阴性）Complete feed（+negative）	（-）	（-）	（-）	（-）

跨国界运输模型transboundary shipping models

- ✓ 储存30天 Stored for 30 days
- ✓ 模拟运输过程环境条件 Simulate the environmental conditions during transportation
- ✓ 初始剂量 Inoculation dose= $10^5 TCID_{50}$

Dee et al., 2018, Plos One

PEDV 在饲料成分中的病毒滴度检测结果
Results of PED (porcine epidemic diarrhea) virus titer in feed ingredients.

DPI 感染后天数	PEDV titer 病毒滴度 (log $TCID_{50}$/mL)							
	Feed 全价料	Plasma 猪血浆	Meat 肉粉	Meat bone 肉骨粉	blood 血粉	SM 豆粕	Core 玉米	DDGS
0	4.28	2.06	2.83	3.06	3.84	3.50	2.84	3.51
7	1.40	0.50	1.51	1.72	1.61	2.83	1.51	1.72
14	0.61	1.06	1.50	1.51	1.51	2.06	0.50	1.51
21	0.50	0.83	1.62	1.51	1.51	1.62	0.50	1.41
28	1.17	0.50	1.51	1.51	1.51	1.51	0.50	0.61
35	0.50	0.50	1.51	1.51	1.51	1.40	0.50	0.50
42	0.50	0.50	1.51	1.51	0.50	0.50	0.50	0.50
49	0.50	0.50	0.50	0.50	0.50	0.72	0.50	0.50
56	0.50	0.50	0.50	0.50	0.50	0.83	0.50	0.50

* Inoculation dose 起始接种量：4.5 logs

Trudeau et al., 2017, Plos One

TGEV 在饲料成分中的病毒滴度检测结果
Results of TGE(Transmissible gastroenteritis) virus titer in feed ingredients

潜伏期（天）	TGEV 病毒滴度 (log $TCID_{50}$/mL)							
	全价料	猪血浆	肉粉	肉骨粉	血粉	豆粕	玉米	DDGS
0	5.61	3.51	6.06	5.28	5.83	7.17	4.51	6.62
7	2.72	3.40	2.51	2.51	3.06	4.95	2.51	2.95
14	2.51	3.51	2.51	2.40	2.29	4.51	3.61	2.51
21	2.51	2.73	1.51	1.51	1.40	5.51	2.18	1.61
28	2.51	2.51	1.51	1.51	1.51	4.73	2.29	2.28
35	2.51	1.51	1.28	1.51	1.51	5.29	1.95	2.06
42	2.51	0.51	0.95	0.61	1.51	4.50	1.61	0.51
49	1.06	0.51	0.51	0.51	0.51	5.17	1.51	0.51
56	0.51	0.51	0.51	0.51	0.51	4.95	0.51	0.51

*Inoculation dose 起始滴度：6.78 logs

Trudeau et al., 2017, Plos One

饲料中病毒最低感染剂量与毒株的关系
Relationship between the viral infection MID and the strains in feed

Pathogen	Feed MID	Source
Indiana PEDV	$10^{1.0}$ $TCID_{50}$	Schumacher et al., 2016
East African ASFV	$10^{2.9}$ HAD_{50}	Parker et al., 1969
Tanzania ASFV	$10^{5.4}$ HAD_{50}	Greig, 1972
Malawi ASFV	$10^{2.0}$ to$10^{5.4}$ HAD_{50} Based on method	Howey et al., 2013
Armenia 08 ASFV	3-25 HAD_{50}	Pietschman et al., 2015
Georgia 2007 ASFV	10^{4} $TCID_{50}$	Niederwerder et al., 2019

中国ASF　Pig/HLJ/18株
China Pig/HLJ/18

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EMi　Taylor & Francis

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Replication and virulence in pigs of the first African swine fever virus isolated in China

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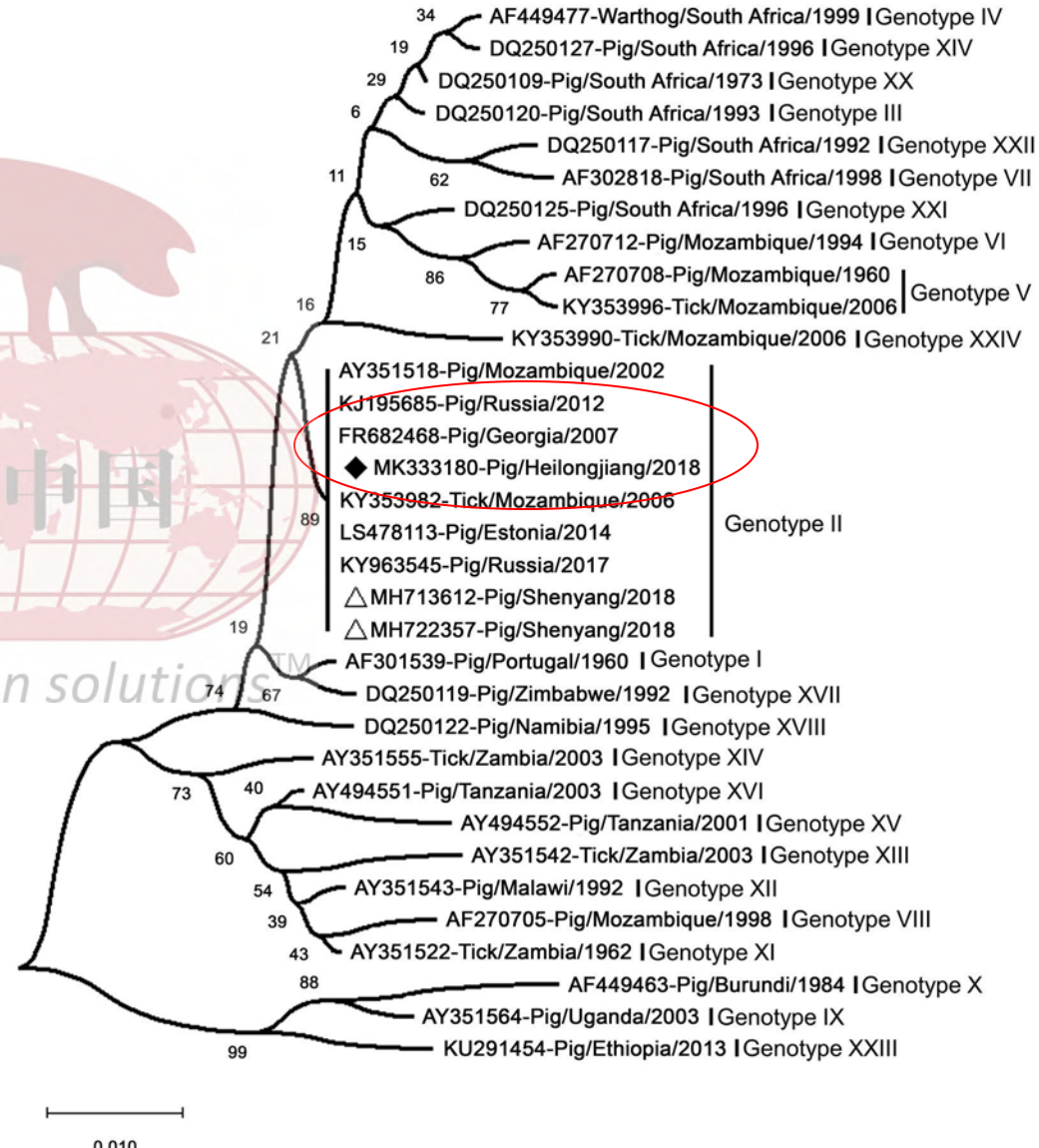
ABSTRACT

African swine fever (ASF) entered China in August 2018 and rapidly spread across the entire country, severely threatening the Chinese domestic pig population, which accounts for more than 50% of the pig population worldwide. In this study, an ASFV isolate, Pig/Heilongjiang/2018 (Pig/HLJ/18), was isolated in primary porcine alveolar macrophages (PAMs) from a pig sample from an ASF outbreak farm. The isolate was characterized by using the haemadsorption (HAD) test, Western blotting and immunofluorescence, and electronic microscopy. Phylogenetic analysis of the viral p72 gene revealed that Pig/HLJ/18 belongs to Genotype II. Infectious titres of virus propagated in primary PAMs and pig marrow macrophages were as high as $10^{7.2}$ HAD_{50}/ml. Specific-pathogen-free pigs intramuscularly inoculated with different virus dosages at $10^{3.5}$–$10^{6.5}$ HAD_{50} showed acute disease with fever and haemorrhagic signs. The incubation periods were 3–5 days for virus-inoculated pigs and 9 days for contact pigs. All virus-inoculated pigs died between 6–9 days post-inoculation (p.i.), and the contact pigs died between 13–14 days post-contact (p.c.). Viremia started on day 2 p.i. in inoculated pigs and on day 9 p.c. in contact pigs. Viral genomic DNA started to be detected from oral and rectal swab samples on 2–5 days p.i. in virus-inoculated pigs, and 6–10 days p.c. in contact pigs. These results indicate that Pig/HLJ/18 is highly virulent and transmissible in domestic pigs. Our study demonstrates the threat of ASFV and emphasizes the need to control and eradicate ASF in China.

ARTICLE HISTORY Received 3 December 2018; Revised 21 January 2019; Accepted 10 February 2019

KEYWORDS African swine fever virus; virus isolation; animal study; pig; transmission



饲料和饮水的风险 Risk in feed & drinking water

Volume 25, Number 5—May 2019

Research

Infectious Dose of African Swine Fever Virus When Consumed Naturally in Liquid or Feed

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Suggested citation for this article

Abstract

African swine fever virus (ASFV) is a contagious, rapidly spreading, transboundary animal disease and a major threat to pork production globally. Although plant-based feed has [illegible] risks for ASFV transmission in feed. We aimed [illegible] oral exposure during natural drinking and feed [illegible] dose ($TCID_{50}$), compared with 10^4 $TCID_{50}$ in feed. The median infectious dose was $10^{1.0}$ $TCID_{50}$ for liquid and $10^{6.8}$ $TCID_{50}$ for feed. Our findings demonstrate that ASFV Georgia 2007 can easily be transmitted orally, although higher doses are required for infection in plant-based feed. These data provide important information that can be incorporated into risk models for ASFV transmission.

(100g 污染的饲料,一次接触
100 grams polluted feed, one touch)

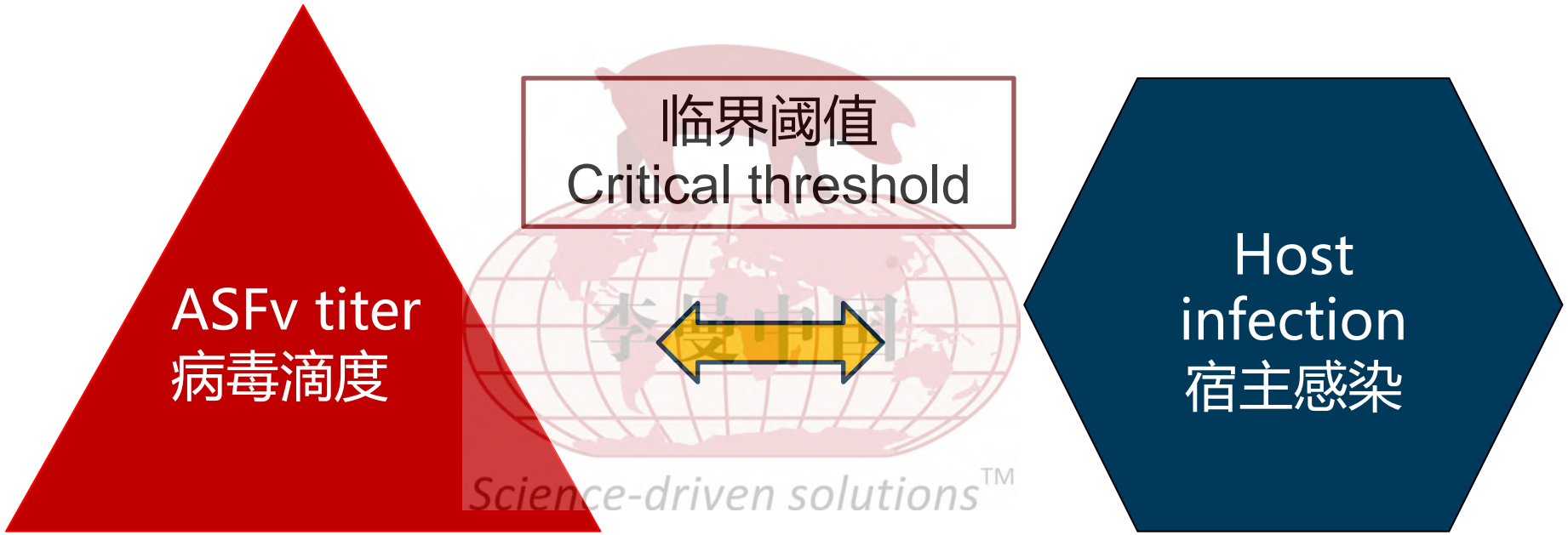
最低感染剂量 Minimum infection dose (MID)

- MID (水源 drinking) = 10^0 $TCID_{50}$
- MID (饲料 feed) = 10^4 $TCID_{50}$

结论 key points

1. 直接证明非洲猪瘟可以通过饮水或者饲喂传播。Direct evidence that ASFv can be transmitted through drinking water and feed
2. 通过水比饲料更容易传染。It is more easily transmitted through water than through feed
3. 接触的频率比剂量更容易导致感染 Frequency of exposure is more likely to lead to infection than dose

病毒活力与宿主感染的关系
Relationship between virus activity and host infection



ASF病毒感染猪发病是需要条件的，和毒株、感染方式和剂量有关
ASF virus infection in pigs requires conditions, depending on the strain, mode of infection, and dosage.

Howey et al., 2013

非瘟下饲料和饮水安全的解决方案

实验1：艾维酸DA®对饲料中非瘟病毒的灭活效果—国内
Exp.1: Inactivation of Activate DA on ASFv in feed- in China

Lab: P4 Lab at the Specific Veterinary Institute;

ASF virus Strain: isolated from China

实验设计 Experimental methods

- **饲料污染模型的建立 Commercial sow feed sample contaminated with ASFv**

  - 饲料表面喷洒10^6 TCID50/ml 的ASFv 病毒液并混和。 Spray ASFV 10ml (10^6 TCID50/ml) on the surface of feed (50g) , mix these well;

- 酸化处理 Treatment group: 添加6kg/t DA到污染的饲料中 0.6% (W/W) DA mixed into the feed, crushed and mixed;

  对照组 Control group: 不添加酸化剂 feed without DA.

- 每周取5g饲料进行实验室检测。take 5g contaminated sample weekly for further testing.

- **实验室检测 Lab tests**

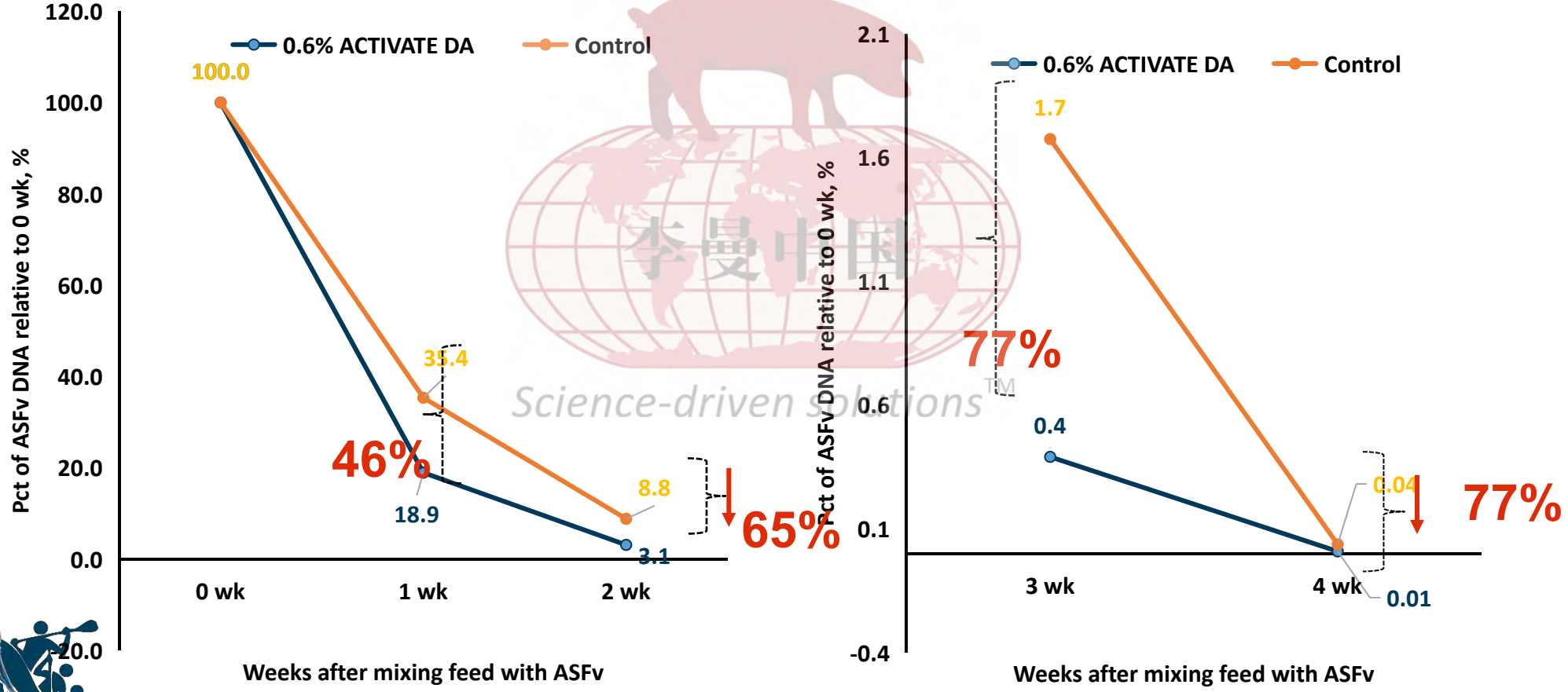
（1）荧光定量测定核酸 Q-PCR test nucleic acid of virus;

（2）在细胞上检测病毒的活力。1 g of the ASFV-contaminated feed sample was added into 9 ml of the medium, after then shaking for 10 min; the supernatant was obtained by centrifugation. The $TCID_{50}$ of virus was determined using PAM cells.

艾维酸DA®可降低饲料中ASFv的核酸
Activate DA can reduce the nucleic acid of ASFv in the feed

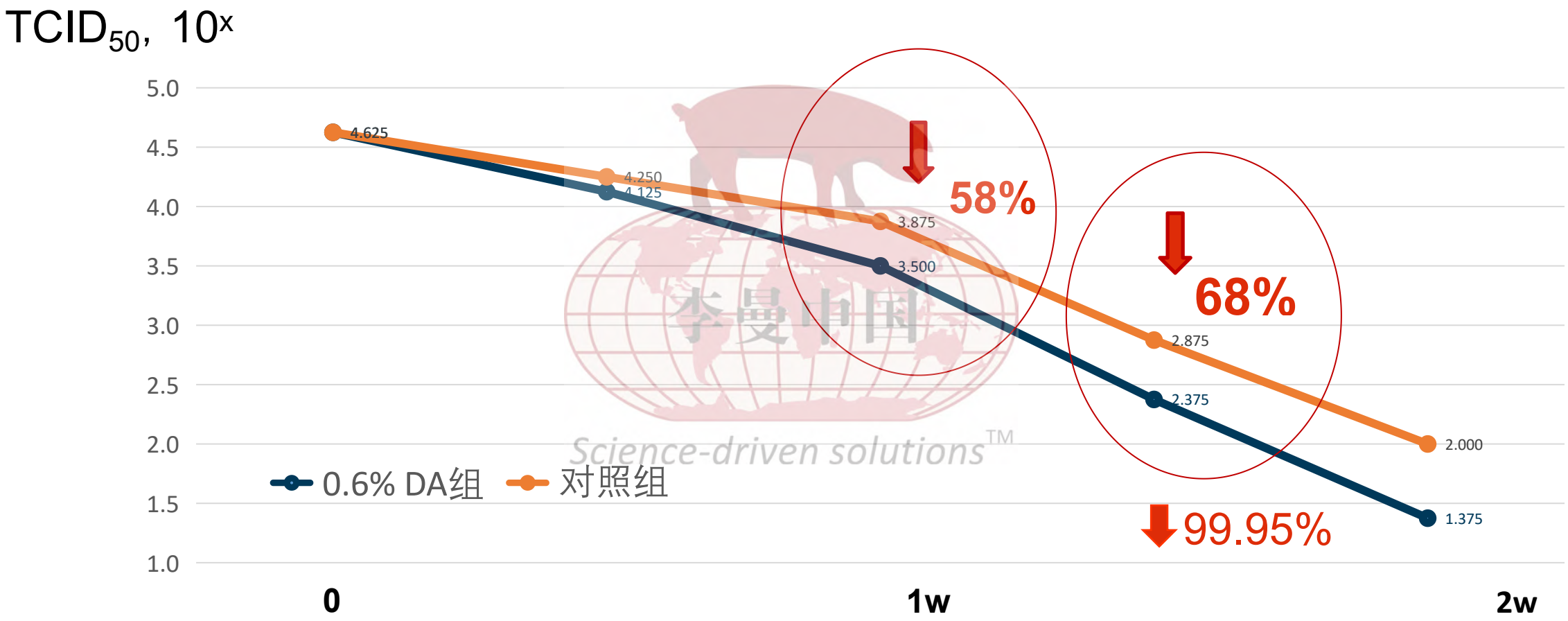
NOVUS SOLUTIONS SERVICE SUSTAINABILITY

病毒核酸下降百分比
Pct of ASFv DNA relative to 0 wk, %



ONE TEAM GOAL ACCELERATE & EXECUTE

艾维酸DA®可降低饲料中非瘟病毒的活力
Activate DA reduces the virulence of ASFv in feed



实验2：艾维酸DA®对饲料中非瘟病毒的灭活效果—越南
Exp.2: Inactivation of Activate DA on ASFv in feed- in Vietnam

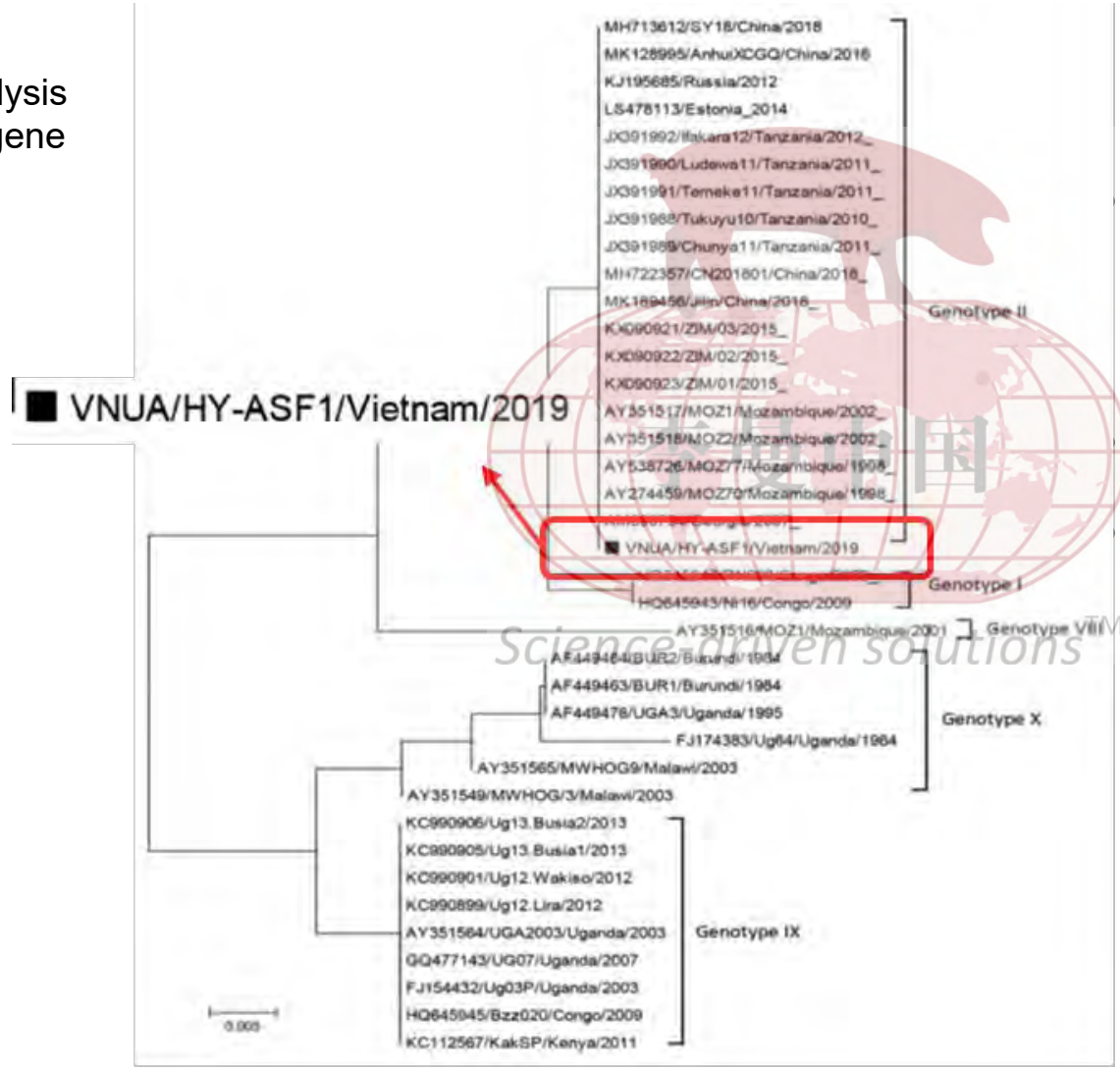
- 越南河内大学兽医生物技术重点实验室 Conducted at Key Laboratory of Veterinary Biotechnology in Hanoi University, Vietnam
- 越南分离的病毒，接种量5 x 10^6 (HAD_{50}) Virus Inoculum: 5 x 10^6 (HAD_{50}) of ASFV isolate (i.e. VNUA/HY-ASF1/Vietnam/2019)
- 样品的采集分别在感染后1，3和7天。Samples were collected at 3 independent times; 1, 3, and 7 days post inoculation (DPI)
- 实验室检测。 荧光定量和活力检测。Using qPCR for viral gene copies and HAD assay for infectivity of ASF virus

实验设计 Experimental Design

Group	Content
负对照 NC	Feed only
正对照 PC (Positive Control)	非瘟污染的饲料 Feed contaminated with ASFV
DA 2	污染的饲料添加DA酸 2.0kg/T Feed treated with Activate DA at 2.0kg/T and contaminated with ASFV
DA 5	污染的饲料添加DA酸 5.0kg/T Feed treated with Activate DA at 5.0kg/T and contaminated with ASFV

毒株 ASFV strain

Fig. 1. Phylogenetic analysis of major capsid protein gene (p72) of ASFv



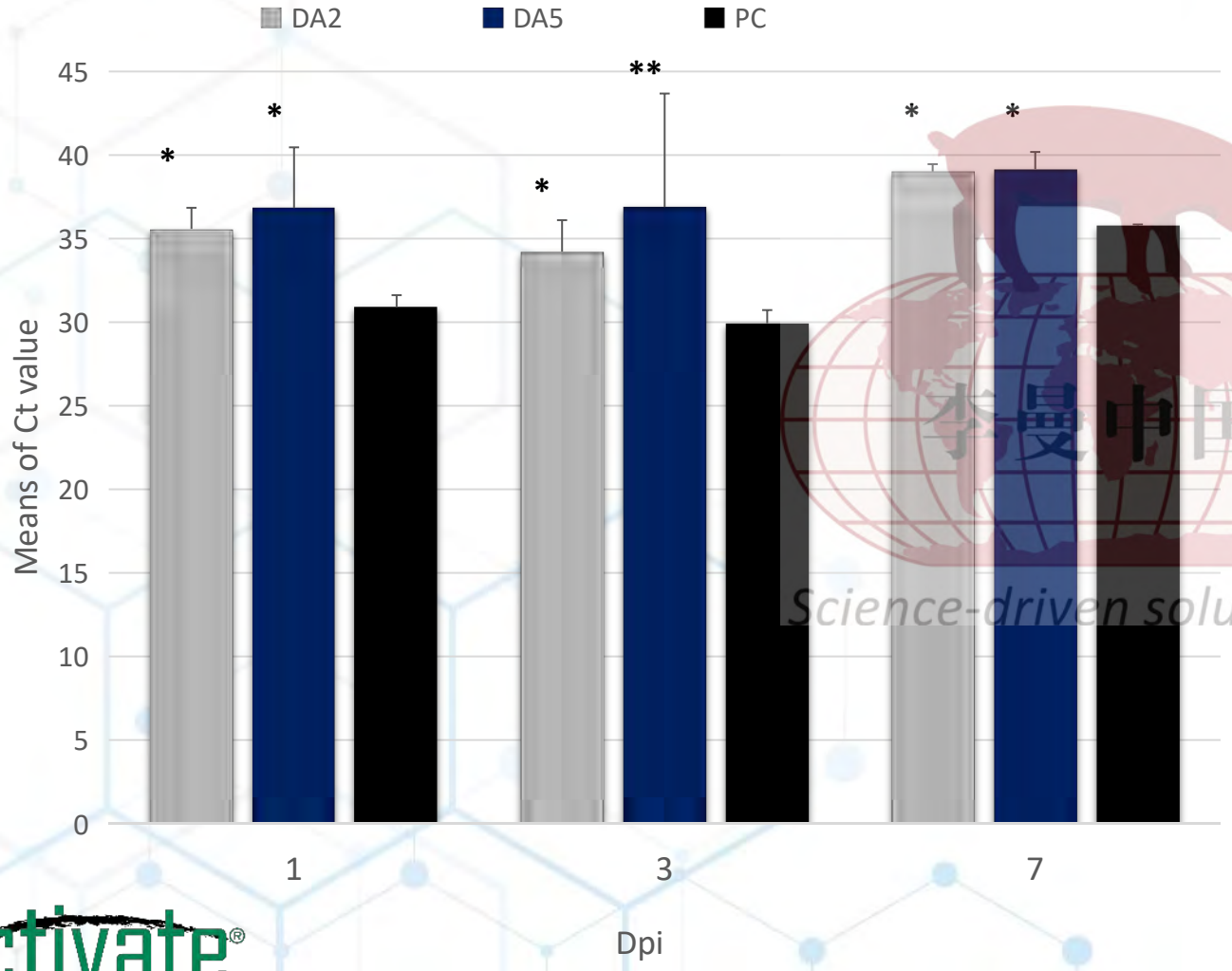
ASFV strain: VNUA/HY-ASF1/Vietnam/2019 isolate in Vietnam, 2019 [2]

该毒株属于ASFv基因II型

和中国分离的 SY18/China/2018 100%同源。

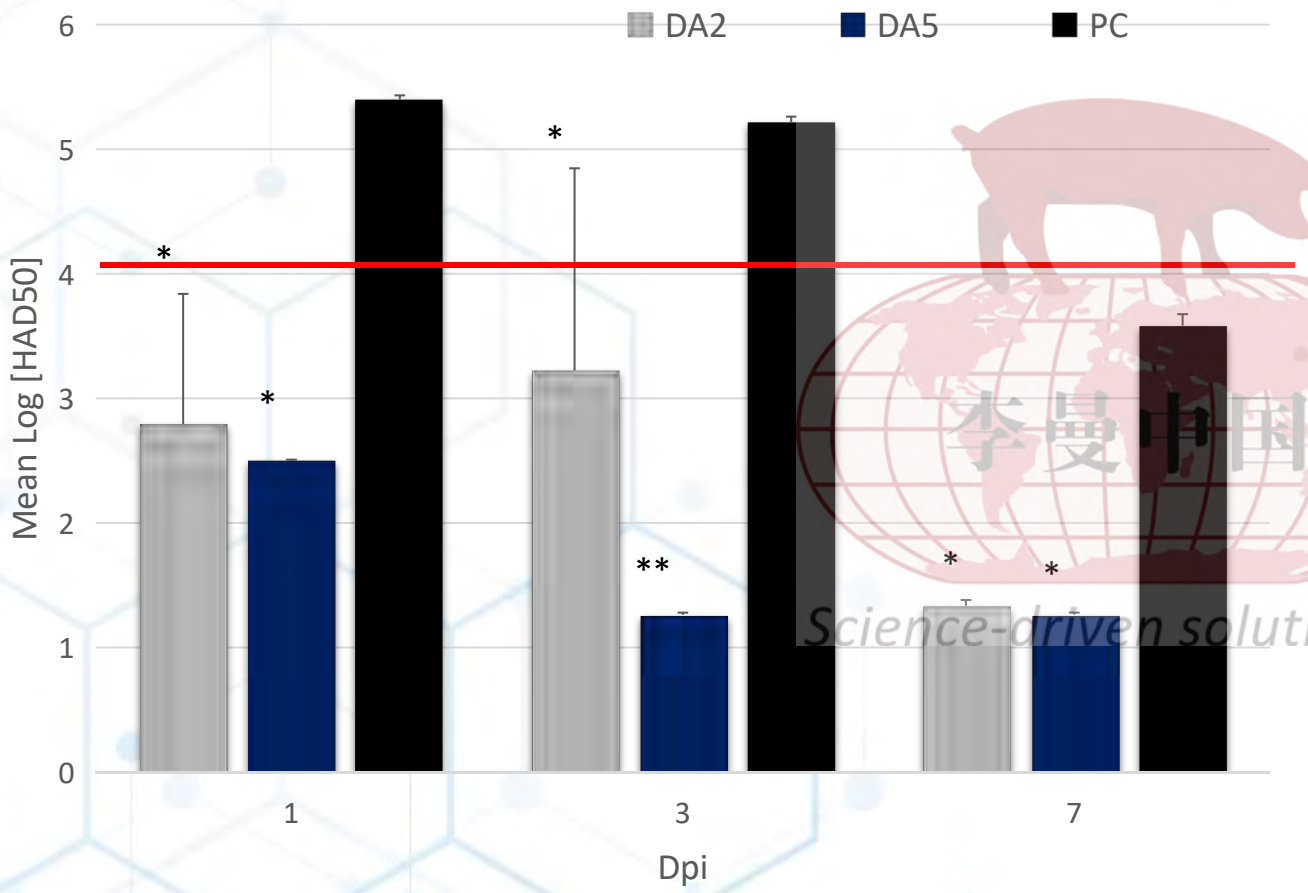
[2] Van Phan Le et al., Emerging infect. Dis. Vol 25 (7), 2019

荧光定量评估非瘟病毒毒力
Assessment of ASFV virulence by qPCR



- 艾维酸DA可明显灭活饲料中的ASFv病毒。 Activate DA showed significant activity against ASFV in feed
- 添加2kg 或5kg 艾维酸DA，仅需要1天，就可降低90%以上的病毒核酸。2kg or 5kg of Activate DA are able to reduce almost 90% of ASFV virus particle within 1 day
- 艾维酸DA可有效杀灭饲料中的ASFv病毒。Activate DA (2kg and 5kg) showed prompt virucidal activity against ASFV in feed

HAD_{50}评估非瘟病毒毒力
Assessment of ASFV virulence by HAD_{50}



- 艾维酸DA可明显降低饲料中ASFv病毒活力。The virucidal activity of Activate DA can significantly decreased the viral activity of ASFV in feed
- 艾维酸DA 可快速杀灭饲料中90%以上的病毒粒子。Activate DA showed faster reaction against ASFV in feed (>90% reduction)
- 添加5kg艾维酸DA，3天就可几乎完全清除饲料中的ASFv病毒活力。5kg of Activate DA completely remove the viral activity of ASFV in fed within 3 days

上述实验的结论
The conclusion of both experiments

- 2kg艾维酸®DA即可降低饲料中非瘟污染的风险。 Activate DA （2kg） can reduce the risk of ASFV contamination in feed.
- 5kg及以上艾维酸®DA不仅能够明显降低饲料中非瘟的核酸拷贝数，还可显著降低病毒活力。5kg and above Activate DA can not only obviously reduce the number of ASFV nucleic acid copies in the feed, but also significantly reduce the virus viability.
- 艾维酸®DA 可有效杀灭饲料中的ASF病毒。Activate DA provides both strong virucidal activity and faster reaction against ASFV in feed.

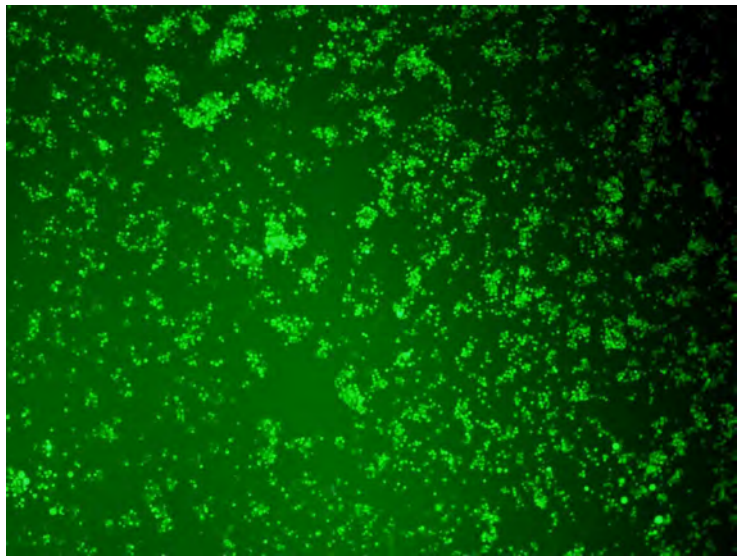
实验3：艾维酸WD®对饮水中非瘟病毒的灭活效果—国内
Exp.3: Inactivation of Activate WD on ASFv in drinking- in China

Lab: P4 Lab at the Specific Veterinary Institute;

ASF virus Strain: isolated from China

实验方法 Experiment methods

- 构建水体污染的模型。 “pollution-disinfection” process with ASFV simulated
- 含有绿色荧光蛋白标签的毒株。Virus selection： green fluorescent protein （GFP）of ASFv indicates Virus is ASFV-GFP, which is epidemic in China;
- 感染的剂量。Infection dose：400-1000$TCID_{50}$/ml
- 艾维酸WD干预。 Activate WD: certain amount to disinfect the water
- 作用时间。Inactivation period：1-120min

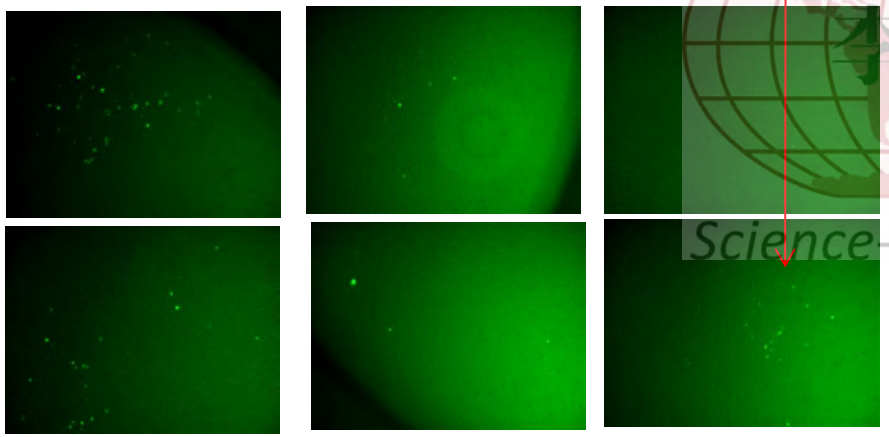


荧光显微镜下观察感染ASFV猪原发性肺巨噬细胞(PAM)
Primary pig lung macrophages（PAM）of swine infected by ASFV under fluorescence microscope

艾维酸WD®对饮水中非瘟病毒的灭活效果
Inactivation of Activate WD on ASFv in drinking water

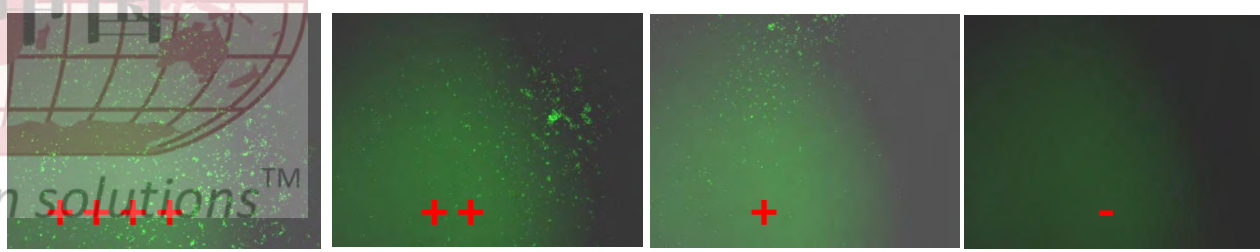
NOVUS SOLUTIONS SERVICE SUSTAINABILITY

Incubation	Concentration of Activate WD in water		
	0	0.05%	0.10%
1min	+++	+	+/-
5min	+++	+	-
10min	+++	+	+/-
30min	+++	+	-



细胞孵育48h后观测的结果。接种剂量：400TCID$_{50}$/ml
Incubation and observation of PAM for 48 hours after inactivation with/without Activate WD

Incubation	Concentration of Activate WD in water			
	0	0.08%	0.10%	0.15%
10min	++++	++++	++++	++/-
30min	++++	++++	++++	- /-
60min	++++	++++	+++	+/-
90min	++++	++++	++/-	-/-
120min	++++	++++	++	-/-



细胞孵育96h后观测的结果。接种剂量：1000TCID$_{50}$/ml
Incubation and observation of PAM for 96 hours after inactivation with/without Activate WD

ONE TEAM GOAL ACCELERATE & EXECUTE

实验结论 Conclusion

- 0.05%及以上浓度的艾维酸WD，仅需1min，对ASFV即有明确的灭活效果。Activate WD with a concentration of 0.05% and above has a clear inactivation effect on ASFV within one minute.
- 0.15%艾维酸 WD作用30min以上可完全灭活病毒ASFV（最低感染量的1000倍）。0.15% Activate WD can completely inactivate the ASF virus (1000 times the MID) for more than 30 minutes.

针对多种病毒和禁抗下饲料安全的解决方案

饲料生物安全也是造成断奶仔猪腹泻的因素之一

- 生理因素：胃酸断奶后被饲料中和，导致pH上升而抗菌性能下降
- 应激因素：断奶、低温、有害气体等
- 营养因素：仔猪断奶前乳糖酶不足、断奶后胰蛋白酶不足、饲料成分不合理、养分消化率低，饲料品质差
- 病原因素：

  病毒性：传染性胃肠炎、流行性腹泻、轮状病毒等

  细菌性：大肠杆菌、沙门氏菌、魏氏梭菌等

  寄生虫：球虫、结节虫等

替抗产品的对比

类型	试验数量	ADG 变化 %	FCR 变化 %
抗生素	13	+8.6	-3.5
有机酸/酸化剂	45	+6.6	-2.3
益生素	13	+2.2	-1.8
精油	23	+2.5	-1.4
酶制剂	9	+2.0	0
寡糖	4	+0.9	-2.2

Lars et al., 2019

Table 2. Percentage of trials reporting a significant increase (+) or decrease (-) in ADG, ADFI, G:F and mortality.

Category	Number of Trials	Response	ADG, %	ADFI, %	G:F, %	Reduced mortality, %
DFM	311	-	1.6	3.2	1.0	0.0
		+	39.9	12.9	25.7	4.8
Yeast	98	-	1.0	1.0	0.0	1.0
		+	23.5	12.2	11.2	1.0
Prebiotics	99	-	0.0	1.0	0.0	0.0
		+	11.1	6.6	9.1	0.0
Oligosaccharides	92	-	4.3	3.3	0.0	0.0
		+	18.5	8.7	20.7	2.2
Starch/Fiber	281	-	15.3	12.5	8.5	0.4
		+	8.9	7.8	6.8	1.4
Organic Acids	151	-	1.3	0.7	0.0	0.0
		+	31.8	12.6	17.9	6.6
Botanicals	365	-	2.2	3.8	1.6	0.3
		+	23.3	9.3	16.4	0.8
Lysozyme	9	-	11.1	0.0	11.1	NR
		+	44.4	11.1	33.3	NR
Zinc/Copper	613	-	0.5	0.8	0.5	0.0
		+	38.7	24.0	19.4	1.8

引自 Gabler & Schweer, 2017,
Proceedings 78th Minnesota Nutrition Conference, Sept 2017

艾维酸DA具有最强的抑制PEDV病毒活力
DA has the strongest inhibit PEDV activity

Additive 添加剂商品名	Active ingredients 主要活性成分	pH	Delta value with additive in days Delta[1] 值（天数）
Activate DA	**organic acids and 2-hydroxy-4-methylthiobutanoic acid** 2-羟基-4甲硫基丁酸（羟基蛋氨酸），富马酸和苯甲酸	5.50	0.44
Product A	**phosphoric, fumaric, lactic, and citric acids** 磷酸、富马酸、乳酸和柠檬酸	5.74	3.28
Product B	**phosphoric, citric, and lactic acids** 磷酸、柠檬酸和乳酸	5.84	7.24
Product C	**orthophosphoric, citric, fumaric, and malic acids** 食用磷酸、柠檬酸、富马酸和苹果酸	5.73	13
Salt	Nacl 氯化钠	5.84	11.42
Control	**None**	5.82	17.23

[1]the first time to reduce the virus titer by 1 log
首次将低一个病毒滴度（log）所用的时间

Trudeau, et, al 2015

艾维酸显著降低SVA病毒滴度
Activate DA obviously reduce the SVA titer

Mitigants 缓解剂	D37 SVA titer * 感染37天后SVA病毒滴度
Activate DA	1.175 logs
Product A	1.5 logs
Product E	1.3 logs
Product I	3.05 logs
Product J	3.3 logs
（+）control	4.5 logs

*: Inoculation dose= 5 logs 接种剂量

Dee, 2018. Leman conference

艾维酸DA®缓解病毒感染----活猪试验
DA mitigated virus infection– in vivo

NOVUS SOLUTIONS SERVICE SUSTAINABILITY

	Infection (pig) 病毒感染			Disease (pen) 临床病变			Gain (pen)增重
Treatment	PRRSV	PEDV	SVA	PRRSV	PEDV	SVA	ADG
0.5% (5kg)	0%	23%	0%	0%	0%	0%	1.65
0.15% (1.5kg)	0%	93%	0%	0%	0%	0%	1.41
(+) control	33%	93%	7%	46%	75%	17%	1.43

= no infection

= virus recovered from animals

= diarrhea, dyspnea rough hair, lameness

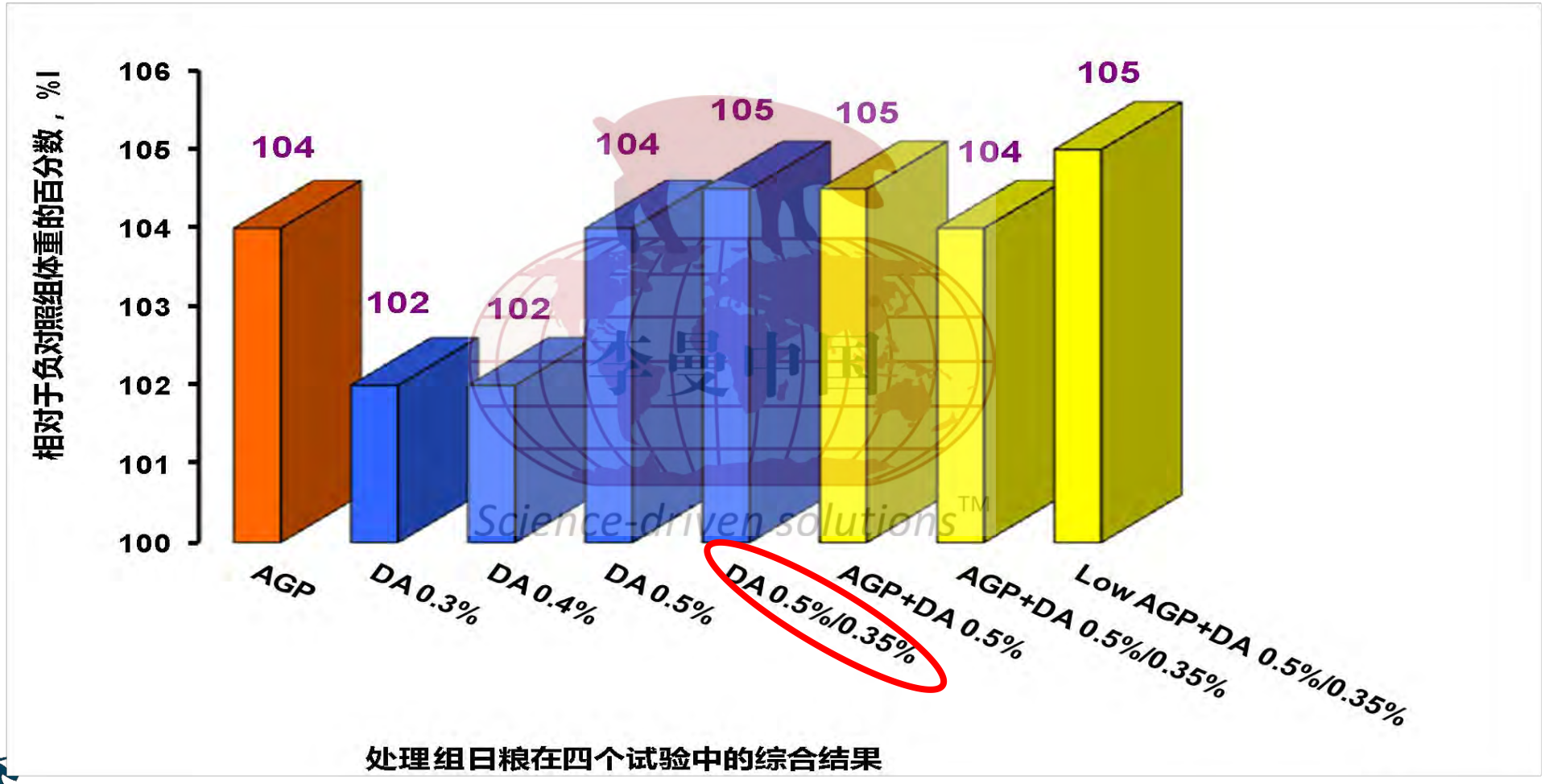
= normal

= lbs/day

1，添加DA干预后，PRRSV和SVA不发病，无任何临床症状；不添加，出现病毒疾病。
2，高剂量DA防控效果好，对生产性能提升显著；低剂量添加，也能起到对病毒性疾病的防御。

ONE TEAM GOAL ACCELERATE & EXECUTE

艾维酸®DA对断奶仔猪生产性能的影响体重提高4-5%（四个试验的结果）



DA 0.3%, 0.4%, & 0.5%: ACTIVATE Starter DA in Phase I to IV; DA 0.5%/0.35%: ACTIVATE Starter DA 0.5% in Phase I & II and 0.35% in Phase III & IV; AGP = 卡巴氧或者 泰乐菌素或金霉素组合.

Gary Alee, 2009

艾维酸®DA显著降低仔猪腹泻

指标	Ⅰ	Ⅱ	Ⅲ	Ⅳ
	复合有机酸组 3kg	缓释型 复合有机酸3kg	包被 复合有机酸3kg	艾维酸®DA 3kg
头均初重	7.36±0.02 a	7.28±0.06 a	7.23±0.01 a	7.39±0.03 a
头均末重	11.38±0.20 a	11.08±0.01 a	11.07±0.20 a	11.63±0.23 a
头均日增重	0.335±0.02 a	0.317±0.01 a	0.320±0.02 a	0.354±0.02 a
头均日采食量	0.383±0.01 a	0.362±0.01 a	0.380±0.02 a	0.415±0.03 a
料重比	1.145±0.04 a	1.140±0.02 a	1.188±0.01 a	1.172±0.03 a
腹泻率	5.27±6.46ab	9.40±4.93 a	10.0±0.00 a	1.27±0.46 b

注：同行间肩标字母相同，差异不显著（P＞0.05）；a，b，差异显著（P＜0.05）。下同。

总结 Summary

关键信息 Take home message

- 加强关注饲料和饮水的生物安全。Pay more attention to the biosecurity of feed and drinking water under ASF & AGP-free.
- 艾维酸®DA双重功效，不仅可以降低饲料中ASFV等多种病毒的风险，还在抗腹泻中发挥重要作用。 Activate DA not only reduces the risk of multiple viruses such as ASFV in feed, but also plays an important role in anti-diarrhea in piglet.
- 艾维酸®WD可快速杀灭饮水中的非瘟病毒，确保水源的生物安全。 Activate WD provides both strong virucidal activity and faster reaction against ASFV in drinking water to enhance the biosecurity of pig farm.


Thank You
Merci
Welalin
Mamana
Salamat
Matondo
Grazie
Kia Ora
Khob Khun
Maake
Gracias
Kiitos
Spasibo
Cam on ban
Multumesc
Vinaka
Arigato
Terima Kasih
Asante
Dhanyavaad
Obrigado
Dank Je
Mochchakkeram
Matur Nuwun
Ua Tsaug Rau Koj
ありがとう
ขอบคุณ
شكرا
谢谢
धन्यवाद
Ευχαριστώ