Metagenomics: Application and implication in a sow abortion case study

母猪群集中流产的病原分析

宏基因组测序技术的临床应用

JY

Chengdu

2024-06

提纲

- 前言与综述
- 病案及问题的提出
- 宏基因组技术应用
- 流行病学分析及确诊
- 总结

1

前言：重大疫情的推动 与 国外经验

1.1 猪病原体核酸检测的临床：问题导向

- 常年从事面向急/难/险/重问题 寻找实际答案的工作
  - 90s年代的临床鉴别诊断
  - 新式猪场工艺设计
  - 场内选育关键技术
  - …
  - 不笑不足以为之道

1.1 猪病原体核酸检测的临床：重大疫情推动

- 快速进入临床应用、在应用中解决技术问题，成为主要路径。
- 集约化养猪的重大疫情，成为猪病原**精准诊断技术**的主要动力，特别是分子诊断技术。
- 2007年开始的猪高致病性蓝耳病，`毁灭性`疫情的首次考验
  - 1998年的虽然凝胶PCR，….
    - 分子水平的抗原确诊
    - 因便利性/经济性问题，未能全面进入临床
  - 2007年，qPCR技术，解决阴性/阳性的高度**准确性及定量**。
  - 2008年，病原体**部分基因组的测序。ORF5测序**
    - 外送检测，但已经有**革命性的**临床意义。
    - 后续用基因序列比较亲缘距离、进化树分析等等，都是我们自行解决的方法

1.1 猪病原体核酸检测的临床：重大疫情推动

- 2018年非洲猪瘟疫情的出现，`毁灭性`疫情的再次考验
  - **在多重高压之下**，抗原检测(阴性vs阳性)成为问题。
  - **qPCR的引物设计；样品合样方法；采样的配对检测；多重检测引物；**
  - **网格化采样；咽拭子工具；…**
  - 每一步都是关键时候开发的出的关键检测方法。
  - 核心还是qPCR广泛进入临床！

1.2 从畜主基因组，到病原体基因组

- 2014年开始进入广泛应用的猪基因组芯片检测→辅助选育；
- 2016年《未来兽医总监系列培训班-**新翼培训班**》引入 下一代测序NGS进入临床尝试。
  - PCV3刚刚发现
  - 美国发现赛尼卡谷病毒
- **宏基因组应用在2014年前，仍属科技前沿**

猪基因组芯片用于选育, 2011
功能基因关联 + 全基因组选择

RESEARCH ARTICLE

Microbiome overview in swine lungs

Franciele Maboni Siqueira[1], Esteban Pérez-Wohlfeil[2], Fabíola Marques Carvalho[3], Oswaldo Trelles[2], Irene Silveira Schrank[1], Ana Tereza Ribeiro Vasconcelos[3], Arnaldo Zaha[1]*

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OPEN ACCESS

Citation: Siqueira FM, Pé... FM, Trelles O, Schrank IS, Vasconcelos ATR, et al. (2017) Microbiome overview in swine lungs. PLoS ONE 12(7): e0181503. https://doi.org/10.1371/journal.pone.0181503

Editor: Gourapura J. Renukaradhya, The Ohio State University, UNITED STATES

Received: January 19, 2017

...nomic distribution showed a great abundance of bacteria. The most common microbial families identified from pneumonic swine's lungs were *Mycoplasmataceae*, *Flavobacteriaceae* and *Pasteurellaceae*, whereas in the carrier swine's lungs the most common families were *Mycoplasmataceae*, *Bradyrhizobiaceae* and *Flavobacteriaceae*. Analysis of community composition in both samples confirmed the high prevalence of *M. hyopneumoniae*. Moreover, the carrier lungs had more diverse family population, which should be related to

猪肺组织中的细菌群, 2016
未明原因肺炎的屠宰场肺组织
DNA细菌群。二代测序宏基因组

nature microbiology

PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16161 | DOI: 10.1038/NM

A reference gene catalogue of the pig gut microbiome

Liang Xiao[1†], Jordi Estellé[2†], Pia Kiilerich[3†], Yuliaxis Ramayo-Caldas[2], Zhongkui Xia[1], Qi... Suisha Liang[1], Anni Øyan Pedersen[4], Niels Jørgen Kjeldsen[4], Chuan Liu[1,5], Emmanuelle Joël Doré[6,7], Nicolas Pons[7], Emmanuelle Le Chatelier[7], Edi Prifti[7‡], Junhua Li[1,8], Huijue J... Xun Xu[1], Stanislav D. Ehrlich[7,9], Lise Madsen[1,3,10], Karsten Kristiansen[1,3*], Claire Rogel-C... and Jun Wang[1,3*]

The pig is a major species for livestock production and is also extensively used as the preferred model species for analyses of a... that sex, age and host genetics are likely to influence the pig gut microbiome. Analysis of the prevalence of antibiotic resistance genes demonstrated the effect of eliminating antibiotics from animal diets and thereby reducing the risk of spreading antibiotic resistance associated with farming systems.

Domesticated pigs are a major food source worldwide and thus important in global food security, but large-scale pig farming faces environmental challenges. Increasing the understanding of the interaction between the gut microbiota and the host has the potential to facilitate knowledge-based development of sustainable pig production by increasing feed efficiency and general health in pig farming. Furthermore, the spread of antibiotic-resistant bacteria and genes is a matter of great global concern, with legislation and research in this area being important issues[6–11]. Hence, an evaluation of the impact of prohibiting the use of antibiotics as growth promotants is needed.

Results

We collected 287 faecal samples from France (100 pigs), Denmark (100 pigs) and China (87 pigs), from 17 breeds or selected lines from 11 farms. The pigs varied in age, sex and antibiotic supplementation (Supplementary Table 1). Deep sequencing of faecal DNA samples generated 1,758 Gb of high-quality data with ... per sample (Supplementary Table 2), and al...

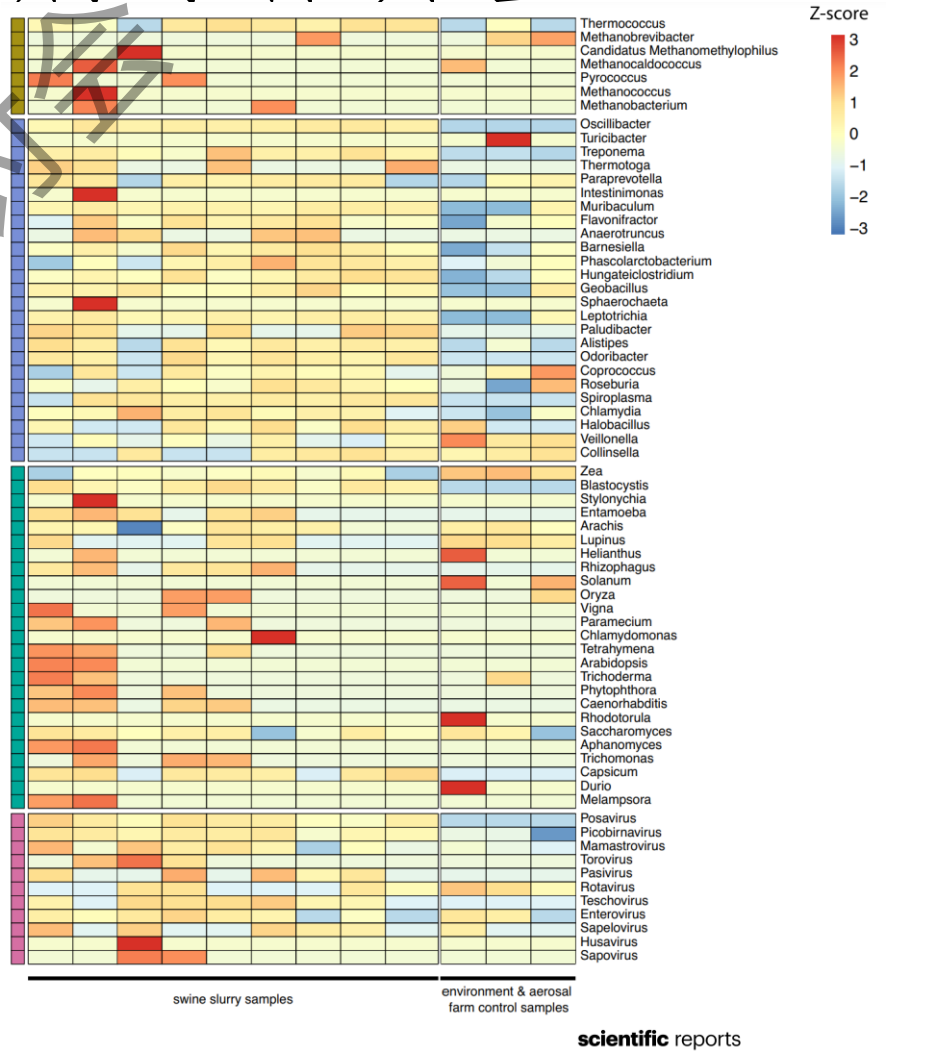
...human reference catalogue could be assigned ... At the genus level, most of the annotated genes ... *Prevotella*, followed by *Bacteroides* (0.80%), ... *Ruminococcus* (0.72%) and *Eubacterium* (0.51... tated metagenomic species (MGS), 497 MGS ... known bacteria, with the remaining 222 MGS ... unknown microbial entities. A total of 353 M... to a phylum, but only 33 and one could be ... and a species, respectively (Supplementary ... MGS, *Prevotella* was the bacterial genera t... (0.97%) could be annotated, followed by *Ru... Eubacterium* (0.42%), *Lactobacillus* (0.42%... (0.41%). Functional classification of annot... MGS using KEGG (Supplementary Fig. ... (Supplementary Fig. 2b) revealed a predon... related to genetic information processing (re... metabolism (carbohydrates, amino acids and ... information processing (membrane transpo... common set of 4,430 NR genes, 36 MGS and ... tated functions shared by 100% of the 287 pi... the existence of a core of genes, species and ... microbiome in pigs (Fig. 1b and Supplemen...

[1]BGI-Shenzhen, 518083 Shenzhen, China. [2]GABI, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France. [3]Laborat... Molecular Biomedicine, Department of Biology, University of Copenhagen, DK-2100 Copenhagen, Denmark. [4]SEGES Pig Research Centre

猪粪中的微生物基因群, 2016
法国/丹麦/中国猪的粪便
DNA细菌群。二代测序宏基因组

1.2 病原体宏基因组→致病病原体筛查

- 宏基因组用于猪群体性健康问题的**致病性微生物病原体**筛查，正在成为趋势。
- 杜克大学/加州大学，2022。2018从**北卡2处育肥舍**的粪便、空气、环境9个样品中，通过二代测序的宏基因组检测，发现了多种可能导致人猪共患、先前未知致猪病的病毒和细菌。
  - 3800存栏；注射器采粪沟样品等
- 怀疑猪粪便及废水可能是病菌传染源。



scientific reports
OPEN
Metagenomic characterization of swine slurry in a North American swine farm operation

1.2 病原体宏基因组→致病病原体筛查

- 在先进企业的示范带领下，新技术的应用，正一如既往地贡献价值；
- **“西湖论坛”**的新贡献！
  - **新冠疫情高压下的首届论坛**
  - **短短两年，**大型、新兴企业的生产模式，迅速开展起场内核心群、4周批、猪病净化，…

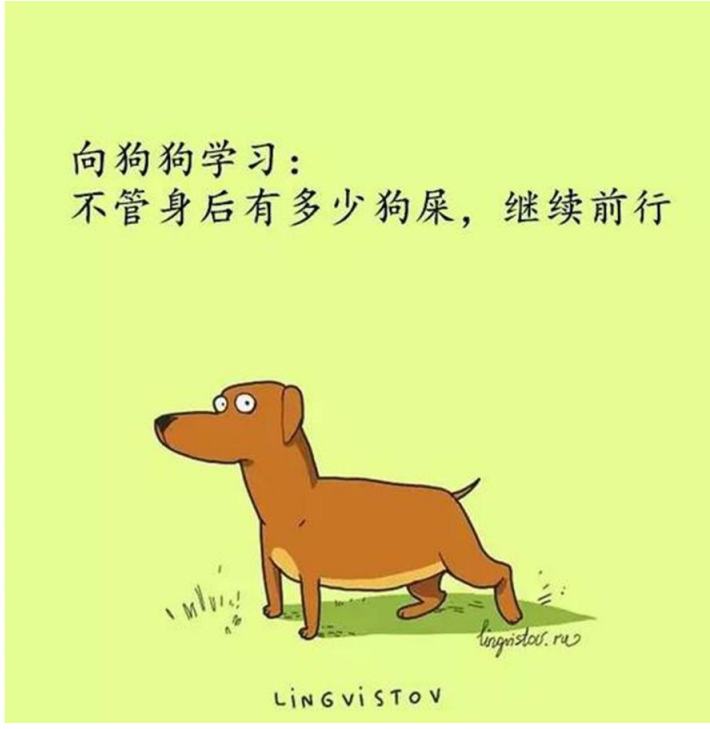
往事可鉴，当下可为，未来可期

西湖论坛
杭州
2022-08

天天新进步

- 宏基因组/全基因组测序
- 大群体28天批次
- 重要疾病病原净化
- 无线快速互联信息技术
- 高度集中后的企业间合作

- 未来可期！



Metagenomics: Application and implication in swine herd health management

基因组检测技术全面进入临床的元年

JY

2024-05

2

母猪群爆发流产的诊断：宏基因组技术的应用

HZ 集中流产与长期分娩率低的致病病原分析

JY

2024-04

2.1 问题的提出：

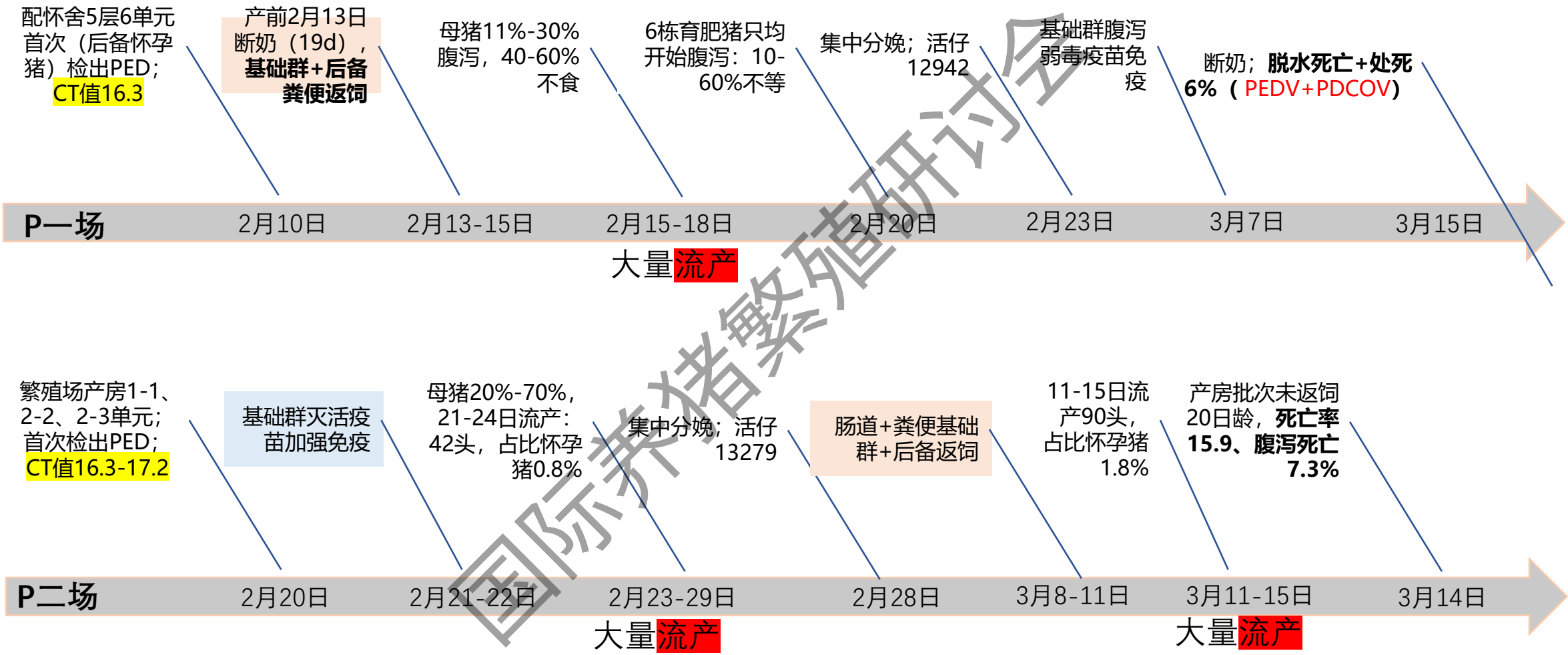
诉：返饲腹泻病料导致大量流产

- 大型**种猪培育-母猪-育肥**一条龙猪场。建群以来，长期分娩率未达目标，主要因批次流产率高。
- **1月中旬发现种猪培育群腹泻**，病料qPCR检测发现PEDV混感PRoV。**2月**11-13日收集腹泻粪便病料及部分腹泻仔猪肠管病料，**返饲所有母猪及后备。**
- **2月13日开始，报告大量母猪流产。返饲病料4天内，流产母猪达存栏母猪的~11%。**
  - qPCR检到流产母猪，部分PRRSV阳性。
    - 但由于是PRRS免疫群，且阳性率低，Ct值高，未能确诊。
- 问：什么问题？怎么办？

提纲

- 材料方法：
  - 问题的界定
  - 流行病学分析
- 宏基因组检测新技术应用
- 结果与讨论

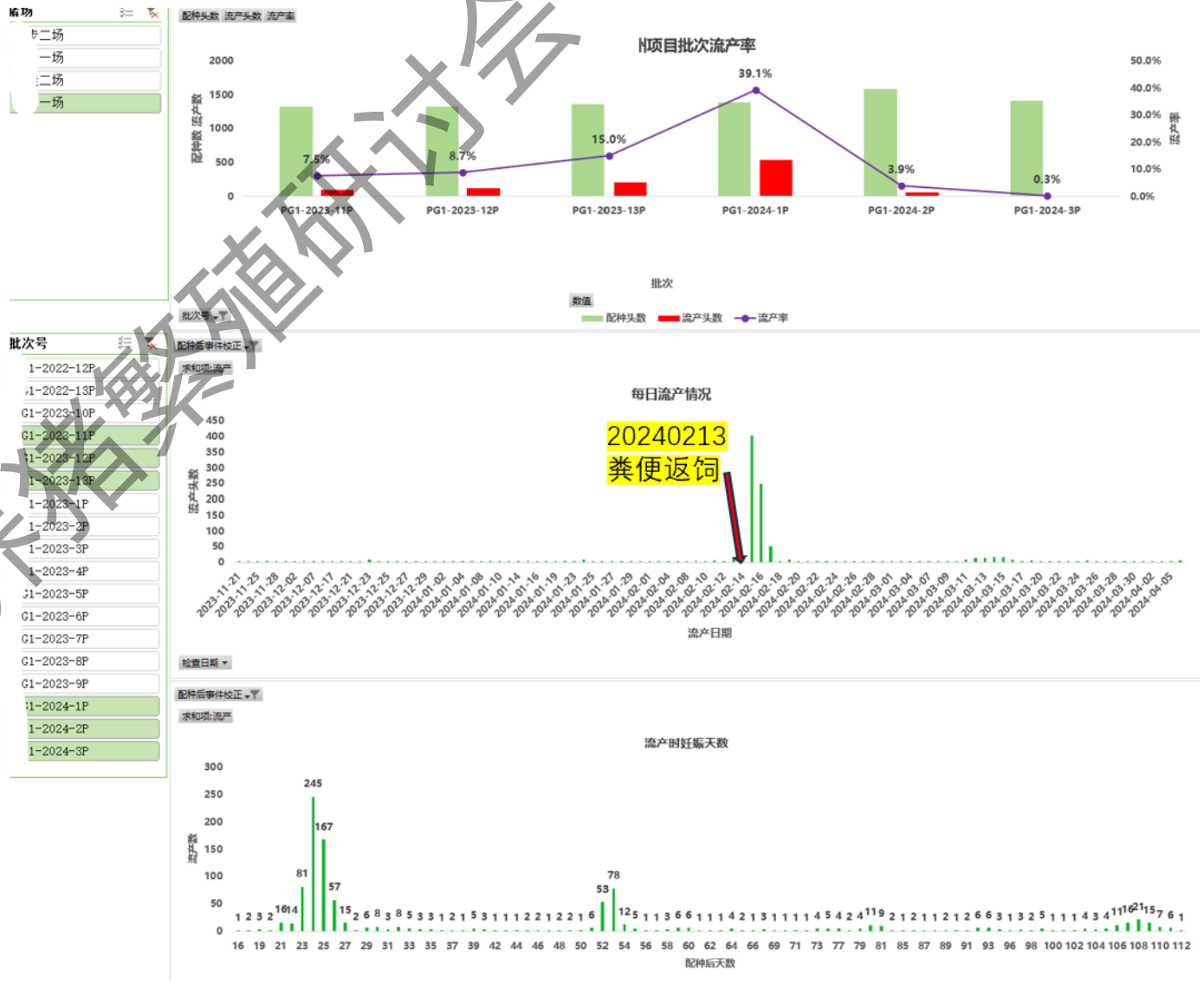
2.1 P场发病过程。 流行病学调查



2.2 问题界定及流行病学分析：P1场

流行病学调查

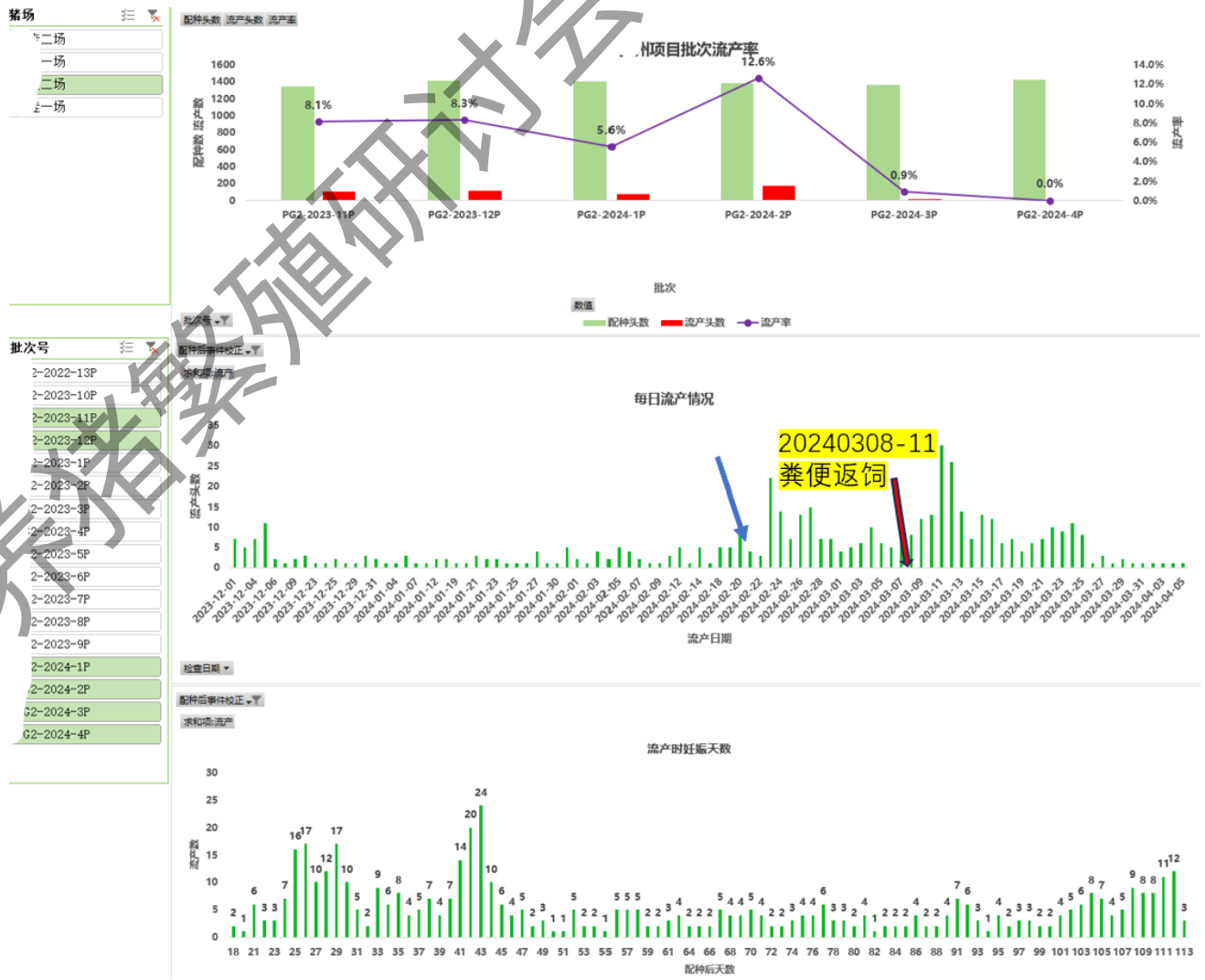
- P1 2024年02月~15~18日集中流产
- 主要是202401P及部分202313P；
- 孕龄23~27天



2.2 问题界定及流行病学分析：P2场

流行病学调查

- P2 2024年02月20~03月25日流产增多。
- 主要是202402P及部分202401P；
- 孕龄23~46天、小部分后期流产



2.2 P1、P2场流产流行病学调查小结

- 两场常年批次配种分娩率偏低，日均流产率高；
- P1场，2024年2月13日母猪群针对PED的返饲粪便后，5天集中流产超标约 670头。
  - 流产**孕龄主要25~28天(约570头)**，也有部分(约100头)；
- P2场，2024年2月22日免疫灭活苗、3月8日返饲粪便和病料后，流产头数增加。
- **两场的流产**
  - 相似点：孕龄主要都为早期、部分中期，很少晚期；窝产总仔、活仔，均未出现异常。可在临床症状和流行病特征上，排除蓝耳病的影响。
  - 不同点：P1场完全集中在返饲后3天内；P2场流产出现实践较分散

2.3 造成母猪流产和胎儿死亡的主要病原体：**研究依据**

- 病毒/细菌/寄生虫
- 不常见的病毒：脑心肌炎病毒；肠病毒/捷申病毒；猪巨细胞病毒；
- 细菌寄生虫：丹毒；钩端螺旋体；

Received: 14 April 2023 | Revised: 20 June 2023 | Accepted: 18 July 2023
DOI: 10.1111/rda.14436

Reproduction in Domestic Animals WILEY

REVIEW

Abortion and fetal death in sows

Dominiek Maes[1] | Olli Peltoniemi[2] | Mateusz Malik[1]

TABLE 1 Main viral pathogens involved in sow abortion and fetal death: clinical signs in sows, lesions in foetuses, appropriate samples and diagnostic tests.[a]

Disease	Clinical signs in sow	Lesions fetus	Sample sow	Sample fetus	Diagnostic test[b]
Aujeszky's disease virus	Generally none	Multifocal white foci (necrosis) in the liver and spleen		Fetal tissue (lung, liver, spleen, brain, kidney)	PCR, VI, IF
Classical swine fever virus	Fever, anorexia, depression, ataxia, cutaneous erythema	Ascites, widespread petechiae, malformations		Fetal tissue (tonsil, kidney, spleen, lung), placenta	PCR
Encephalomyocarditis virus	None	Chalky white foci in heart, hydrothorax, ascites		Fetal tissue (heart, lung, spleen, kidney, brain)	PCR, VI
Enterovirus, Teschovirus	None	None		Fetal tissue (lung, spleen, kidney, heart)	PCR, VI, IF
Porcine circovirus type 2 and 3	None	Dilated and hypertrophied heart; congested liver		Fetal tissue (heart, lung)	PCR, IHC (heart)
Porcine cytomegalovirus	None	Pulmonary edema		Fetal tissue (lung, spleen, kidney)	PCR, VI, IF
Porcine parvovirus	None	Mummification		Mummified fetal tissue (lung) or body fluid	PCR, IF (fetal tissue) Serology (body fluid)
Porcine pestiviruses A, B and D[c]	None	Cerebellar hypoplasia		Fetal tissue (brain, spleen, lung, kidney)	PCR, VI, IF
Porcine reproductive and respiratory syndrome virus	Mild depression, anorexia, fever	Umbilical cord edema, segmental haemorrhage	Serum (acute phase)	Thoracic fluid, fetal and placenta tissue	PCR
Swine influenza A virus	Fever, lethargy, respiratory signs	None	Nasal swabs (acute phase), paired serum samples		PCR, VI (swabs) HI test (serum)

[a]Table adapted from Althouse et al. (2019).
[b]PCR polymerase chain reaction; VI virus isolation; IF immunofluorescence testing; IHC immunohistochemistry; in all cases next generation sequencing (NGS) can be used.
[c]Bovine viral diarrhoea virus (pestivirus A and B), border disease virus (pestivirus D).

TABLE 2 Main bacterial and parasitic microorganisms involved in sow abortion and fetal death: clinical signs in sows, lesions in foetuses, appropriate samples and diagnostic tests.[a]

Disease	Clinical signs in sow	Lesions fetus	Sample sow	Sample fetus	Diagnostic test[b]
Brucella suis	None	Placentitis	Blood	Fetal tissue (liver, stomach content, lung), placenta	Bacterial culture serology
Chlamydia sp.	None	None		Fetal tissue (liver), placenta	PCR, Ag-ELISA, IHC
Erysipelothrix rhusiopathiae	Fever, anorexia, depression, skin lesions	None	Blood or other tissues		Bacterial culture, PCR
Leptospira sp.	Transient fever, anorexia, depression	Occasional fetal jaundice	Blood	Fetal tissue (kidney)	PCR, IF, IHC (kidney) serology
Toxoplasma gondii	None	None		Fetal tissue (blood)	Serology

[a]Table adapted from Althouse et al. (2019).
[b]PCR polymerase chain reaction; IF immunofluorescence testing; IHC immunohistochemistry.

2.3 宏基因组测序：材料方法

2.3 宏基因组测序：材料方法

- 1. 根据临床症状，群体条件下的流行病学数据分析；
  - 日期；孕龄；胎次；比例；
  - 趋势
- 2. POC 关注点采样。根据流行病学特征，
  - 母猪流产物：　　孕前期，孕后期；
  - 前期流产母猪：　咽/阴拭子；血清；
  - 流产母猪临近母猪：　　咽/阴拭子；
  - 发烧不食母猪：　咽/阴拭子；
  - **返饲病料样品：**
- 3. 宏基因组测序后病原体丰度分析
  - 未扩增reads数
- 4. 3家不同检测机构

Point-of-care testing, also known as **near-patient testing**, typically means carrying out a test using a device or test kit in the presence of the patient and without the need to send a sample to a laboratory.

POC testing （POC采样法）

在最容易出现病人的地方采样。例，仅采集那些有类似非洲猪瘟症状的猪只。

2.4 宏基因组新技术的应用：结果分析

POC采样，只采流产相关

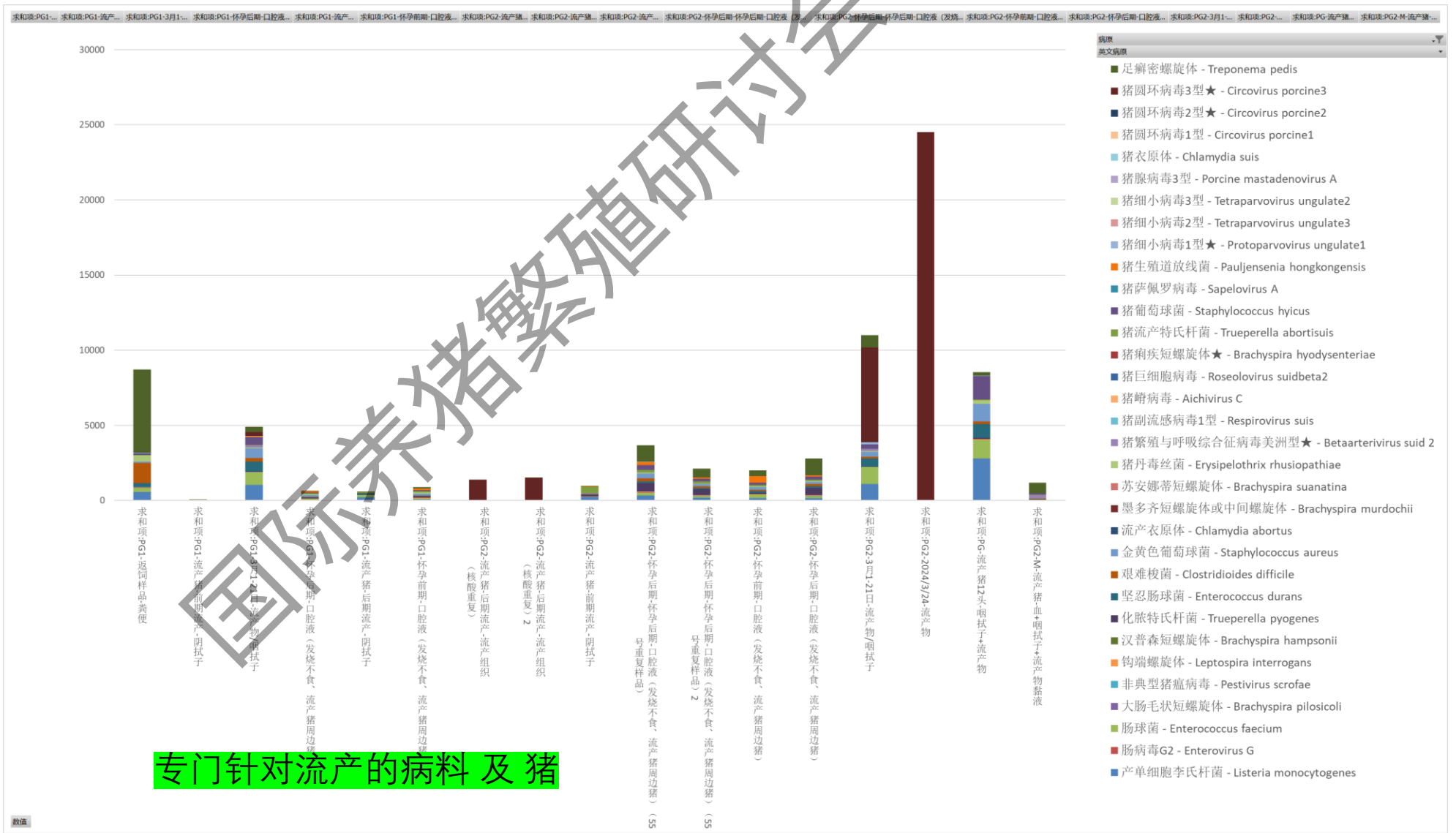
- P1与P2明显有区别；
- 流产物与返饲病料中，病原体含量及类型，无关联；
- 不同流产物样本中，病原体含量及类型，无关联；
- 流产母猪样品中，病原体含量及类型，无关联；

专门针对流产的病料 及 猪

2.4 宏基因组新技术的应用：结果分析

POC采样，只采流产相关

- PG1与PG2明显有区别；
- 去掉明显无关的大肠杆菌、球虫、罗森菌、等等。
- 丁型冠状病毒在粪便中检出高，但微量在其它流产相关猪样品中

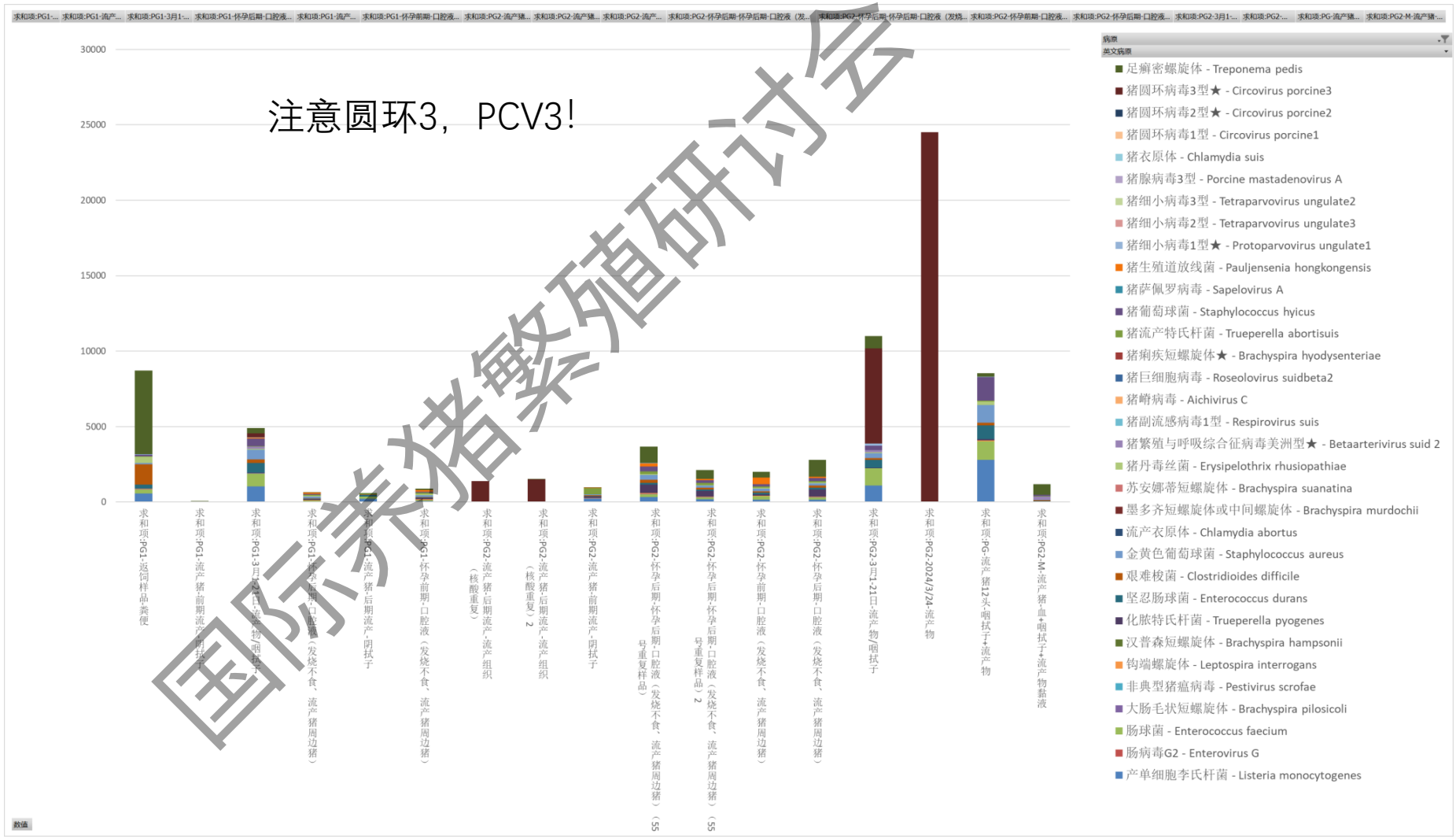


专门针对流产的病料 及 猪

2.4 宏基因组新技术的应用：结果分析

POC采样，只采流产相关

- PG1与PG2明显有区别；
- 去掉明显无关的大肠杆菌、球虫、罗森菌、等等。
- 丁型冠状病毒在粪便中检出高，但微量在其它流产相关猪样品中
- **只选对流产重要的病原**

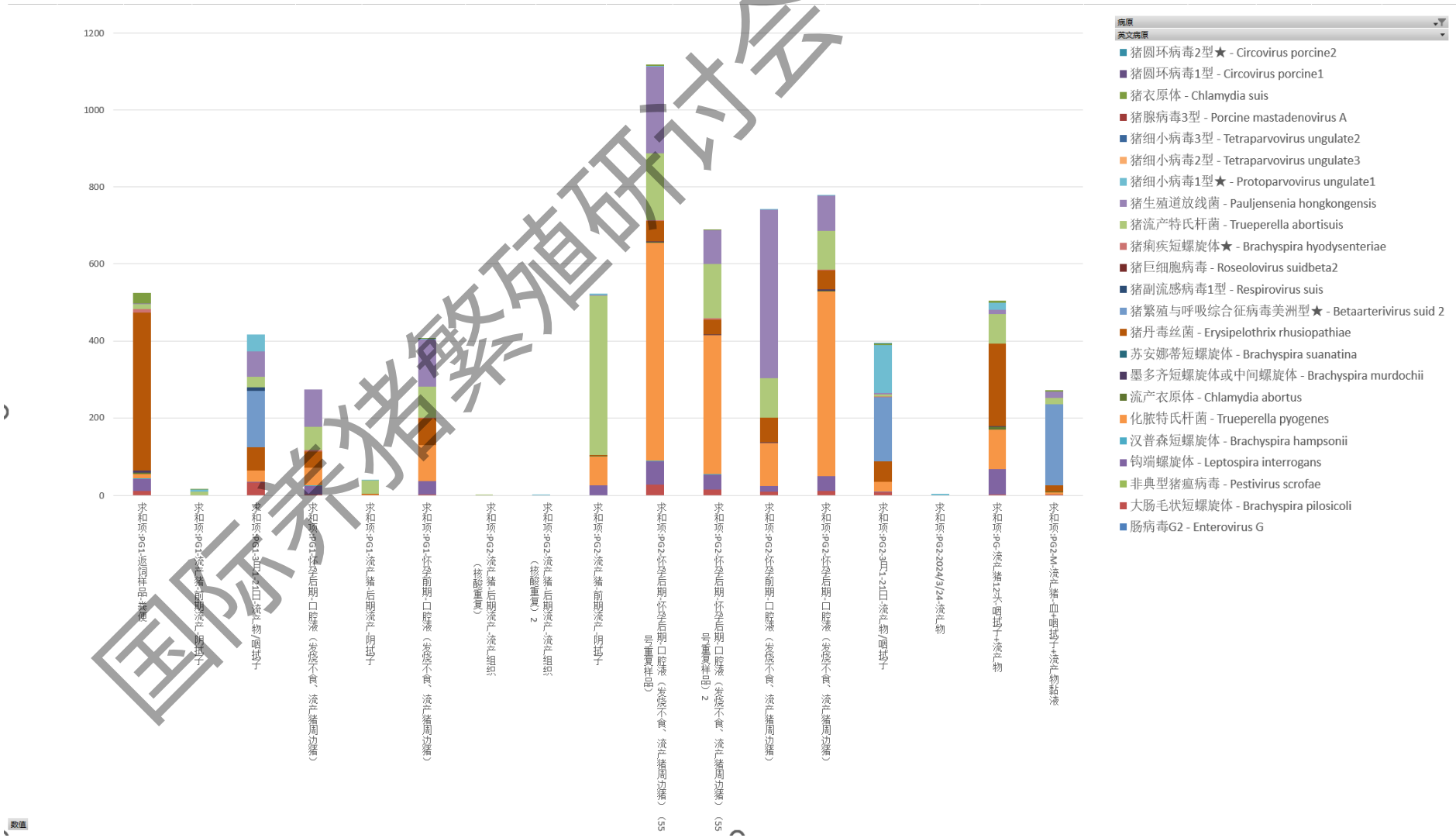


专门针对流产的病料 及 猪

2.4 宏基因组新技术的应用：结果分析

POC采样，只采流产相关

- PG1与PG2明显有区别；
- 只留下对流产最重要的病原
- 由于圆环病毒类/PRRSV会影响窝产总仔和活仔，不符合本案。
- 应更加关注
  - 密螺旋体，钩端螺旋体
  - 猪流产特氏杆，猪化脓特氏杆菌
  - 但均不可能造成急性、集中大量流产



2.5 讨论：PCV2/PCV3的繁殖影响

- PCV2：PCV-RD. 不常见急性；
- PCV3：木乃伊； 死胎；

Among four PCVs discovered so far, PCV1 is considered as non-pathogenic, while the other three PCVs, i.e., PCV2, PCV3, and PCV4, are known to be pathogenic. PCV1, PCV2, and PCV3 are distributed throughout the global pig population, while the distribution of PCV4 is yet to be known [80]. PCV2 is the main etiological agent of several diseases in pigs, collectively known as porcine circovirus-associated diseases (PCVADs) in North America [81] or porcine circovirus diseases (PCVDs) in Europe [82]. These diseases include PCV2-systemic disease (PCV2-SD) formerly known as postweaning multisystemic wasting syndrome (PMWS), PCV2-reproductive disease (PCV2-RD), PCV2-lung disease (PCV2-LD), PCV2-enteric disease (PCV2-ED), porcine dermatitis and nephropathy syndrome (PDNS), PCV2-subclinical infection (PCV2-SI) [83], and acute pulmonary edema [2]. PCV2-SD affects pigs between 8 and 16 weeks of age [84] and is clinically characterized by wasting,

vaccines

MDPI

Review

Revisiting Porcine Circovirus Infection: Recent Insights and Its Significance in the Piggery Sector

Hemanta Kumar Maity [1,†], Kartik Samanta [1,†], Rajib Deb [2,*] and Vivek Kumar Gupta [2,*]

PCV3 has been detected in pigs showing the symptoms of systemic disease, reproductive disease [104], PDNS [23,105]), respiratory disease [106], GI disorder [107], PFTS [108], and cardiac and multisystemic inflammation [24]. The major clinical signs include mummified and stillborn fetuses [104,109], weight loss, dyspnea, rectal prolapse [24], abdominal breathing, diarrhea [107], anorexia, fever, icterus [110], congenital tremor [111], and wasting [112]. Pathological lesions that are associated with PCV3 infection include lymphoid depletion in the spleen and lymph nodes [113]; periarteritis and arteritis in the heart, kidney, spleen, lung, and/or stomach [112,114]; bronchointerstitial pneumonia in the lung [23]; epicarditis, myocarditis, and endocarditis in the heart [104,113,115]; atrophic, shortened villi and decreased depth of crypt in the intestine [64]; and mild lymphoplasmacytic meningoencephalitis, nephritis, periportal hepatitis, and rhinitis [112]. Recently, a study also demonstrated the potential role of PCV3 in subclinical infection, which is characterized by lack of evident clinical signs but presence of detectable prolonged viremia, viral replication in tissues, and multisystemic inflammation [116]. PCV3 can infect animals of different age groups and production phases, being found in fetuses, nursery pigs, fattening pigs, stillborn, and sows [17,117]. PCV3 has been detected not only in pigs with signs and symptoms of different clinical diseases [23,24], but also in healthy animals [104]. Moreover, co-infection of PCV3 with other viruses such as PCV2 [118], PRRSV [106], PPV [108], CSFV [118], and TTSuVs [119] has been reported. Thus, the detection of PCV3 in samples alone does not guarantee its association with disease casualty [114]. Under experimental conditions, an infectious PCV3 DNA clone was able to induce development of PDNS-like signs in specific-pathogen-free (SPF) piglets [120]. However, in another study, no clinical disease was developed in caesarean-derived, colostrum-deprived (CD/CD) pigs inoculated with PCV3. However, four out of eight PCV3-infected pigs showed histological lesions such as lymphoplasmacytic myocarditis and perivasculitis, which are consistent with multisystemic inflammation [109]. Similar results were found in PCV3-inoculated CD/CD pigs where no significant clinical signs were observed, except histological lesions that resembled multisystemic inflammation. Thus, the pathobiology of PCV3 is complex and of multifactorial nature [116]. Further investigation is needed to reveal the pathogenic efficacy of PCV3 along with the role of other co-infecting pathogens. PCV3 infects both domestic pigs as well as wild boars. However, there is still no evidence regarding the association of PCV3 infection and development of clinical disease in wild boar [121].

2.5讨论：钩端螺旋体

- 不明原因流产
- 但不会造成急性、集中流产

Ellis WA. Leptospirosis. In: Zimmermann JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. Diseases of swine. Wiley; 2012. p. 1562–79.

Strutzberg-Minder *et al. Porcine Health Management* (2018) 4:10
https://doi.org/10.1186/s40813-018-0086-5

Porcine Health Management

RESEARCH Open Access

Passive surveillance of *Leptospira* infection in swine in Germany

Katrin Strutzberg-Minder[1*], Astrid Tschentscher[1], Martin Beyerbach[2], Matthias Homuth[1] and Lothar Kreienbrock[2]

Table 1 Collection of swine serum samples tested for leptospires by MAT

	Reasons for testing of individual samples			Total	Number of farms
	Monitoring	Reproductive problems	Unknown reasons		
2011	392	2514	3002	5908	856
2012	400	2422	2650	5472	797
2013	338	2249	2981	5568	718
2014	298	2087	2721	5106	659
2015	300	1582	2851	4733	529
2016 / 01 to 09	85	1163	1794	3042	394
Total	1813	12,017	15,999	29,829	3953[1]

[1]Because some farms sent samples in more than one year, this number of farms is higher than that of the different farms tested in the total study period

Table 2 Results of swine serum samples tested for leptospires by MAT. Number (n) of samples examined, numbers (n) and percentage (%) of positive samples and farms with at least one positive sample per farm and year from January 2011 to September 2016, and totals

Year	Number of serum samples	Positives[a]		Number of farms	Positives[b]	
		n	%		n	%
2011	5908	964	16.3	856	508	59.3
2012	5472	1042	19.0	797	509	63.9
2013	5568	1076	19.3	718	434	60.4
2014	5106	993	19.5	659	416	63.1
2015	4733	1009	21.3	529	375	70.9
2016 / 01 to 09	3042	941	30.9	394	308	78.2
Total	29,829	6025	20.2	3953	2550	64.5

[a]Samples with a titre ≥100
[b]farms with at least one positive sample per year

2.5 讨论： 猪流产特氏杆菌 *Trureperella Abortisuis*

- 近年来，英国、美国时有报道 时导致母猪流产的原因。
- 但都不是集中流产。只是较零星的不明原因流产

PEER REVIEWED CASE STUDY

Placentitis and abortion in domestic pigs (*Sus scrofa domesticus*) associated with *Trueperella abortisuis* on US swine farms

Alexandra K. Ford, DVM; Rachel M. Palinski, PhD; Brian V. Lubbers, DVM, PhD, DACVCP; Lisa Tokach, DVM, DABVP Swine Health Management; A. Giselle Cino-Ozuna, DVM, PhD, DACVP

Table 1: Bacteria isolates identified from samples submitted by 3 Midwest US swine farms experiencing increased abortions

Case number and date	Submitted tissues	Gross lesions	Microscopic lesions	Bacteriology
Case 1; November 2017	Uterine fluid*	NA	NA	Abundant *Trueperella abortisuis* isolated from uterine fluid, *Trueperella pyogenes*, *Pasteurella multocida*, *Streptococcus dysgalactiae*, *Enterococcus gallinarum*, *Escherichia coli* (non-hemolytic and hemolytic), *Proteus mirabilis*, *Streptococcus suis*, *Streptococcus* species (beta-hemolytic), and *Clostridium perfringens*
Case 1; November 2017	Uterine fluid*	NA	NA	*T abortisuis* isolated from uterine fluid, *Actinobacillus rossii*, *E coli* (non-hemolytic), *Streptococcus hyointestinalis*, *Aerococcus viridans*, gram-negative cocci (unable to identify), *Acinetobacter lwoffii*, *Lactococcus raffinolactis*, *Aeromonas bestiarum*, *Streptococcus parauberis*, *Acinetobacter johnsonii*, and *C perfringens*
Case 2; December 2017	Five aborted fetuses† and five placentas	Mild-moderate placental thickening and hemorrhage; pleural effusion and subcutaneous hemorrhage	Suppurative placentitis and intra-trophoblast gram-positive bacteria; hepatic congestion and renal hemorrhage	Abundant *T abortisuis* isolated from stomach contents, *A rossii*, *E coli* (non-hemolytic), *S hyointestinalis*, *A viridans*, gram-negative cocci (unable to identify), *A lwoffii*, *L raffinolactis*, *A bestiarum*, *S parauberis*, *A johnsonii*, and *C perfringens*
Case 2; December 2017	One aborted fetus‡ and one placenta	Diffuse placental thickening, multifocal tan-brown discolorations and roughening	Suppurative necrotizing placentitis, trophoblast sloughing, gram-positive and negative coccobacilli in airways and placenta	Abundant *T abortisuis* isolated from a fetal swab, *E coli* (non-hemolytic), *Citrobacter gilleni*, *Aeromonas* species, *Lactococcus garvieae*, *Enterococcus faecium*, *A viridans*, *Enterococcus hirae*, gram-negative cocci (unable to identify), *S suis*, *Streptococcus* species (Alpha hemolytic), *Staphylococcus chromogenes*, *Streptococcus alactolyticus*, *S parauberis*, *L raffinolactis*, *Lactobacillus ruminis*, *Enterococcus hirae*, and *C perfringens*
Case 3; May 2020	Three aborted fetuses§ and one placenta	Moderate amount of tan-yellow exudate covering allantois; umbilical cord and allantois hemorrhage	Suppurative gram-negative and gram-positive bacterial placentitis; suppurative, fibrinous omphalitis	Abundant *T abortisuis* isolated from placenta, *E coli* (non-hemolytic), and *A viridans*

* Unknown parity, age, or health status.
† Crown-rump length of fetuses 150-180 mm (approximately 60-70 days of gestation).
‡ Crown to rump length of fetus 70 mm (approximately 40-50 days of gestation).
§ Crown-rump length of fetuses 50-60 mm (approximately 40 days of gestation).
NA = not available or analyzed.

集中流产案例:症状/诊断与处置:

- PG1: 1)与返饲操作有关联的集中流产: 可基本排除主要的造成流产的病毒性抗原; 如果是，也似只与返饲病料中高浓度致病菌有关(钩端螺旋体/密螺旋体/猪流产特氏杆菌/化脓杆菌/等)+捷申/PCV3;
  - 证据：仅早/中期流产，未明显影响产仔成绩。
  - 2)长期分娩率低 + 长期流产率高: 疑似高量细菌性感染。
  - 3)长期断奶前成活率低: 鉴别诊断病毒性腹泻; PRRSV;PCV3
- PG2:
  - 1) 似与免疫及返饲有关的流产头数增加: 怀疑细菌性污染。
    - 证据：仅早/中期流产，未明显影响产仔成绩。
  - 2) 长期断奶前成活率低: 鉴别诊断病毒性腹泻; PRRSV;PCV3;

- **结论：宏基因组检测结果，未发现可造成急性、集中流产的传染性病原体**

3

回到现实世界：事出反常必有妖！

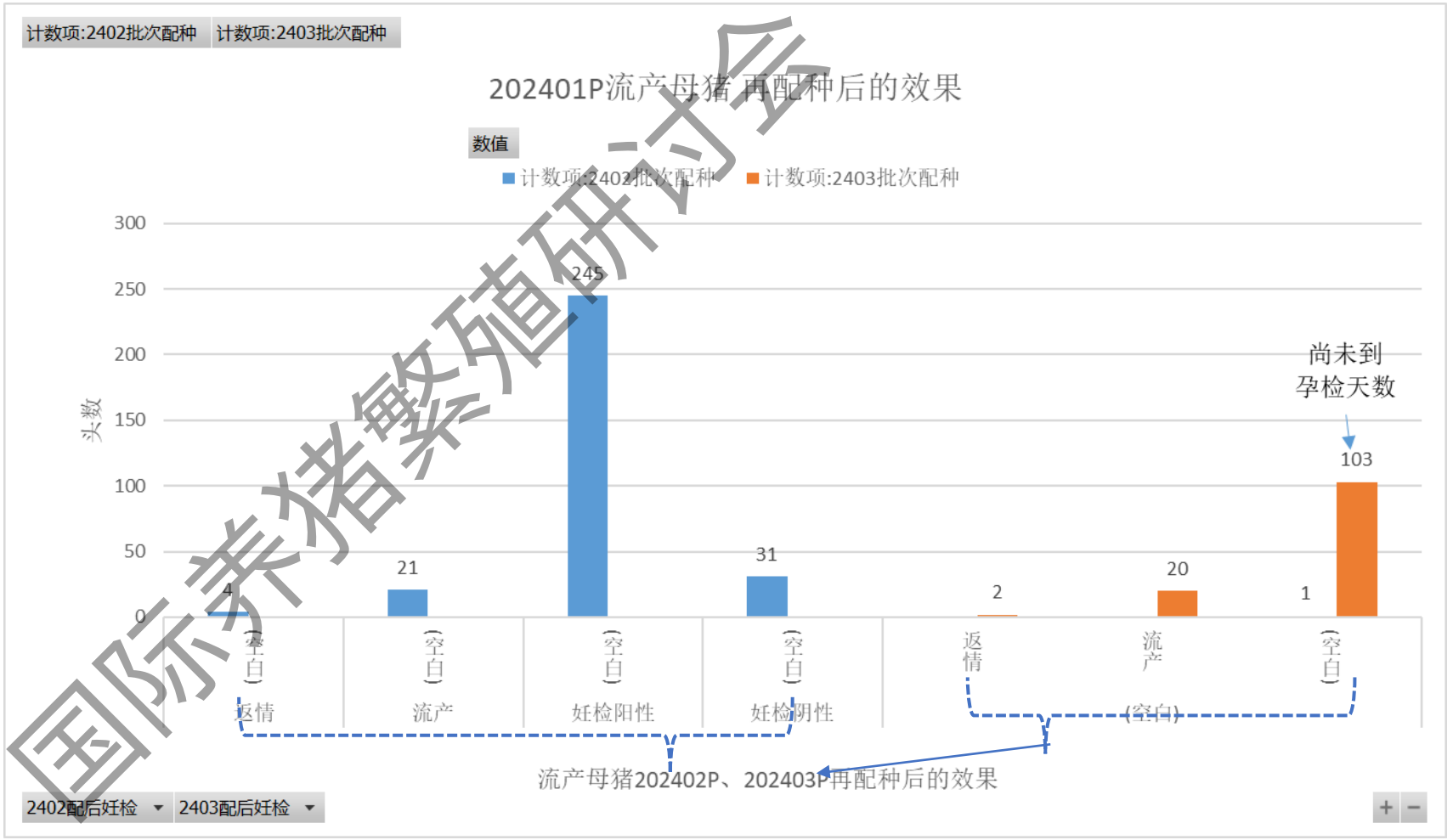
即使直接肌注、接种有关病毒、细菌，都不可能造成 P1场次日至4日内的集中流产，或P2场不同孕龄母猪持续流产40天

3 针对P1场，返饲后所有流产母猪：后续事件分析

- P1场2024年2月11~13日集中返饲粪便 后，15~18日流产母猪509头，
  - 其中302头在流产后0~5天实现配种，245头孕检阳性。
  - **流产母猪几乎是不可能实现的！**

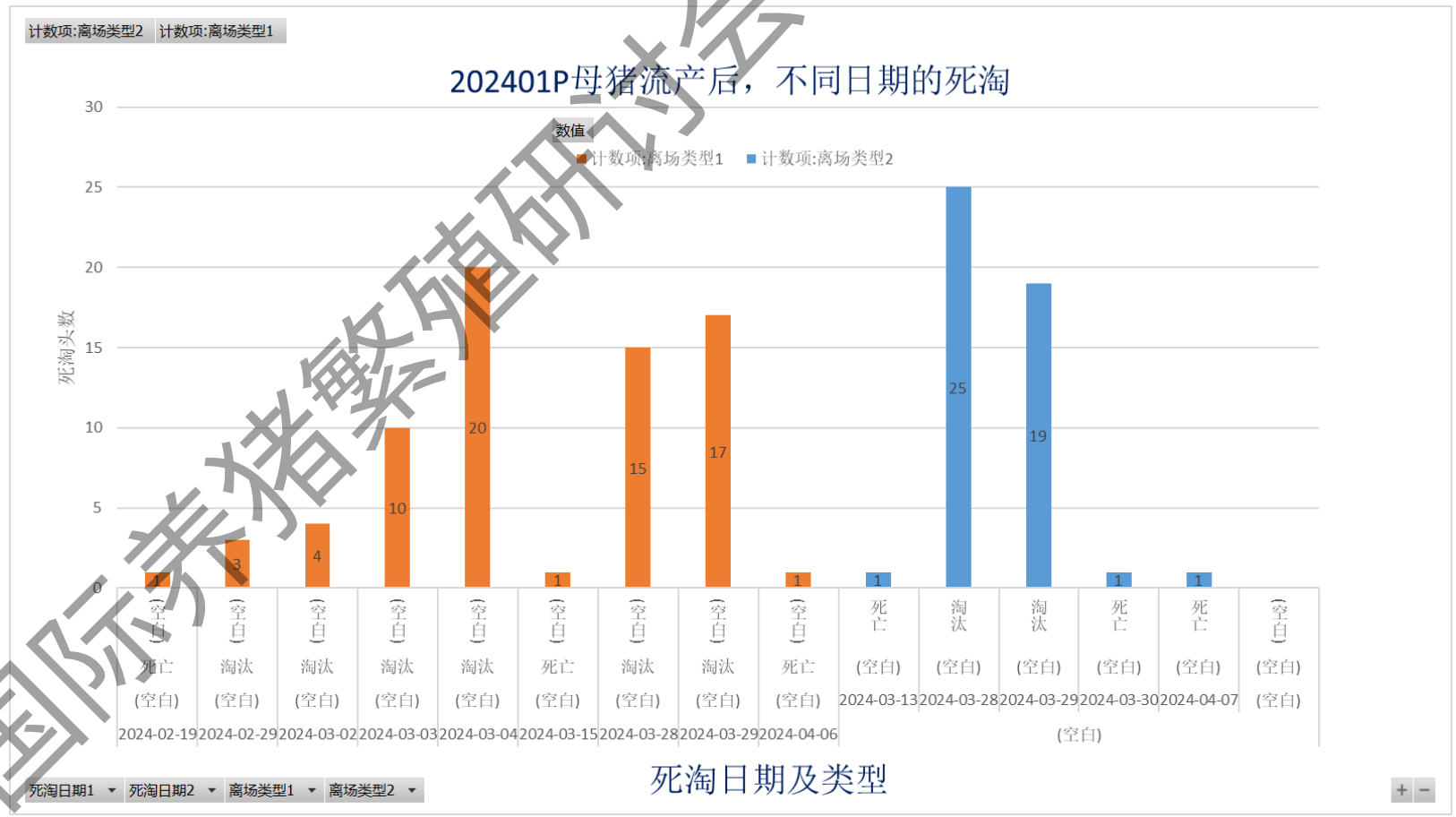
3 针对P1场，返饲后所有流产母猪：后续事件分析

- P1场2024年2月11~13日集中返饲粪便 后，15~18日流产母猪509头，
  - 其中302头在流产后0~5天实现配种，245头孕检阳性。
  - **流产母猪几乎是不可能实现的！**
- **`流产母猪`再配种后返情、流产很少**



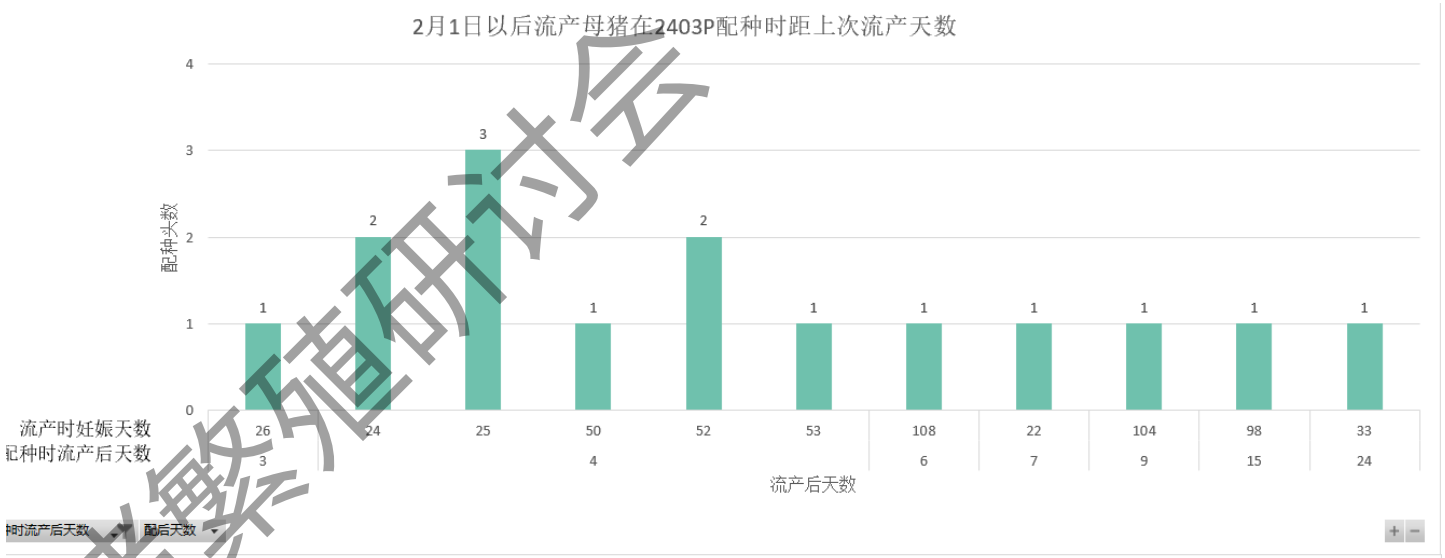
3 针对P1场，返饲后所有流产母猪：后续事件分析

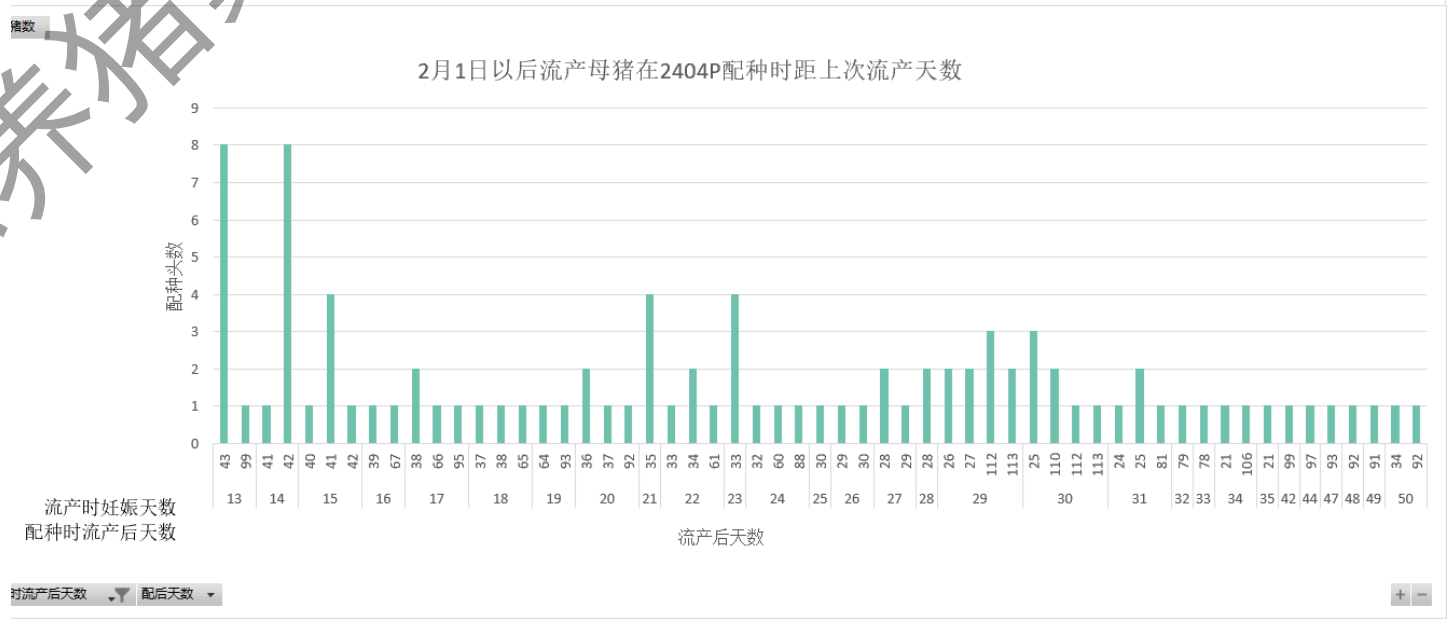
- P1场2024年2月11~13日集中返饲粪便 后，15~18日流产母猪509头，
  - `流产母猪`死、淘很少。



3 针对P2场，返饲后所有流产母猪 所有事件分析

- P2场2024年2月集中返饲粪便后，流产母猪312头，
- 少部分流产母猪93头在下面批次配种 距离流产日期均超过
- 多数(215头)淘汰





总结

- 两大型母猪-育肥场后备母猪群腹泻检出PED，全群母猪返饲了粪便、肠管等病料,现场报告出现大量“母猪流产”。
  - P1场的01P配种批，509头母猪2024年2月15~18日**集中**记录为`流产`。P2场流产母猪分布在30天期间，流产母猪孕龄**不集中**。
- 将流产母猪流产物、不同孕龄流产母猪口腔液/阴拭子/粪便、流产母猪临近猪只的病料专门化、针对性采样样品；在不同的实验室，使用宏基因组测序新技术，检测所有致猪病的病原体。结果均未发现可导致大量母猪流产的病原体基因。
  - 病料中检出的少量流产关联病原体，均不会造成**短时间**、大量、集中的流产。

总结

- 回归到猪流产的严格流行病学研究，发现期间分娩母猪产仔均正常，流产母猪孕龄多为早期、部分中期、少量晚期；针对P1场01P所记录的509头`流产母猪`进行逐头跟踪分析，发现其中302头在流产后0~5天成功实现配种，且245头孕检阳性，未见异常淘汰和死亡。P2场流产母猪部分延至一个情期后配种，但超半数淘汰离场。
  - **现场重新调查核验，发现流产母猪数据为数据记录不当和错误。给大型猪群的生产经营和管理造成误导。**
- 宏基因组测序给本次严重问题的分析、调查和解决，提供了有力佐证。

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谢谢！