2024 International PRRS SYMPOSIUM YANTAI

会刊 PROCEEDINGS

The International PRRS Symposium (IPRRSS 2024)

国际猪繁殖与呼吸综合征学术会议

2024年8月7-9日 August 7-9, 2024

中国·山东烟台 Yantai, China

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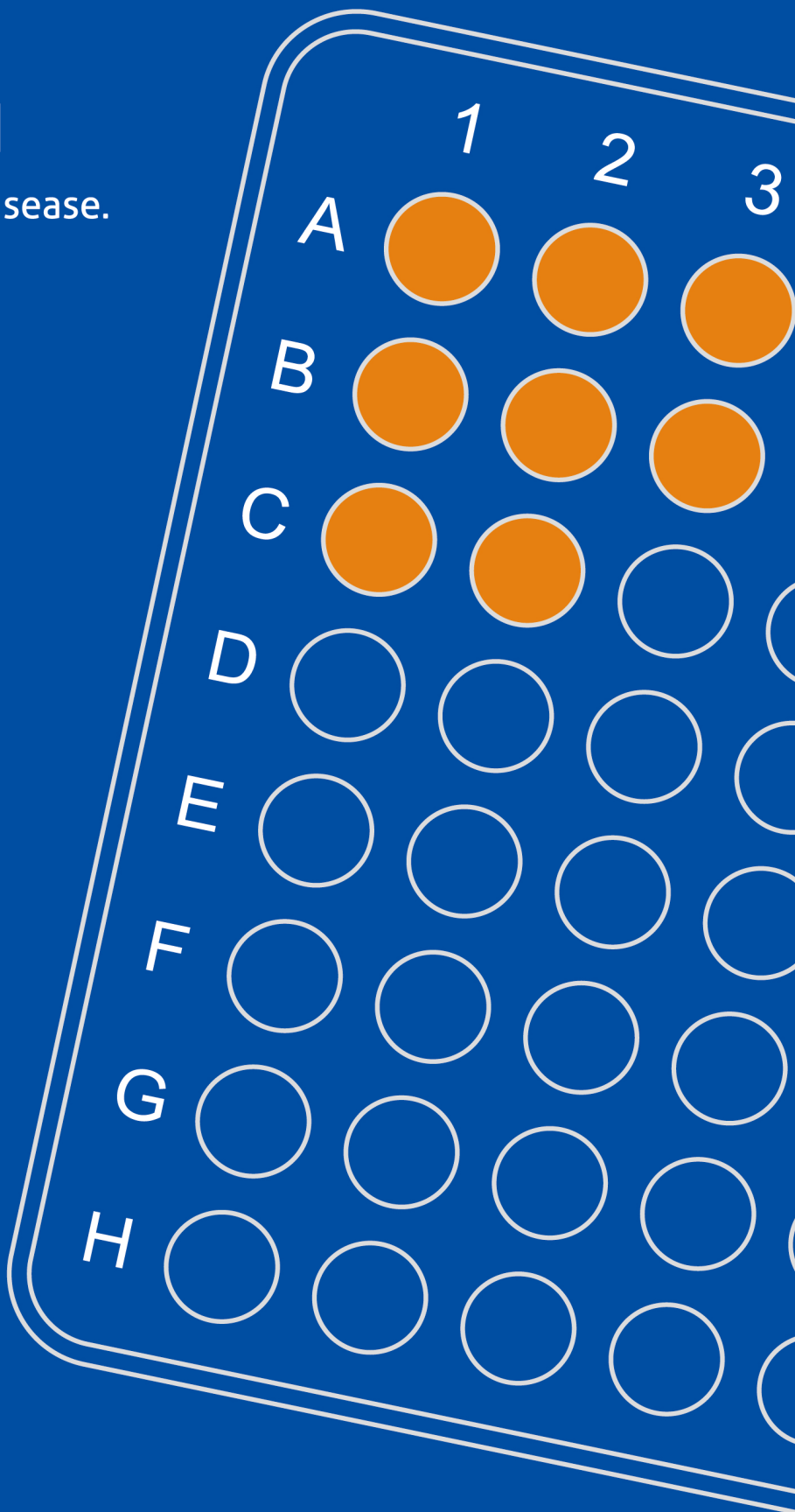

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A company that focuses solely on rapid stabilization and eradication services for PRRS

湖南科赛安生物科技公司是由多名教授和博士等高科技人才联合创办的一家科技创新与技术服务型企业，主要开展天然药物、高效消毒剂、免疫分子与诊断试剂的研制，以项目化管理模式打造“净蓝增效200工程”，帮助猪场实现“净化PRRSV，增效200元/头出栏”的目标，做最值得信赖的猪蓝耳病快速稳定与净化服务团队。

Hunan Coscian Biotechnology Co., Ltd. is a technology innovation and service enterprise jointly founded by multiple professors, PhDs and other high-tech talents. We mainly develop natural medicines, efficient disinfectants, immune molecules and diagnostic reagents.Promotion the "PRRSV Eradication Project" through project-based management mode, assisting pig farms in achieving the goal of eradication PRRSV and increasing benefits. Our mission is to be the most reliable and professional team of providing rapid stabilization and eradication services of PRRS.

净蓝服务主要内容
MAIN SERVICES ↘

01 ▶ **核心产品** **科赛安1号**，加速清除猪群PRRSV病毒血症及组织带毒

Core product：Coscian I, accelerating the clearance of PRRSV viremia and tissue carriers in pig herds

02 ▶ **系统方案** 净蓝4招8步，一场一方案

System solution: Coscian 4 Measures and 8 Steps PRRSV Eradication Plan, one farm/program, one plan

03 ▶ **项目管理** 项目经理负责制，以目标导向6步快速落地PRRSV稳定净化方案

Project management: manager responsibility system of a goal oriented 6-step rapid implementation of PRRSV stable eradication schem

主要服务伙伴
MAIN SERVICE PARTNERS↘

目前已与正大集团、新五丰股份、唐人神股份等大型养猪集团，以及数十家中大型规模化养猪企业建立合作关系。

At present, we have established partnerships with large pig farming groups such as Charoen Pokphand Group (CP Group), Hunan New Wellful Group and Tangrenshen Group

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The evaluation of the Pig Disease Research Laboratory at China Agricultural University .

Jialanqing In vivo toxicity challenge stud

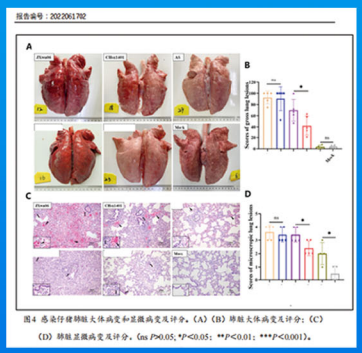


图4 感染仔猪肺脏大体病变和显微病变及评分。(A)（B）肺脏大体病变及评分；(C)（D）肺脏显微病变及评分。(ns P>0.05; *P<0.05; **P<0.01; ***P<0.001)。

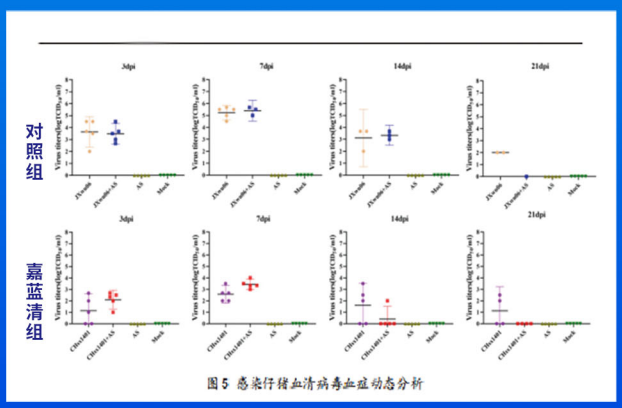


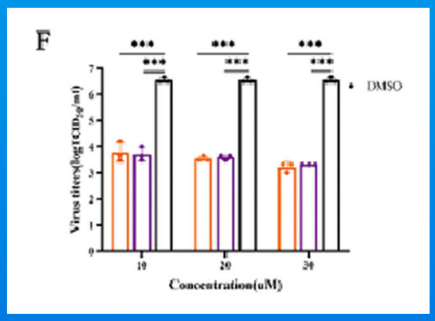
图5 感染仔猪血清病毒血症动态分析

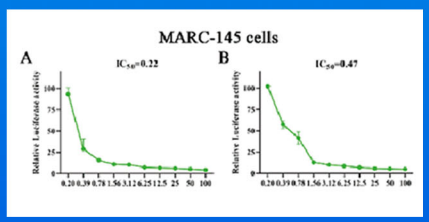
The virus titers in the serum of each group were measured on the 3rd, 7th, 14th, and 21st day after virus challenge to analyze the replication of the virus in the body.

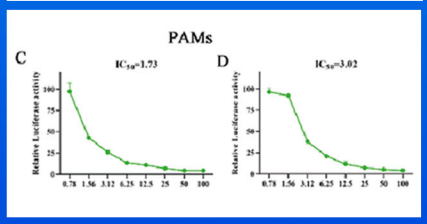
The results showed that the replication of JXwn06 and CHsx1401 in the body could be detected on the 3rd day after virus challenge, and both reached the peak of replication on the 7th day. The virus in the Jialanqing group was cleared on the 14th day.

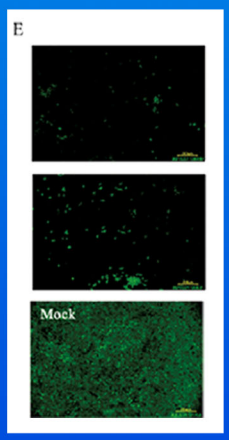
Rapidly clear viremia and accelerate outcome.

Jialanqing In vitro screening experiments









Immunofluorescence showed that when infected at a concentration of MOI = 0.1, the fluorescence signal in the Jialanqing treatment group was significantly reduced by more than 95%.The core components of Jialanqing have significant anti-PRRSV activity in vitro.

Sow during farrowing

Control infection in the farrowing room, reduce the positive rate of piglets, and increase the number of weaned piglets

Pregnant sow

Reduce the rate of abortion, decrease the number of stillbirths, and improve the mating and farrowing rate

Weaner pig

Reduce the number of medication times, lower the morbidity and mortality rate, and decrease the feed conversion ratio

Replacement gilts into the herd

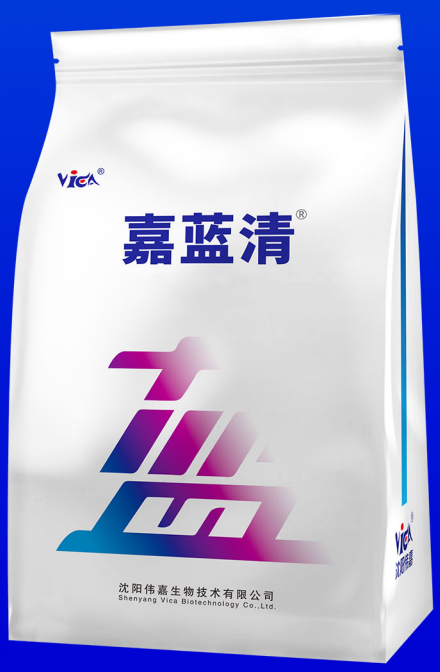
Eliminate viremia to prevent virus excretion

Two weeks after vaccination

elimination of viremia and reduction of recombination risk.

Piglet

Increase the average number of weaned piglets per litter, reduce morbidity rates, and enhance weaning weights



Scan the QR code to learn more details

We sincerely invite partner companies to collaborate.

目录 Catalogue

大会主席致辞

我们非常荣幸地邀请大家参加 2024 国际猪繁殖与呼吸综合征学术会议（IPRRSS 2024）。IPRRSS 是猪繁殖与呼吸综合征研究领域学术水平最高的专业国际会议，将于 2024 年 8 月 7 日至 9 日在中国烟台举行。

猪繁殖与呼吸综合征（俗称：猪蓝耳病）依然是影响全球猪业的一个重要的病毒性疾病。IPRRSS 曾经于 2013 年和 2018 年在中国举行过。本届 IPRRSS 2024 将汇聚来自世界各地猪病领域相关的专家、学者和从业者。大会的会议内容包括 PRRS（猪蓝耳病）的病毒复制、致病机制、疫苗、临床疾病控制、诊断与流行病学、宿主遗传学以及新发病毒等相关研究和最新科研成果。会议设有专家特邀报告、口头报告、壁报交流及展览环节，到场嘉宾可享用当地特色美食。

我们希望这次会议能为您的专业研究及与业界其他企业的交流合作提供新的视角和新的机会。

期待在烟台与您相见！

杨汉春

2024 年 7 月

Welcome IPRRSS 2024

Dear Friends,

With deep honor and great pleasure, we would like to invite you to The International Porcine Reproductive and Respiratory Syndrome Symposium (IPRRSS 2024). This conference, a highest-level international conference in the field of PRRS, will be held in Yantai, China, during August 7-9, 2024.

PRRS remains an important viral disease impacting global swine industry. The IPRRSS were held in China in 2013 and 2018. The IPRRSS 2024 will bring together experts, scholars, and practitioners working on swine disease from all over the world. Plenary scientific sessions include PRRS virus replication, pathogenesis, vaccines, field disease control, diagnostics and epidemiology, host genetics, and emerging viruses. We will have keynote presentations, selected orals and posters, exhibition and sumptuous banquet to enjoy the local food.

We hope this conference will provide new insights and new opportunities for your research andcollaboration.

We look forward to meeting you at Yantai!

Hanchun Yang

IPRRSS 2024 Chair and Organizing Committee

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日程简表

日期	时间	会议内容	地点
8 月 5—7 日	08:30-18:00	第六届中国猪业科技大会	八角湾厅 金沙滩厅
8 月 7 日	09:00-18:00	注册报到 / 展览展示 / 壁报交流	东登录厅
8 月 8 日	08:30-08:40	开幕式	八角湾厅
	08:40-10:00	病毒复制专题	八角湾厅
	10:00-10:30	休息 / 壁报交流	东登录厅
	10:30-12:00	致病机制专题	八角湾厅
	12:00-13:30	午餐	A1 展厅
	13:30-15:00	疫苗与免疫专题	八角湾厅
	15:00-15:30	休息 / 壁报交流	东登录厅
	15:30-17:30	实践论坛	八角湾厅
	15:30-17:30	铂金卫星会（勃林格殷格翰）	金沙滩厅
	18:00-21:30	Banquet	烟台鑫广万豪酒店户外草坪
8 月 9 日	08:30-10:00	临床疾病控制专题	八角湾厅
	10:00-10:30	休息 / 壁报交流	东登录厅
	10:30-12:00	诊断与流行病学专题	八角湾厅
	12:00-13:30	午餐	A1 展厅
	13:30-15:20	宿主遗传学及宿主与病毒相互作用专题	八角湾厅
	15:20-15:50	休息 / 壁报交流	东登录厅
	15:50-17:50	新发病毒专题	八角湾厅
	17:50-18:00	闭幕式	八角湾厅

Conference Agenda

Date	Time	Plenary Sessions	Location
August 5-7, 2024	08:30-18:00	The 6th China Swine Science Conference (CSSC 2024)	Multi-functional Hall Golden Beach Hall
Wednesday, August7, 2024	09:00-18:00	Registration/Exhibition / Posters Communication	East Entry Hall
Thursday, August 8, 2024	08:30-08:40	Opening Ceremony	Multi-functional Hall
	08:40-10:00	Virus Replication	Multi-functional Hall
	10:00-10:30	Break/Posters Communication	East Entry Hall
	10:30-12:00	Pathogenesis	Multi-functional Hall
	12:00-13:30	Launch/Break	Hall A1
	13:30-15:00	Vaccines and Immunity	Multi-functional Hall
	15:00-15:30	Break/Posters Communication	East Entry Hall
	15:30-17:30	Practice Forum	Multi-functional Hall
	15:30-17:30	Satellite Symposium - Boehringer Ingelheim	Golden Beach Hall
	18:00-21:30	Banquet	Yantai Marriott Hotel Outdoor Lawn
Friday, August 9, 2024	08:30-10:00	Field Disease Control	Multi-functional Hall
	10:00-10:30	Break/Posters Communication	East Entry Hall
	10:30-12:00	Diagnostics-Epidemiology	Multi-functional Hall
	12:00-13:30	Launch/Break	Hall A1
	13:30-15:20	Host Genetics	Multi-functional Hall
	15:20-15:50	Break/Posters Communication	East Entry Hall
	15:50-17:50	Emerging Viruses	Multi-functional Hall
	17:50-18:00	Closing Ceremony	Multi-functional Hall

大会日程
Scientific Program

8 月 8 日，山东省烟台市八角湾国际会展中心八角湾厅
August 8，Multi-functional Hall, Yantai Bajiaowan International Convention & Exhibition Center

08:30　大会开幕式　Opening Ceremony

病毒复制专题　Virus Replication

主持人（Moderators）：
Hans Nauwynck 比利时根特大学教授 (Ghent University, Belgium)
翁长江 中国农业科学院哈尔滨兽医研究所研究员
(**Changjiang Weng** Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China)

08:40　猪繁殖与呼吸综合征病毒复制机制研究进展
The Art of PRRSV Replication
Ying Fang 美国伊利诺伊大学厄巴纳 - 香槟分校教授
University of Illinois at Urbana-Champaign, USA

09:10　猪繁殖与呼吸综合征病毒非结构蛋白 nsp2 的多重生物学功能：一个神秘、不断趋异演化的复制酶蛋白
The multifaceted functions of PRRSV nsp2: a mysterious replicase with divergent genetic evolution
韩军　中国农业大学教授
Jun Han China Agricultural University, China

09:40　PRRSV GP2b (E) 促进 GP2/3/4 复合物在质膜的表达
Glycoprotein 2b (E) of porcine reproductive and respiratory syndrome virus (PRRSV) enhances the expression of the GP2/3/4 complex on the plasma membrane.
任晓磊 比利时根特大学
Xiaolei Ren Ghent University, Belgium

09:47　PRRSV nsp1α 二聚化对亚基因组 RNA 合成起重要作用
The dimerization of porcine reproductive and respiratory syndrome virus nsp1α is important for viral subgenomic RNA synthesis
李燕华　扬州大学
Yanhua Li Yangzhou University, China

09:54 PRRSV nsp2 促进接头蛋白 SH3KBP1 的自噬降解从而增强 RIG-I K63 位的多聚泛素化以拮抗宿主的先天性免疫反应展
Porcine reproductive and respiratory syndrome virus nonstructural protein 2 promotes the autophagic degradation of adaptor protein SH3KBP1 to antagonize host innate immune responses by enhancing K63-linked polyubiquitination of RIG-I
张婧 中国农业科学院兰州兽医研究所
Jing Zhang Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

10:00-10:30 休息 / 壁报交流（Break/Posters Communication）

致病机制专题 Pathogenesis

主持人（Moderators）：
Ying Fang 美国伊利诺伊大学厄巴纳 - 香槟分校教授 (University of Illinois at Urbana-Champaign, USA)
Jianqiang Zhang 美国爱荷华州立大学教授 (Iowa State University, USA)

10:30 巨噬细胞随组织差异和环境变化而发生 " 变色龙式 " 适应，以推动 PRRSV-1 演化
Tissue differences and environmental changes give rise to ‘chameleon-like’ adaptation of macrophages, which drives PRRSV-1 evolution
Hans Nauwynck 比利时根特大学教授
Ghent University, Belgium

11:00 通过病毒与宿主蛋白质互作来阐释猪繁殖与呼吸综合征病毒的免疫逃逸机制
Elucidating the immune evasion mechanism of PRRSV through viral protein-host protein interactions
翁长江 中国农业科学院哈尔滨兽医研究所研究员
Changjiang Weng Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

11:30 中国 PRRSV 类 NADC34 毒株的致病性及商用疫苗的保护效果
Pathogenicity of Chinese NADC34-like PRRSV and commercial PRRSV vaccine efficacy against its infection
李向东 扬州大学
Xiangdong Li Yangzhou University, China

11:37 PDCD4 以 eIF4A 依赖性方式限制 PRRSV 复制，并被 nsp9 拮抗
PDCD4 restricts PRRSV replication in an eIF4A-dependent manner and is antagonized by the viral nonstructural protein 9
郭春和 华南农业大学
Chunhe Guo South China Agricultural University, China

11:44 PRRSV JXwn06 毒株 5' UTR 存在一个调节细胞炎症反应的 uORF
The 5'UTR of porcine reproductive and respiratory syndrome virus strain JXwn06 harbors an uORF that regulates cellular inflammation
刘腾 中国农业大学
Teng Liu China Agricultural University, China

12:00-13:30 自助午餐，地点：烟台八角湾国际会展中心 A1 展厅
Lunch, Hall A1, Yantai Bajiaowan International Convention & Exhibition Center

疫苗与免疫专题 Vaccines and Immunity

主持人（Moderators）：

Xiang-Jin (X.J.) Meng 美国弗吉尼亚理工学院暨州立大学教授 (Virginia Polytechnic Institute and State University, USA)

韩军 中国农业大学教授 (**Jun Han** China Agricultural University, China)

13:30 猪繁殖与呼吸综合征疫苗的现状与未来
Present and future of PRRS vaccines
Enric Mateu 西班牙巴塞罗那自治大学教授
Autonomous University of Barcelona, Spain

14:00 猪繁殖与呼吸综合征疫苗不断优化的三十年进程
Three-decade of marching towards a better PRRS vaccine
袁世山 中国农业科学院兰州兽医研究所深圳生物制品前沿创新中心研究员
Shishan Yuan Shenzhen Center of Excellence on Veterinary Biologics, Lanzhou Vet Research Institute, CAAS

14:30 PRRSV GP5 A 表位在猪体内的免疫反应及其在病毒中和中的作用
Porcine antibody response to epitope A on porcine reproductive and respiratory syndrome virus glycoprotein 5 and its role in virus neutralization
Jing Huang 美国明尼苏达大学
University of Minnesota, USA

14:37 PRRSV 特异性 IgM 用作 PRRSV 灭活苗新型佐剂
PRRSV-specific IgM as a novel adjuvant for inactive PRRSV vaccine
南雨辰 西北农林科技大学
Yuchen Nan Northwest A&F University, China

14:44 谱系 1 PRRSV MLV 可为仔猪提供同源和异源毒株的广泛交叉保护
Lineage 1 Porcine Reproductive and Respiratory Syndrome Virus Attenuated Live Vaccine Provides Broad Cross-Protection against Homologous and Heterologous NADC30-Like Virus Challenge in Piglets
张洪亮 中国农业科学院哈尔滨兽医研究所
Hongliang Zhang Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

14:51 剖析 PRRSV 中和抗体反应——确定新型疫苗靶标
Dissecting the neutralizing antibody response to porcine reproductive and respiratory syndrome virus to identify novel vaccine targets
Rory Fortes de Brito 英国皮尔布莱特研究所
The Pirbright Institute, UK

15:00-15:30 休息 / 壁报交流（Break/Posters Communication）

实践论坛 Practical Forum

主持人（Moderators）：

周磊 中国农业大学教授 (**Lei Zhou** China Agricultural University, China)

刘从敏 PIC 亚太区技术服务总监 (**Congmin Liu** PIC, Asia Regional Technical Service Director)

15:30 **集约化养猪场消除疫病的要点**
Key points of diseases elimination in intensive pig farm
李金龙 东北农业大学教授（中英合资 伊科拜克邀请）
Jinlong Li Ph.D/DVM/Professor, Northeast Agricultural University
(Invited by Zhejiang ECO-BIOK Animal Health Products Co., Ltd)

15:50 **从抗体角度看猪场 PRRSV 防控与净化实践**
Prevention, Control and Eradication Practice of PRRSV in Pig Farms from the Perspective of Antibody
吴星亮 北京金诺百泰生物技术有限公司技术总监
Xingliang Wu Technical director of Beijing Jinnuo Biotech Co.,Ltd.

16:10 **最佳案例分享：通过持续的健康计划提高 PRRSV 感染场的生猪生产性能**
Best practise sharing：Improve pig production performance of a PRRS infected farm via constant health program
胡群山 硕腾中国猪业务团队 KA 技术服务经理
Qunshan Hu KA Technical Service Manager, Zoetis China

16:30 **不同药物干预日龄对保育猪蓝耳感染情况的差异分析**
Analysis of PRRSV infection situation in nursed piglets at the difference age of drug intervention
田伟普 瑞普生物家畜 CBG 解决方案部专家
Weipu Tian Ringpu-Domestic animal CBG Solution expert

16:50 **困扰蓝耳病防控的十个重要问题**
Top ten questions perplexing PRRS control
曾容愚 天康制药股份有限公司技术支持总监
Rongyu Zeng Director of technical support in TECON Pharmaceutical Co.Ltd.

17:10 **猪蓝耳病防控实践与思考**
Practice and Reflection on Prevention and Control of PRRSV
刘汉平 国家企业技术中心、国家地方联合实验室，华派生物技术（集团副总裁）
Hanping Liu Vice President MD of Huapai Biological Group

18:00-21:30 Banquet

大会日程
Detail Program

8 月 9 日，山东省烟台市八角湾国际会展中心八角湾厅
August 9，Multi-functional Hall, Yantai Bajiaowan International Convention & Exhibition Center

临床疾病控制专题 Field Disease Control

主持人（Moderators）：
Tomasz Stadejek 波兰华沙生命科学大学教授 (Warsaw University of Life Sciences, Poland)
Enric Mateu 西班牙巴塞罗那自治大学教授 (Autonomous University of Barcelona, Spain)

08:30 猪繁殖与呼吸综合征病毒变异株的出现：前瞻性监测的示例与思考
PRRSV variant emergence: example and considerations for prospective monitoring
Mariana Kikuti 美国明尼苏达大学研究员
University of Minnesota, USA

09:00 中国规模化猪场蓝耳病的净化实践与启示
The practice and enlightenment of PRRS elimination in large scale pig farm of China
刘从敏 PIC 亚太区技术服务总监
Congmin Liu PIC Asia Regional Technical Service Director

09:30 PRRSV 类 NADC30 毒株感染后种猪群主要生产参数的变化评估
Assessment of main productive parameters changes of a breeding herd following a NADC30-like PRRSV infection
方奎 唐人神集团股份有限公司
Kui Fang Tang Ren Shen Group Co., Ltd

09:37 实施定点清除策略以快速清除猪场中 PRRSV 的案例研究
Implementing Unit Elimination Strategies for Rapid Elimination of Porcine Reproductive and Respiratory Syndrome in Swine Farms: A Case Study
李月田 威海华育米山养殖有限公司
Yuetian Li Weihai Huayu Mishan Breeding Co., Ltd

09:44 降低 PRRS MLV 免疫剂量对中国类 NADC30 和类 NADC34 毒株保护效果的影响
Protective efficacy of PRRS MLV vaccine with reduced immunization dosage against either NADC30-like or NADC34-like strain prevalence in China
Nanfang Zeng Gianstar research institute

09:51 一种潜在的 PRRSV 感染抑制剂：硝唑尼特
Nitazoxanide as a Potential Inhibitor of PRRSV Infection
崔占鼎 中国农业科学院兰州兽医研究所
Zhanding Cui Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

10:00-10:30 休息 / 壁报交流（Break/Posters Communication）

诊断与流行病学专题 Diagnostics-Epidemiology

主持人（Moderators）：
Mariana Kikuti 美国明尼苏达大学研究员 (University of Minnesota, USA;)
周磊 中国农业大学教授 (**Lei Zhou** China Agricultural University, China)

10:30 猪繁殖与呼吸综合征病毒 -1：基因亚型的含义
PRRSV-1: the meaning of genetic subtypes
Tomasz Stadejek 波兰华沙生命科学大学教授
Warsaw University of Life Sciences, Poland

11:00 美国猪繁殖与呼吸综合征病毒诊断与分子流行病学研究进展
Overview of PRRSV diagnostics and molecular epidemiology in the USA
Jianqiang Zhang 美国爱荷华州立大学教授
Iowa State University, USA

11:30 美国 PRRSV 遗传变异：谱系、亚群和毒株如何帮助我们理解疾病的流行病学
PRRSV genetic variability in the U.S.: how can lineages, sub-lineages and variants help us understand disease epidemiology
Igor Paploski 美国明尼苏达大学
University of Minnesota, USA

11:37 中国新发高致死性 PRRSV RFLP 1-4-4 L1C 重组毒株
Emerging of highly lethal PRRSV RFLP 1-4-4 L1C recombinant variant in China
安同庆 中国农业科学院哈尔滨兽医研究所
Tongqing An Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

11:44 剖析中国 PRRSV-1 的遗传多样性和演化趋势以及致病性
Dissecting genetic diversity, evolutionary trends and pathogenicity of Chinese PRRSV-1
龚帮俊 中国农业科学院哈尔滨兽医研究所
Bangjun Gong Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China

11:51 基于扁桃体刮片和 TOSc 结果——单头母猪随时间变化的 PRRSV RNA 检测模式
PRRSV RNA detection patterns in individual sows over time based on tonsil scraping and TOSc results
李鹏 美国爱荷华州立大学
Peng Li Iowa State University, USA

12:00-13:30 自助午餐，地点：烟台八角湾国际会展中心 A1 展厅
Lunch, Hall A1, Yantai Bajiaowan International Convention & Exhibition

宿主遗传学及宿主与病毒相互作用专题 Host Genetics and Host virus interactions

主持人（Moderators）：

Laura Miller 美国堪萨斯州立大学教授 (Kansas State University, USA)

黄耀伟 华南农业大学教授 (**Yaowei Huang** South China Agricultural University, China)

13:30 抗猪繁殖与呼吸综合征病毒基因编辑猪的创制和培育
Creation and breeding of gene edited pigs resistant to porcine reproductive and respiratory syndrome virus
李奎 中国农业科学院深圳农业基因组研究所教授
Kui Li Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, China

14:00 生长育肥猪疾病抗性的遗传学研究
Genetics of Disease Resilience in Grow-Finish Pigs
Jack Dekkers 美国爱荷华州立大学教授
Iowa State University, USA

14:30 CD163 SRCR5 结构域部分缺失的两广小花猪对 PRRSV-2 感染的抵抗力评估
Evaluation of the resistance of Liang Guang small Spotted pigs with partial deletion of the CD163 SRCR5 Domain to Porcine Reproductive and Respiratory Syndrome Virus 2 Infection
吴雨 中山大学
Yu Wu Sun Yat-sen University, China

14:37 切换免疫靶点：应用 MJPRRS 分类法来表征 PRRSV GP5 C 表位随时间的变化
Switching immune target: applying MJPRRS classifications to characterize how PRRSV GP5-epitope C changes over time
Julia Baker 美国明尼苏达大学
University of Minnesota, USA

14:44 PRRSV 类 NADC34 毒株的分离、鉴定及致病性分析
Isolation, identification and pathogenicity analysed of a NADC34-like porcine reproductive and respiratory syndrome virus
王开 吉林农业大学
Kai Wang Jilin Agricultural University, China

14:51 一种全新 PRRSV-1 和 PRRSV-2 多重 qPCR 方法检测性能的田间试验评估
Performance of a newly designed qPCR for multiplex detection of PRRSV-1 and PRRSV-2: field study evaluation
Álvaro Hidalgo 爱德士
IDEXX

14:58 PRRSVseq：基于多重 PCR 的 PRRSV 全基因组测序
PRRSVSeq：Multiplex PCR-Based Whole-Genome Sequencing for Porcine Reproductive and Respiratory Syndrome Virus PRRSV
郭静静 华中农业大学
Jingjing Guo Huazhong Agricultural University, China

15:05 HP-PRRSV 与类 NADC30 毒株复制保真性差异分析
Fidelity Characterization of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus and NADC30-like Strain
高翔 中国农业大学
Xiang Gao China Agricultural University, China

15:12 PRRSV 通过多重机制调节自噬以促进病毒增殖
PRRSV modulates autophagy via multiple mechanisms to optimize viral proliferation
周艳荣 华中农业大学
Yanrong Zhou Huazhong Agricultural University, China

15:20-15:50 休息 / 壁报交流（Break/Posters Communication）

新发病毒专题 Emerging Viruses

主持人（Moderators）：
韩军 中国农业大学教授 (**Jun Han** China Agricultural University, China)
袁世山 中国农业科学院兰州兽医研究所深圳生物制品前沿创新中心研究员
(**Shishan Yuan**, Shenzhen Center of Excell ence on Veterinary Biologics, Lanzhou Vet Research Institute, CAAS)

15:50 猪流行性腹泻病毒新型候选疫苗
Novel vaccine candidates against porcine epidemic diarrhea virus
Xiang-Jin X.J.) Meng 美国弗吉尼亚理工学院暨州立大学教授
Virginia Polytechnic Institute and State University, USA

16:20 揭示猪的抗病毒免疫：基于 PRRSV 感染后的转录组分析以制定靶向策略
Transcriptomic Profiling of PRRSV Infection for Targeted Strategies
Laura Miller 美国堪萨斯州立大学教授
Kansas State University, USA

16:50 抗重组 PEDV 疫苗的创制
Develop recombination-resistant PEDV vaccines
王秋红 美国俄亥俄州立大学教授
Qiuhong Wang The Ohio State University, USA

17:20 猪肠道冠状病毒 PEDV 非结构蛋白 2 是一种新的毒力因子——联接天然免疫与自噬
Enteric coronavirus PEDV non-structural protein 2 acts as a novel virulence determinant by linking innate antiviral immunity and autophagy
黄耀伟 华南农业大学教授
Yaowei Huang South China Agricultural University, China

17:50-18:00 大会闭幕式 (Closing Ceremony)

勃林格殷格翰卫星会
Satellite Symposium

8 月 8 日，山东省烟台市八角湾国际会展中心金沙滩厅
August 8, Golden Beach Hall, Yantai Bajiaowan International Convention& Exhibition Center

经典有道 创行无界 - 暨蓝福莱全球上市 30 周年会议
IPRRS 2024 - Boehringer Ingelheim Satellite Symposium

15:30 开场致词 Opening speech
杨汉春 中国农业大学教授
Hanchun Yang China Agricultural University, China
陈镇鸿 勃林格殷格翰中国猪业务负责人
Ray Head of BI China Swine Business

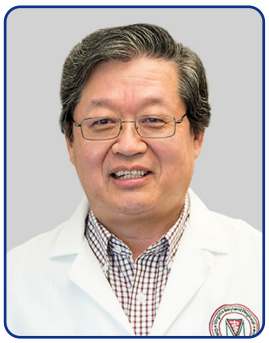
15:45 PRRS 流行病学调查及诊断决策树
PRRS epidemiological investigation and diagnostic decision tree
张建强，美国爱荷华州立大学教授
Jianqiang Zhang Iowa State University, USA

16:25 新一代测序在蓝耳病监控中的实践探索
Practice of next-generation sequencing in PRRS surveillance
王衡，华南农业大学教授
Heng Wang South China Agricultural University, China

17:00 PRRS 防控 2.0 全球解决方案
PRRS control 2.0 global holistic solution
李超斯博士，勃林格殷格翰市场部蓝耳 & 猪瘟技术经理
Dr. Chaosi Li PRRS&CSF Tech Manager of BI China Swine, China

17:30 会议总结 Wrap-up
樊爱华，勃林格殷格翰中国猪业务市场部负责人
Fiona Head of BI China Swine Marketing

嘉宾介绍
Introduction of Speakers



Xiang-Jin(X.J.)Meng

美国国家发明家学会，院士；美国微生物科学院，院士；美国科学促进会，院士；
弗吉尼亚理工学院暨州立大学，教授

Elected Fellow of National Academy of Inventors;Elected Fellow of the American Academy of Microbiology;Elected Fellow of the American Association for the Advancement of Science;Virginia Polytechnic Institute and State University, Professor

题目：猪流行性腹泻病毒新型候选疫苗

Title: Novel vaccine candidates against porcine epidemic diarrhea virus

Meng 博士是弗吉尼亚马里兰兽医学院、弗吉尼亚理工学院暨州立大学的杰出教授、美国国家科学院院士、美国国家发明家学会院士、美国微生物科学院院士以及美国科学促进会的当选院士。他在 1999 年加入弗吉尼亚理工大学之前，是美国国立卫生研究院 (NIH) 传染病实验室的高级研究员，他在爱荷华州立大学兽医学院获得了免疫生物学博士学位。目前担任弗吉尼亚理工大学 NIH T32 post-dvm 研究生培训项目的主任 (2006- 至今)。

也是许多著名奖项的获得者，包括弗吉尼亚理工大学终身成就奖，爱荷华州立大学首届洛林 J. 霍夫曼研究生校友奖，以及弗吉尼亚州高等教育国务院杰出教师奖。

Meng 博士研究新兴和人畜共患病毒，并开发有效的病毒疫苗。他发表了 360 余篇同行评议论文 [>37,107 次引用，h 指数 = 100]，是 22 项获得美国病毒疫苗专利的发明人。Meng 博士目前担任四家国际期刊 (PNAS, mBio, Virus Research 和 Veterinary Microbiology) 的编辑或主编。

Dr. X.J. Meng is a University Distinguished Professor at the Virginia-Maryland College of Veterinary Medicine, Virginia Polytechnic Institute and State University (Virginia Tech) in Blacksburg, VA. Dr. Meng is an elected member of the U.S. National Academy of Sciences, elected Fellow of the National Academy of Inventors, elected Fellow of the American Academy of Microbiology, and elected Fellow of the American Association for the Advancement of Science. Prior to joining the faculty at Virginia Tech in 1999, Dr. Meng was a Senior Staff Scientist in the Laboratory of Infectious Diseases at the U.S. National Institutes of Health (NIH). Dr. Meng received his PhD in immunobiology from Iowa State University College of Veterinary Medicine in Ames, Iowa. Dr. Meng is Director of the NIH T32 post-DVM graduate training program at Virginia Tech (2006-present).

He is the recipient of many prestigious awards, including the Virginia Tech Lifetime Achievement Award for Innovation, the Inaugural Lorraine J. Hoffman Graduate Alumni Award from Iowa State University, and the Outstanding Faculty Award from State Council of Higher Education for Virginia. Dr. Meng studies emerging and zoonotic viruses and develops effective viral vaccines. He has published more than 360 peer-reviewed papers [>37,107 citations, h-index = 100], and is an inventor of 22 awarded U.S. patents on virus vaccines. Dr. Meng currently serves as Editor or Editor-in-Chief for four international journals (PNAS, mBio, Virus Research, and Veterinary Microbiology).

Ying Fang

美国伊利诺伊大学厄巴纳 - 香槟分校 教授

University of Illinois at Urbana-Champaign, Professor

题目：猪繁殖与呼吸综合征病毒复制机制研究进展

Title: The Art of PRRSV Replication

Fang 教授现任美国伊利诺伊大学厄巴纳 - 香槟分校病理生物学系的全职教授。她是全球研究 PRRSV 复制和发病机制的先驱和知名领导者。她首次构建了美国 PRRSV-1 株的感染性克隆并且引领了 PRRSV 非结构蛋白在病毒复制、致病性以及宿主免疫应答过程中作用的相关研究。她的实验室率先对 PRRSV 复制酶的结构 - 功能进行了深入的研究，这使得她后来发现了新的 PRRSV 蛋白和前所未有的病毒复制机制。除了对基础科学的贡献外，方教授的研究还产生了商业产品和技术，直接使畜牧业受益。为了表彰她对研究和专业团体的贡献，她获得了各种奖项，包括 2023 年和 2017 年 Zoetis Animal Health 卓越研究奖，2013 年杰出研究员奖，2010 年知识产权转化奖，2006 年院长研究奖等。

Prof. Ying Fang is a Full Professor at the Department of Pathobiology, University of Illinois at Urbana-Champaign. She is a pioneer and world-renowned leader in studying PRRSV replication and pathogenesis. She constructed the first reverse genetic system of an emerging North American PRRSV-1 and developed diagnostic assays and vaccines in aid of disease control and prevention programs. Her laboratory was the first to conduct in-depth studies on the structure-function of PRRSV replicases, which led to her later discoveries of novel PRRSV proteins and unprecedented viral replication mechanisms.

Besides her contributions to basic science, Prof. Fang’ s research has also resulted in commercial products and technologies that have directly benefited the livestock industry. In recognition of her contribution to research and professional societies, she has been recognized with various awards, including Zoetis Animal Health Award for Research Excellence in 2023 and 2017, Distinguish Researcher Award in 2013, Intellectual Property Commercialization Award in 2010, Dean’ s Research Award in 2006, etc.

韩军 Jun Han

中国农业大学 教授

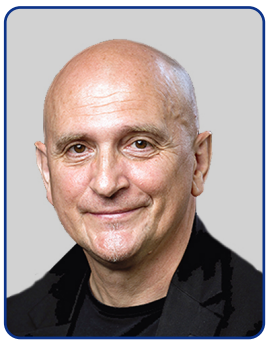
China Agricultural University, Professor

题目：猪繁殖与呼吸综合征病毒非结构蛋白 nsp2 的多重生物学功能：一个神秘、不断趋异演化的复制酶蛋白

Title: The multifaceted functions of PRRSV nsp2: a mysterious replicase with divergent genetic evolution

韩军，中国农业大学动物医学院教授，副院长，国家杰出青年基金获得者。任兽医公共卫生安全全国重点实验室副主任、农业农村部动物流行病学重点实验室主任、农业农村部转基因安全委员会委员、中国畜牧兽医学会兽医公共卫生学分会副理事长、J Vet Sci 期刊编辑，Frontier in Microbiology 编委。长期从事动物分子病毒学研究工作，聚焦猪繁殖与呼吸综合征病毒与非洲猪瘟病毒的病原学与致病机制，承担国家自然科学基金、国家重点研发计划等多项国家级课题，现任国家十四五重点研发计划《非洲猪瘟病毒的病原生物学及致病机制》项目负责人，主要研究成果以第一或通讯作者（含共同）发表于 PNAS、PLoS Pathog、J Virol 等国际期刊。

Dr. Han is a professor of virology and associate Dean for Research and International Programs at the China Agricultural University College of Veterinary Medicine. He is the recipient of the National Distinguished Young Investigator award from National Natural Science Foundation. He is currently an associate director of State Key Laboratory for Veterinary Public Health and Safety, Director of Key Laboratory of Animal Epidemiology of the Ministry and Agriculture and Rural Affairs, a member of the GMO Safety Evaluation Committee of the Ministry of Agriculture and Rural Affairs, and the vice chairman of the Veterinary Public Health Branch of the Chinese Animal Husbandry and Veterinary Medical Association. He also serves as the editor of J Vet Sci and on the editorial board of Frontiers in Microbiology. Dr. Han’ s current research interests focus on the replication and pathogenesis of ASFV and Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) with an aim to promote swine health through better understanding of the viruses. He is currently the chief Principal Investigator of the national joint program “the Molecular Biology and Pathogenesis of African Swine Fever Virus (ASFV)”. His findings are mainly published in peer-reviewed prestigious journals, including PNAS, PLoS Pathogens, and Journal of Virology.



Hans Nauwynck

比利时根特大学 教授

Ghent University, Professor

题目：巨噬细胞随组织差异和环境变化而发生＂变色龙式＂适应，以推动 PRRSV-1 演化

Title: Tissue differences and environmental changes give rise to 'chameleon-like' adaptation of macrophages, which drives PRRSV-1 evolution

Hans Nauwynck 博士于 1993 年在比利时根特大学取得博士学位并被直接提名为教授。在 2004 年，他成为根特大学兽医学院病毒研究室主任并获得欧洲猪健康管理学院认证资质。目前他开设了多门有关哺乳动物、鱼类和甲壳类病毒性疾病的课程。他的研究主要集中在感染动物或人的病毒的细胞或分子致病机制，特别是关于病毒如何进入宿主细胞、病毒如何通过白细胞或沿着神经元跨越机体屏障以及病毒如何免疫逃避等方面的机制研究。他是 483 篇同行评审论文的（共同）作者（15,800 次引用，h-index=63（Web of Science）），是 78 位博士的前任导师，拥有 12 项专利，并创办了两家衍生公司（Imaqua（虾健康服务中心）和 Pathosense（基于纳米孔测序的新一代诊断平台；已在 4 个欧洲国家开展业务））。

Hans Nauwynck is a DVM, who did his PhD and directly became nominated as professor at Ghent University in 1993. In 2004, he took the lead of the Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University and became Diplomate at the European College of Pig Health Management. At present, he lectures several courses on viral diseases in mammals, fish and shellfish. His research focuses on the cellular and molecular pathogenesis of viral diseases in humans and animals (including shrimp and honeybees), with special emphasis on (i) the entry of the virus in its host cell, (ii) the invasion of the virus in its host through barriers, via leukocytes and along neurons and (iii) the escape of the virus from immunity. He is (co-)author of 483 peer reviewed papers (15,800 times cited and h-index=63 (Web of Science)), past promoter of 78 PhDs, owner of 12 patents and founder of two spin-off companies (Imaqua (shrimp health service center) & Pathosense (new generation diagnostic platform, based on nanopore sequencing; already active in 4 European countries)).

翁长江 Changjiang Weng

中国农业科学院哈尔滨兽医研究所 研究员

Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, chief scientist

题目：通过病毒与宿主蛋白质互作来阐释猪繁殖与呼吸综合征病毒的免疫逃逸机制

Title: Elucidating the immune evasion mechanism of PRRSV through viral protein-host protein interactions

翁教授是基础免疫学研究室首席科学家，黑龙江省兽医免疫学重点实验室主任。翁教授一直从事非洲猪瘟病毒 (ASFV)、猪繁殖与呼吸综合征病毒 (PRRSV) 和其他动物病毒的研究。主要研究方向为病毒感染、病毒免疫逃逸及其发病机制。在 Cell Rep、PLoS Pathog、J Biol Chem、J Immunol 等知名期刊发表学术论文 40 余篇。他的研究被世界各地的同行高度引用。

Prof. Weng is the chief scientist of the Division of Fundamental Immunology and the director of the Key Laboratory of Veterinary Immunology of Heilongjiang Province. Dr. Weng has been studying African Swine Fever Virus (ASFV), Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), and other animal viruses. His work focuses on viral infection, viral immune escape, and pathogenesis. Prof. Weng has published more than 40 scientific papers in prestigious journals such as Cell Rep, PLoS Pathog, J Biol Chem, J Immunol and others. His research has been highly cited by peers worldwide.

Enric Mateu

西班牙巴塞罗那自治大学 教授

Autonomous University of Barcelona, Professor

题目：猪繁殖与呼吸综合征疫苗的现状与未来

Title: Present and future of PRRS vaccines

巴塞罗那自治大学动物健康教授。于 1989 年获得兽医学位，1993 年获得博士学位。1994-1995 年在伊利诺伊大学厄巴纳 - 香槟分校做博士后研究员。整个学术生涯都集中在传染性动物疾病领域内，特别是在猪繁殖和呼吸综合征的研究上，并在国际期刊上发表了多篇论文。主要兴趣是了解猪繁殖和呼吸综合征的发病机制和控制方法。

Professor of Animal Health at the Autonomous University of Barcelona. I obtained my veterinary degree in 1989, and my doctorate in 1993. Post-doctoral researcher at the University of Illinois at Urbana-Champaign during 1994-95. My entire academic career has focused on infectious animal diseases, especially in the study of porcine reproductive and respiratory syndrome on which I have published numerous papers in international journals. My main interest is in understanding the pathogenesis of the disease and the methods we can use to control this infection.

袁世山 Shishan Yuan

中国农业科学院兰州兽医研究所深圳生物制品前沿创新中心 研究员

Shenzhen Center of Excellence on Veterinary Biologics, Lanzhou Vet Research Institute, CAAS, Chief Scientific Officer,Researcher

题目：猪繁殖与呼吸综合征疫苗不断优化的三十年进程

Title: Three-ecade of marching towards a better PRRS vaccine

1987 年获青岛农业大学兽医学学士学位，2001 年获明尼苏达大学分子兽医生物科学博士学位。1989-1991 年北京大学植物遗传与蛋白质工程国家重点实验室博士后。1990-1996 年在中国农业科学院哈尔滨兽医研究所兽医生物技术国家重点实验室工作。2000-2004 年，他在明尼苏达州圣保罗的 proteatek International Inc. 担任高级科学家 / 病毒学研发负责人。2005 年起任中国农业科学院上海兽医研究所猪传染病科主任，并获农业部首批“精英科学家计划”资助。2011 年 5 月 1 日加入勃林格殷格翰中国亚洲兽医研究中心 (AVRDC)，担任疫苗研发负责人，AVRDC 负责人 (2017-2021)，兽医事务负责人 (2021-2024)。自 4 月 1 日起，任中国农业科学院兰州兽医研究所深圳生物制品前沿创新中心首席科学官。主要研究方向为猪 RNA 病毒的分子进化与流行病学、RNA 合成机制、猪繁殖与呼吸病毒 (PRRSV) 蛋白结构与功能关系、新一代疫苗开发。

He received bachelor degree of veterinary medicine from Qingdao Agricultural University in 1987 and Ph.D. in Molecular Veterinary Biosciences at University of Minnesota in 2001. He was a post-doctoral fellow at the State Key Laboratory of Plant Genetic and Protein Engineering at Peking University during 1989-1991. He had worked at the State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, CAAS (1990-1996). He worked as the Senior scientist/virology leader of R&D at the ProtaTek International Inc., St. Paul, Minnesota (2000-2004). He was appointed as the director of the Department of Swine Infectious Diseases at Shanghai Veterinary Research Institute, CAAS, and was sponsored by the Ministry of Agriculture as the first tier “elite scientist program” since 2005. Since May 1, 2011, he joined the Asian veterinary research center (AVRDC) at Boehringer Ingelheim AH China as the head of vaccine R&D, and head of AVRDC (2017-2021), and Head of Veterinary Affairs (2021-2024). Starting from April 1, he serves as the Chief Scientific Officer of the Shenzhen Center of Excellence on Vet Biologics, Lanzhou Vet Res Inst, CAAS. His team is mainly focused on the molecular evolution and epidemiology of porcine RNA viruses, mechanisms of RNA synthesis, protein structure-function relationship of porcine reproductive and respiratory virus (PRRSV), and development of next-generation of vaccines.

李金龙 JinLong Li

东北农业大学教授（中英合资 伊科拜克邀请）

Ph.D/DVM/Professor, Northeast Agricultural University（Invited by Zhejiang ECO-BIOK Animal Health Products Co., Ltd）

题目：集约化养猪场消除疫病的要点

Title: Key points of diseases elimination in intensive pig farm

李金龙，东北农业大学，教授 / 博导，入选国家百千万人才工程、国家新世纪优秀人才、龙江学者特聘教授，黑龙江省杰出青年，黑龙江省新世纪优秀人才等，被授予“国务院特殊津贴专家、中国有突出贡献的中青年专家、全模范教师、黑龙江省畜牧科技推广先进个人”等称号。被聘为《Sci Rep》高级编委，《Ecotoxicol Environ Saf》、《Food Chem Toxicol》、《猪业科学》等编委委员。主要从事猪病防控与猪场健康管理、畜禽营养代谢病与中毒病等教学和科研工作。

Jinlong Li, Ph.D/DVM/Professor, is a Dean of College of Veterinary Medicine at Northeast Agricultural University. He received honorary titles such as National Million Talents Program, National New Century Excellent Talents Program, Distinguished Professor of Longjiang Scholars, Outstanding Young Person of Heilongjiang Province. He also has been awarded the title of Special Allowance Expert of the State Council, Expert of Outstanding Contributions to China by Middle-Aged and Young People, Excellent Model Teacher in Heilongjiang Province and Advanced Individual in the Promotion of Animal Husbandry Technology in Heilongjiang Province. He worked as Senior editorial member of Sci Rep, Editorial board member of Ecotoxicol Environ Saf, Food Chem Toxicol, Pig Science, etc. His work in research, teaching and extension has been in prevention and control of pig diseases, focusing on health management, nutrition and metabolic and poisoning diseases in pig farm.

吴星亮 Xingliang Wu

北京金诺百泰生物技术有限公司技术总监

Technical director of Beijing Jinnuo Biotech Co.,Ltd.

题目：从抗体角度看猪场 PRRSV 防控与净化实践

Title: Prevention, Control and Eradication Practice of PRRSV in Pig Farms from the Perspective of Antibody

姓名：吴星亮

现工作单位：北京金诺百泰生物技术有限公司

现职务：技术总监

中国农业科学院哈尔滨兽医研究所硕士，执业兽医。从业 11 年。

猪场生产管理方面：曾负责猪场生产方面的工作，在生产方案制定及批次化生产方面具有一线实践经验。

猪群健康管理方面：曾负责 3.5 万头基础母猪健康管理方面的工作，对引种投产和蓝耳、PEDV、伪狂犬、非洲猪瘟等重大疾病的诊断与防控方面具有一线实践经验。

试剂盒开发与应用方面：拥有发明专利两项，目前主要从事技术服务工作，负责“金诺诊断动物疫病检测技术实训项目交流会”项目，交流会常年开设，旨在提升实验室技术员管理、操作、分析水平。

Name： Xingliang Wu

Profile：

Master, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences

Practicing veterinarian，11 years of working experience

Pig farm production management: Responsible for the work of pig farm production, with front-line practical experience in production plan made and batch production of pigs.

pig health management: responsible for the health management of 35,000 sows, and have front-line practical experience in the gilts introduction,the diagnosis and control of major diseases， such as PRRSV, PEDV,PRV and ASFV.

Kit development and application: have two invention patents, currently mainly engaged in technical services, responsible for the "Animal Disease Detection Technology Training Project Exchange Meeting of Jinnuo Company" project, exchange meeting is held all year round, aimed at improving the management, operation and analysis level of laboratory technicians.

胡群山 Qunshan Hu

硕腾中国猪业务团队 KA 技术服务经理

KA Technical Service Manager, Zoetis China

题目：最佳案例分享：通过持续的健康计划提高 PRRSV 感染场的生猪生产性能

Title: Best practise sharing : Improve pig production performance of a PRRS infected farm via constant health program

胡群山，农村农业部专家库成员，执业兽医毕业于中国农业大学预防兽医学专业多年规模化猪场生物安全、疾病防控及生产管理经验硕腾中国猪业务团队 KA 技术服务经理

Mr. Hu Qunshan, an experienced veterinary expert with a PhD from China Agricultural University, has comprehensive knowledge in farm biosecurity, porcine disease control and production management of big farms. A member of the Expert Panel of the Ministry of Agriculture and Rural Affairs and a licensed veterinarian, he provides valuable insights and guidance for the pig farming industry. Currently, Dr. Hu serves as KA Technical Service Manager in Zoetis China.

田伟普 Weipu Tian

瑞普生物家畜 CBG 解决方案部专家

Ringpu-Domestic animal CBG Solution expert

题目：不同药物干预日龄对保育猪蓝耳感染情况的差异分析

Title: Analysis of PRRSV infection situation in nursed piglets at the difference age of drug intervention

田伟普

瑞普生物 -- 家畜 CBG

技术解决方案部 -- 蓝耳方案负责人

工作履历

一线管理经验

十年大型规模猪场管理经验

六年牧场生产技术总负责人经验

六年牧场一线技术服务经验

Weipu Tian

Ringpu --Domestic animal CBG

Technical Solutions Department--Head of PRRS Solutions--Tian Weipu

Work resume:

Frontline management experience

Ten years of experience in large-scale pig farm management

Six years of pasture experience in Production Technology Director

Six years of pasture experience in front-line technical service

曾容愚 Rongyu Zeng

天康制药股份有限公司技术支持总监

Director of technical support in TECON Pharmaceutical Co.Ltd.

题目：困扰蓝耳病防控的十个重要问题

Title: Top ten questions perplexing PRRS control

曾容愚

天康制药股份有限公司技术支持总监。

南京农业大学动物营养硕士、预防兽医学硕士、马里兰大学免疫学博士，在 Nature Biotechnology, PNAS, The Journal of Immunology，J.Dairy Science 发文多篇。

从事畜禽营养研究与饲料配方制作 7 年、猪和牛新型疫苗研发和推广 20 年。在猪临床免疫学领域，提出伪狂犬“三剑合一”免疫净化方案、蓝耳“五精”防控方案、PEDV 乳源免疫免疫力最大化方案。兼职上海交通大学等 11 大学的专硕导师。中国兽医学会专家委员会副主任委员，兽医公共卫生学会委员。从事畜牧兽医行业国际会议翻译 10 多年。参与翻译 6 本行业书籍和教材。

Rongyu Zeng MS.MS.Ph.D

Director of technical support in TECON Pharmaceutical Co.Ltd.

Trained in Animal Nutrition and Preventive Veterinary Medicine respectively as M.S students at Nanjing Agricultural University; in Immunology as PhD student at University of Maryland. Published decent papers in Nature Biotechnology, PNAS, The Journal of Immunology，J.Dairy Science,etc.

Worked as a nutritionist for pigs, poultry, aquaculture and ruminants for 7 years; Engaged in vaccine development and application against major diseases in pigs and cows for 20 years. In the field of clinical immunology in pigs, proposed and practised innovative solutions to major diseases control and eradication including "three swords in one" approach for pseudo rabies eradication, "five essence" for PRRSV prevention, and maximizing milk-derived immunity to control PEDV.

Served as adjunct Master degree pursuing graduate student supervisor in veterinary medicine in 11 universities including Shanghai Jiaotong University, Vice Chairman of the Expert Committee of the China Veterinary Association, and Committee Member of the Veterinary Public Health Society,Simultaneous interpreter for International meetings in the area of animal husbandry and veterinary medicine for 13yrs. Participated in translating 6 industrial books and university textbooks.

刘汉平 Hanping Liu

国家企业技术中心、国家地方联合实验室，华派生物技术（集团）副总裁

Vice President MD of Huapai Biological Group

题目：猪蓝耳病防控实践与思考

Title: Practice and Reflection on Prevention and Control of PRRSV

刘汉平 , 男 , 生于 1974 年 3 月 ,2008 年 7 月毕业于国药中生集团武汉生物制品研究所 , 免疫学专业，获医学博士学位。

曾在武汉生物制品研究所，国药动保，海利生物，成都史纪工作，从事人用和动物疫苗的研发，生产及动物疫苗企业整体运营管理工作。历任研发工程师，研发部经理，技术副总裁，总经理。在国药动保工作期间，领衔独立自主开发了国产第一支圆环病毒 2 型杆状病毒基因工程病毒样颗粒疫苗。现任华派生物技术（集团）股份有限公司副总裁，负责集团公司猪和家禽疫苗业务的整体运营。

Mr.Liu Hanping is a professional with extensive experience in the field of biopharmaceuticals. His career began in 2008 after he earned his Ph.D.in Immunology from the Wuhan Institute of Biological Products, under the China National Pharmaceutical Group. Since then, he has held various positiong including research and development engineer, R & D department manager, vice president of technology,and general manager in renowned biopharmaceutical companies,working on the research and development and production of vaccines for human and animal use, as well as the overall operational management of animal vaccine enterprises.

Throughout his career, Mr.Liu Hanping has achieved significant accomplishments, most notably during his time at Sinopharm Animal Health, where he led the independent development of China’ s first domestically produced PCV2 VLPs vaccine. This breakthrough has had a profound impact on China’ s animal vaccine industry.

Currently,Mr.Liu Hanping serves as the vice president of HuaPai Biotechnology(Group) Co.,Ltd., where he is responsible for the overall operation of the company’ s pig and poultry vaccine business. His expertise and wealth of experience undoubtedly bring tremendous value to the company.

Mariana Kikuti

美国明尼苏达大学 研究员

University of Minnesota, Researcher

题目：猪繁殖与呼吸综合征病毒变异株的出现：前瞻性监测的示例与思考

Title: PRRSV variant emergence: example and considerations for prospective monitoring

Mariana Kikuti 是一名兽医专业毕业生，作为兽医学院医师，参加过人畜共患病和公共卫生项目，进行了人畜共患疾病的关键技术研究和实验室诊断。在攻读公共卫生流行病学硕士和博士学位期间，她主要侧重于调查人类虫媒病毒的暴发，并评估在多种共循环虫媒病毒的情况下诊断测试的准确性。

在她的职业生涯中，她加入了美国明尼苏达大学的莫里森猪健康监测项目，并担任研究员。她的工作集中在养猪业的流行病学调查，重点关注关键的健康结果，如母猪死亡率、PRRS、PED 和 PDCoV，使用分子流行病学方法在疾病发生调查中确定病例定义。

Mariana Kikuti, a Veterinary Medicine graduate, delved into specialized training as a fellow of the Veterinary Medicine Residency Program in Zoonosis and Public Health. Here, she conducted pivotal research and laboratory diagnosis of zoonotic diseases. Her academic journey advanced as she pursued both her MPH and PhD in public health epidemiology, focusing primarily on investigating human arbovirus outbreaks and assessing the field accuracy of diagnostic tests in the context of multiple co-circulating arboviruses.

Transitioning into her professional career, Mariana joined the Morrison Swine Health Monitoring Project at the University of Minnesota, USA, where she currently serves as a Researcher. Her work centers on epidemiological investigations within the swine industry, focusing on key health outcomes like sow mortality, PRRS, PED, and PDCoV using molecular epidemiology approaches to ascertain case definitions in investigations of disease occurrence.

刘从敏 Congmin Liu

PIC 亚太区技术服务总监

PIC, Asia Regional Technical Service Director

题目：中国规模化猪场蓝耳病的净化实践与启示

Title: The practice and enlightenment of PRRS elimination in large scale pig farm of China

刘从敏，PIC 亚太区技术服务总监，带领技术服务团队为 PIC 中国亚洲客户提供现场和远程服务。2009 年毕业于中国农业大学，获兽医学硕士学位。2009 年至 2012 年，他在 VICA 开始了他的职业生涯，担任动物保健产品的技术服务经理。2012 年 5 月加入 PIC 中国后，他曾在 PIC - PN 猪场作为健康保证兽医工作了 3 年，管理高健康状况猪场 (PRRS, PED 和 MH 阴性猪场)。他从 2015 年 5 月开始担任 PIC 中国的技术服务经理，为客户提供支持。他带领健康服务团队为客户成功建立生物安全体系，包括现场生物安全评估、养殖场布局设计、饲料厂审核和生物安全审核等。同时，他在消灭 PED 和 PRRS 方面也有很多经验。

Congmin Liu, PIC Asia Regional Technical Service Director, leads technical service team to provide onsite and remote services to PIC customers in Asia.

Congmin Liu graduated China Agricultural University and got his Master degree of Veterinary Medicine in 2009. He started his career from VICA as the technical service manager of animal health products from 2009 to 2012. Congmin joined PIC China in May 2012. He had worked in the PIC PN herd farms as health assurance vet for 3 years to manage high health status farm (PRRS, PED and MH negative herd). He started to be the technical service manager to support customers of PIC China since May 2015. He leads health service team to work with customers to set up their biosecurity system successfully, including site biosecurity evaluation, farm layout design, feed mill audit and biosecurity audit etc. Congmin also has good experiences in PED and PRRS elimination.

Tomasz Stadejek

波兰华沙生命科学大学 教授

Warsaw University of Life Sciences, Professor

题目：猪繁殖与呼吸综合征病毒 -1: 基因亚型的含义

Title: PRRSV-1: the meaning of genetic subtypes

Tomasz Stadejek 于 1990 年毕业于波兰卢布林生命科学大学兽医学院。1991 年至 2011 年，他在波兰普拉维国家兽医研究所猪病部门工作。1996 年获博士学位，2002 年获理学博士学位。他曾在美国爱荷华州艾姆斯的国家动物疾病中心和国家兽医服务实验室、瑞典乌普萨拉的国家兽医研究所和丹麦林德霍尔姆的国家兽医研究所担任客座研究员。

2007 年，他被世界动物卫生组织 (OIE) 任命为 PRRS 专家，并于 2007-2011 年担任 OIE PRRS 参考实验室主任。他也是国际病毒分类委员会 (ICTV) 动脉炎病毒研究组成员。

2008 年，他获得了欧洲猪健康管理学院 (ECPHM) 的文凭，2011 年至 2013 年，他担任该学院的董事会成员和秘书。

自 2012 年以来，他作为全职教授任职于华沙生命科学大学兽医学院。他目前的研究重点是 PRRSV, IAV, PCV2, PCV3 和新近流行的 PPV 的诊断和流行病学。

Tomasz Stadejek graduated from the Faculty of Veterinary Medicine at the University of Life Sciences in Lublin, Poland in 1990. From 1991 to 2011 he worked at the Department of Swine Diseases of the National Veterinary Research Institute in Pulawy, Poland. He obtained PhD degree in 1996 and DSc in 2002. He worked as a guest researcher at the National Animal Disease Centre and National Veterinary Services Laboratories in Ames, Iowa, USA, National Veterinary Institute in Uppsala, Sweden and the National Veterinary Institute, Lindholm, Denmark.

In 2007 he was appointed by the World Organization for Animal Health (OIE) as an expert for PRRS, and in 2007-2011 he was the head of the OIE Reference Laboratory for PRRS. He is a member of Arterivirus Study Group of the International Committee on Taxonomy of Viruses (ICTV).

In 2008 he obtained the diploma of the European College of Porcine Health Management (ECPHM) and from 2011 to 2013 he was a board member and the secretary of the college.

Since 2012 he is full professor at the Faculty of Veterinary Medicine at the Warsaw University of Life Sciences. His current research is focused on diagnostic and epidemiology of PRRSV, IAV, PCV2, PCV3 and emerging porcine parvoviruses.

Jianqiang Zhang

美国爱荷华州立大学 教授

Iowa State University, Professor

题目：美国猪繁殖与呼吸综合征病毒诊断与分子流行病学研究进展

Title: Overview of PRRSV diagnostics and molecular epidemiology in the USA

Zhang 博士是爱荷华州立大学兽医诊断实验室病毒学 / 分子诊断部门的教授和病毒学家。他目前的研究重点是兽医病毒的诊断，发病机制和疫苗开发。他有 160 篇同行评审的文章及书籍章节。他是《猪病学》（英文第 11 版和 12 版）的主编之一以及 JVDI 期刊病毒学的编辑。

Dr. Jianqiang (JQ) Zhang is currently a professor and virologist at the Iowa State University Veterinary Diagnostic Laboratory. He is focused on diagnostics, pathogenesis, and vaccine development of viruses of veterinary significance. He has ~160 refereed journal publications and book chapters. He has served as one of the Editors of the book Diseases of Swine (11th and 12th editions) and the Virology Session Editor of Journal of Veterinary Diagnostic Investigation (JVDI).

李奎 Kui Li

中国农业科学院深圳农业基因组研究所教授

Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Professor

题目：抗猪繁殖与呼吸综合征病毒基因编辑猪的创制和培育

Title: Creation and breeding of gene edited pigs resistant to reproductive and respiratory syndrome virus

李奎，中国农业科学院农业基因组研究所教授，研究员委员会主任，国家杰出青年科学基金获得者，973 首席科学家。主要研究方向为动物遗传育种，多年来一直围绕猪基因组育种、猪基因工程育种、实验用小型猪及医学模型等方面开展研发工作。主持和参与培育了多个猪新品种、配套系，以及农用基因编辑猪育种新材料和医学模型猪。以通讯作者（含共同）在 Nature Genetics, Nature Communications, National Science Review, Genome Biology 等刊物发表论文 200 余篇，授权专利 100 余件，软件著作权 10 余件件，制订国家和地方标准 10 余件项，主持获得国家技术发明二等奖、中国专利银奖和优秀奖。兼任《 Journal of Integrative Agriculture 》执行主编、《农业生物技术学报》和《生物技术通报》副主编、《 Journal of Animal Science and Biotechnology》等刊物编委。

Li Kui, Professor at Agricultural Genomic Institute at Shenzhen, Chinese Academy of Agricultural Sciences. He received his bachelor's degree, master's degree and doctoral degree from Wuhan University. From 1996 to 2003, he served as a Professor at Huazhong(Central China) Agricultural University. From 2004 to 2020, he served as a Professor at the Institute of Animal Science, Chinese Academy of Agricultural Sciences. He also used to be the visiting scientist at University of Sydney, Australia, and full-time visiting Professor at Cornell University, USA.

He also serves as the executive editor in chief of Journal of Integrated Agriculture, deputy editor in chief of Journal of Agricultural Biotechnology. He has been granted three US patents and more than 100 national invention patents in China. He has published over 200 Chinese and English papers as the first author or corresponding author in journals such as Nature Genetics, Nature Communications, National Science Review and Genome Biology.

His main research direction is animal genetics and breeding, and for many years, his research and development work has been carried out around pig genome breeding, pig genetic engineering breeding, experimental miniature pigs, and medical models. He has led and participated in the cultivation of multiple new pig breeds, supporting lines, as well as new genome editing pig breeding materials for agriculture and medical models. At present, his main research work is breeding pigs for resistance to epidemic diseases.

Jack Dekkers

美国爱荷华州立大学 教授

Iowa State University, Professor

题目：生长育肥猪疾病抗性的遗传学研究

Title: Genetics of Disease Resilience in Grow-Finish Pigs

Dekkers 博士在荷兰长大，在瓦赫宁根农业大学获得动物科学学士和硕士学位，在威斯康星大学获得乳业科学博士学位，主要研究动物育种和遗传学。1989 年至 1997 年，他在圭尔夫大学任教，与加拿大奶牛业密切合作，致力于奶牛的遗传改良。1997 年，他来到爱荷华州立大学，现任 C.F. Curtiss 特聘教授。他在动物育种和遗传学的几个关键领域建立并开展了卓有成效的研究为动物育种和遗传学及其应用做出了重要贡献。他最初的工作是关于奶牛育种，包括育种项目的设计和经济评估、育种目标和经济指标的制定、遗传分析和功能性评估以及随机回归模型的开发。随后，他的研究重点是猪的饲料效率遗传学以及猪和家禽的抗病性。在他的职业生涯中，他还为将分子遗传学和基因组学整合到动物育种中做出了重要贡献，包括 QTL 检测、标记辅助选择和基因组选择。他的贡献得到了国际认可，以基础研究和创新为特征，同时在应用上又具有敏锐的眼光。

Dr. Jack Dekkers grew up in the Netherlands and received B.Sc. and M.Sc. degrees in animal science from the Wageningen Agricultural University and a Ph.D. in dairy science with a focus on animal breeding and genetics from the University of Wisconsin. From 1989 to 1997 he was on faculty at the University of Guelph, working closely with the Canadian industry on genetic improvement of dairy cattle. He moved to Iowa State University in 1997, where he currently is a C.F. Curtiss Distinguished Professor. Dr. Dekkers has established and conducted highly productive research programs in several key areas in animal breeding and genetics and has thereby made important contributions to the science of animal breeding and genetics and its application. His initial work was on dairy cattle breeding, including design and economic evaluation of breeding programs, development of breeding objectives and economic indexes, genetic analysis and evaluation of functional traits, and development of random regression models. Subsequent contributions have focused on genetics of feed efficiency of pigs and disease resistance in pigs and poultry. Throughout his career, he has also made important contributions to the integration of molecular genetics and genomics in animal breeding, including QTL detection, marker-assisted selection, and genomic selection. His contributions are internationally recognised and characterized by fundamental strength and innovation but with a keen eye for application.

Laura Miller

堪萨斯州立大学，副教授

Kansas State University Associate Professor

题目：" 揭示猪的抗病毒免疫：基于 PRRSV 感染后的转录组分析以制定靶向策略 "

Title: Unveiling Porcine Antiviral Immunity: Transcriptomic Profiling of PRRSV Infection for Targeted Strategies

Miller 博士于 2023 年来到堪萨斯州立大学，担任兽医病毒学副教授。她以优异成绩获得苏格兰爱丁堡大学医学微生物学理学学士学位和英国雷丁大学皮尔布莱特动物健康研究所分子病毒学博士学位。2001 年英国口蹄疫疫情期间，米勒博士在皮尔布莱特担任血清学检测负责人，之后他前往美国内布拉斯加州克雷中心的美国肉用动物研究中心担任微生物学家。2007 年，Miller 博士被美国农业部调往爱荷华州艾姆斯的国家动物疾病中心。她已发表超过 65 篇经同行评审的文章，引用次数达 1839 次。她目前担任美国兽医免疫学家协会前任主席、NC-229 猪繁殖与呼吸综合征病毒和猪新发病毒性疾病多州检测与控制委员会成员，以及 NSRP-8 国家动物基因组研究计划项目主任。

Dr. Miller came to Kansas State University in 2023 as an Associate Professor in Veterinary Virology. She received her Bachelor of Science with Honors in Medical Microbiology from the University of Edinburgh, Scotland, and a Ph.D. in Molecular Virology from the Institute for Animal Health, Pirbright, conferred by the University of Reading, England. After serving as a leader of the serological testing at Pirbright during the UK Foot-and-Mouth Disease Epidemic of 2001, Dr. Miller moved to the United States as a Research Microbiologist at the US Meat Animal Research Center, Clay Center, Nebraska. In 2007, Dr. Miller moved within the USDA to the National Animal Disease Center in Ames, Iowa. She has published more than 65 peer-reviewed manuscripts, with 1839 citations. She currently serves as Past-President of the American Association of Veterinary Immunologists, Member-At-Large NC-229 Multistate Committee for Detection and Control of Porcine Reproductive and Respiratory Syndrome Virus and Emerging Viral Diseases of Swine, and a Project Director of the NSRP-8 National Animal Genome Research Program.

王秋红 Qiuhong Wang

美国俄亥俄州立大学 教授

The Ohio State University, Professor

题目：抗重组 PEDV 疫苗的创制

Title: Develop recombination-resistant PEDV vaccines

1994 年本科毕业于北京医科大学预防医学系。

1994-1998 年在中国预防医学科学院 (现中国疾病预防控制中心) 病毒学研究所方肇寅教授的肠道组工作，参与甲型肝炎病毒 (Hepatitis A virus) ，轮状病毒 (rotavirus) ，诺如病毒 (Norovirus)，和脊髓灰质炎病毒 (poliovirus) 的研究项目。

1998-2000 年在日本东京大学师从于牛岛广志 (Hiroshi Ushijima) 教授，研究星状病毒 (astrovirus)， 获得硕士学位。

2000-2005 年师从于俄亥俄州立大学的 Linda Saif 教授，从事杯状病毒（calicivirus）的研究，获得博士学位。

2006-2007 年在威斯康辛医学院 Amy Hudson 教授的实验室做博士后，研究疱疹病毒 (herpesvirus) 的免疫逃避机制。

2008 年回到俄亥俄州立大学任教职，从 Research Scientist 做起，2013-2018 任 Assistant Professor (助理教授)， 2018-2023 任 tenured Associate Professor (副教授)， 于 2023 年 5 月起，升为教授 (Professor)。

目前的研究重点是 RNA 病毒，包括 BSL2 和 BSL3 病原体，例如猪和牛冠状病毒 (CoV) 和 SARSCoV-2。其实验室进行分子流行病学研究，利用细胞培养进新病毒分离，研究其在动物中的发病机制和免疫反应，并开发疫苗。掌握了反向遗传学技术，这为在研究这些病毒的毒力决定基因、研究跨物种传播机制和开发疫苗方面提供了独特的优势。

2006 – 2007 Postdoctoral Training - Medical College of Wisconsin

2000 – 2005 PhD - The Ohio State University, Columbus, OH

1998 - 2000 MS - University of Tokyo, Japan

1989 - 1994 BM - Peking University (Previous: Beijing Medical University), Beijing, China

Current research activities: My research focus is on RNA viruses, including both BSL2 and BSL3 pathogens, such as porcine and bovine coronaviruses (CoVs), and SARS-CoV-2. My laboratory performs molecular epidemiology studies, isolates new viruses in cell culture, studies their pathogenesis and immune responses in animals, and develops vaccines. We mastered reverse genetics technology and generated infectious clones for several animal CoVs. These give us distinct advantages in studying the virulence determining genes of these viruses, investigating the mechanisms of interspecies transmissions, and developing vaccines.

黄耀伟 Yaowei Huang

华南农业大学 教授

South China Agricultural University, Professor

题目：猪肠道冠状病毒 PEDV 非结构蛋白 2 是一种新的毒力因子：联接天然免疫与自噬

Title: Enteric coronavirus PEDV non-structural protein 2 acts as a novel virulence determinant by linking innate antiviral immunity and autophagy

华南农业大学兽医学院教授，博导。曾任职浙江大学动物医学系教授，系主任；美国弗吉尼亚理工大学兽医学院博士后、研究助理教授。南京大学生物学学士，浙江大学生物医学工程学博士。入选国家万人计划科技创新领军人才、青年千人计划、浙江省千人计划、浙江省杰出青年基金项目等。Veterinary Microbiology、Virus Research、Virology 等国际期刊编委。主要研究方向为动物冠状病毒和戊型肝炎病毒的病原生物学与抗感染免疫。在 J Hepatology, mBio, PLoS Pathogens, Autophagy, J Virol, JBC 等病毒学相关主要期刊发表 SCI 论文 100 余篇，参编《Fields Virology》第 7 版“圆环病毒与细环病毒”章节。近年主持国家自然科学基金联合基金重点、面上、专项、重大研究计划培育等项目、科技部十三五、十四五重点研发计划（课题或子课题负责人）等多项研究课题。

Dr. Yao-Wei Huang is currently a professor in College of Veterinary Medicine of South China Agricultural University. Dr. Huang received his B.S. degree in Biology from Nanjing University and his Ph.D. degree from Department of Biomedical Engineering of Zhejiang University in China. He then joined College of Veterinary Medicine, Virginia Tech, in 2003, as a Postdoctoral Associate and later as a Research Assistant Professor, focusing on molecular mechanism of replication and pathogenesis of hepatitis E virus (HEV) and Torque teno sus virus. From September 2013 to 2023, Dr. Huang worked as a professor in Department of Veterinary Medicine of Zhejiang University, China. Dr. Huang has authored or co-authored 110 publications in peer review journals such as Journal of Hepatology, Autophagy, mBio, PLoS Pathogens and Journal of Virology, and co-authored a book chapter in Fields Virology (7th Edition, 2021). Dr. Huang is the Editorial Board Member of Virology, Virus Research, and Veterinary Microbiology. His current research is mainly focused on pathobiology, mechanism of interspecies transmission, and vaccine development of emerged swine enteric coronaviruses (PEDV, PDCoV and SADS-CoV) as well as the molecular biology of HEV. His major contributions in the filed include discovery of SADS-CoV, identification of the cellular receptor for PDCoV and avian deltacoronaviruses, and recognized phenotyping of PEDV classical and prevalent strains.

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- 暨蓝福莱全球上市 30 周年会议

IPRRS 2024 - Boehringer Ingelheim Satellite Symposium

嘉宾介绍
Introduction of Speakers

王衡 Heng Wang

华南农业大学 教授

South China Agricultural University, China

题目：新一代测序在蓝耳病监控中的实践探索

Title: Practice of next-generation sequencing in PRRS surveillance

王衡，博士，华南农业大学兽医学院副教授，博士研究生导师。主要从事猪病诊断与防控、猪场生物安全、重要病毒性猪病发病机制等研究。

Heng Wang, PhD, Associate Professor and Doctoral Supervisor at the College of Veterinary Medicine, South China Agricultural University. Mainly engaged in research on pig disease diagnosis and prevention, pig farm biosecurity, and the pathogenesis of important viral pig diseases.

李超斯 Chaosi Li

勃林格殷格翰市场部蓝耳 & 猪瘟技术经理

PRRS&CSF Tech Manager of BI China Swine

题目：PRRS 防控 2.0 全球解决方案

Title: PRRS control 2.0 global holistic solution

嘉宾介绍
Introduction of Speakers

李超斯博士，

勃林格殷格翰猪蓝耳病 & 猪瘟经理；中国农业大学动物医学博士；职业兽医师，

Dr. Chaosi Li

Associated technical manager in BI, got PhD degree of veterinary science in China Agricultural University, NVLE.

同传译员介绍
Introduction of Simultaneous Interpreters

杨振 Zhen Yang

杨振，南京农业大学副教授，硕士生导师，中国、美国执业兽医，国际兽医检测诊断大会组委会成员，现任中国兽医协会猪兽医分会副秘书长、中国兽医分会文化分会理事、中国兽医协会动物福利分会理事，“一带一路” 国际重大跨境动物疫病诊断与免疫专业创新院协调员。2014 年毕业于华南农业大学动物医学专业获农学学士学位，2012 年至 2013 年在深圳华大基因研究院传染病组进修。2014 年至 2015 年在美国堪萨斯州立大学农学院进修。2019 年毕业于美国明尼苏达大学大学兽医学院获得兽医学博士学位和明尼苏达大学猪医学证书，2022 年获得明尼苏达大学兽医科学硕士学位。2019 年至 2020 年，赴联合国粮食及农业组织总部动物卫生司访学，参与非洲猪瘟等跨境动物传染病和新冠肺炎等相关工作。研究领域包括规模猪场、牛场生产医学，跨境动物传染病和 One Health 研究，担任国内多家集团养殖公司和动物保健公司顾问。

Zhen Yang is an Associate Professor and Master's Supervisor at Nanjing Agricultural University, a licensed veterinarian in both China and the United States, and a member of the Organizing Committee for the AVDC. He currently serves as the Deputy Secretary-General of the Swine Veterinary Branch of the Chinese Veterinary Association, a committee member of the Cultural Branch of the Chinese Veterinary Association, and a committee member of the Animal Welfare Branch of the Chinese Veterinary Association. He is also the coordinator for the Belt and Road Initiative's Innovation Institute of Major Transboundary Animal Disease Diagnosis and Immunology. Dr.Yang graduated in 2014 with a Bachelor of Agriculture in Veterinary Medicine from South China Agricultural University and furthered his studies in the Infectious Diseases Group at BGI Shenzhen from 2012 to 2013. He continued his studies at Kansas State University's College of Agriculture from 2014 to 2015. In 2019, he earned a Doctorate of Veterinary Medicine and a Swine Medicine Certificate from the University of Minnesota, followed by a Master of Veterinary Science in 2022 from the same university. From 2019 to 2020, he visited the Animal Health Division of the FAO headquarters, participating in work related to transboundary animal diseases like African Swine Fever and COVID-19. His research areas include production medicine for large-scale pig and cattle farms, transboundary animal diseases, and One Health studies, and he serves as a consultant for several production, vet clinics and pharmaceutical companies.

曾容愚 Rongyu Zeng

曾容愚

天康制药股份有限公司技术支持总监。

南京农业大学动物营养硕士、预防兽医学硕士、马里兰大学免疫学博士，在 Nature Biotechnology, PNAS, The Journal of Immunology，J.Dairy Science 发文多篇。

从事畜禽营养研究与饲料配方制作 7 年、猪和牛新型疫苗研发和推广 20 年。在猪临床免疫学领域，提出伪狂犬“三剑合一”免疫净化方案、蓝耳“五精”防控方案、PEDV 乳源免疫免疫力最大化方案。兼职上海交通大学等 11 大学的专硕导师。中国兽医学会专家委员会副主任委员，兽医公共卫生学会委员。从事畜牧兽医行业国际会议翻译 10 多年。参与翻译 6 本行业书籍和教材。

RONGYU ZENG MS.MS.Ph.D

Director of technical support in TECON Pharmaceutical Co.Ltd.

Trained in Animal Nutrition and Preventive Veterinary Medicine respectively as M.S students at Nanjing Agricultural University; in Immunology as PhD student at University of Maryland. Published decent papers in Nature Biotechnology, PNAS, The Journal of Immunology，J.Dairy Science,etc.

Worked as a nutritionist for pigs, poultry, aquaculture and ruminants for 7 years; Engaged in vaccine development and application against major diseases in pigs and cows for 20 years. In the field of clinical immunology in pigs, proposed and practised innovative solutions to major diseases control and eradication including "three swords in one" approach for pseudo rabies eradication, "five essence" for PRRSV prevention, and maximizing milk-derived immunity to control PEDV.

Served as adjunct Master degree pursuing graduate student supervisor in veterinary medicine in 11 universities including Shanghai Jiaotong University, Vice Chairman of the Expert Committee of the China Veterinary Association, and Committee Member of the Veterinary Public Health Society,Simultaneous interpreter for International meetings in the area of animal husbandry and veterinary medicine for 13yrs. Participated in translating 6 industrial books and university textbooks.

史兰广 Languang Shi

硕腾公司战略产品注册负责人

本科毕业于东北农业大学动物医学专业，研究生毕业于中国兽医药品监察所预防兽医学专业；先后从事疫苗研发、生猪养殖场和屠宰场审核以及进口兽用生物制品注册等工作。

Strategic Product Registration Lead of Zoetis China

Bachelor of Veterinary Medicine, NEAU; Master of Preventive Veterinary, IVDC; Career involved in R&D of vaccine, auditing of pig farms and slaughterhouses, and registration of import veterinary biologics.

洪浩舟 Reid Hong

勃林格殷格翰动物保健有限公司 技术经理

现任勃林格殷格翰动物保健有限公司产品技术经理。曾在迦太基兽医服务公司（中国）、国内某养猪集团公司工作多年，并担任兽医主管、兽医负责人等职务。

Boehringer Ingelheilm Animal Health Technical Manager

Currently woks as technical manager at Boehringer Ingelheilm Animal Health.

Many years of working experience at Carthage Veterinary Service (China), Chinese Pig companies as Veterinary director, Chief Veterinarian.

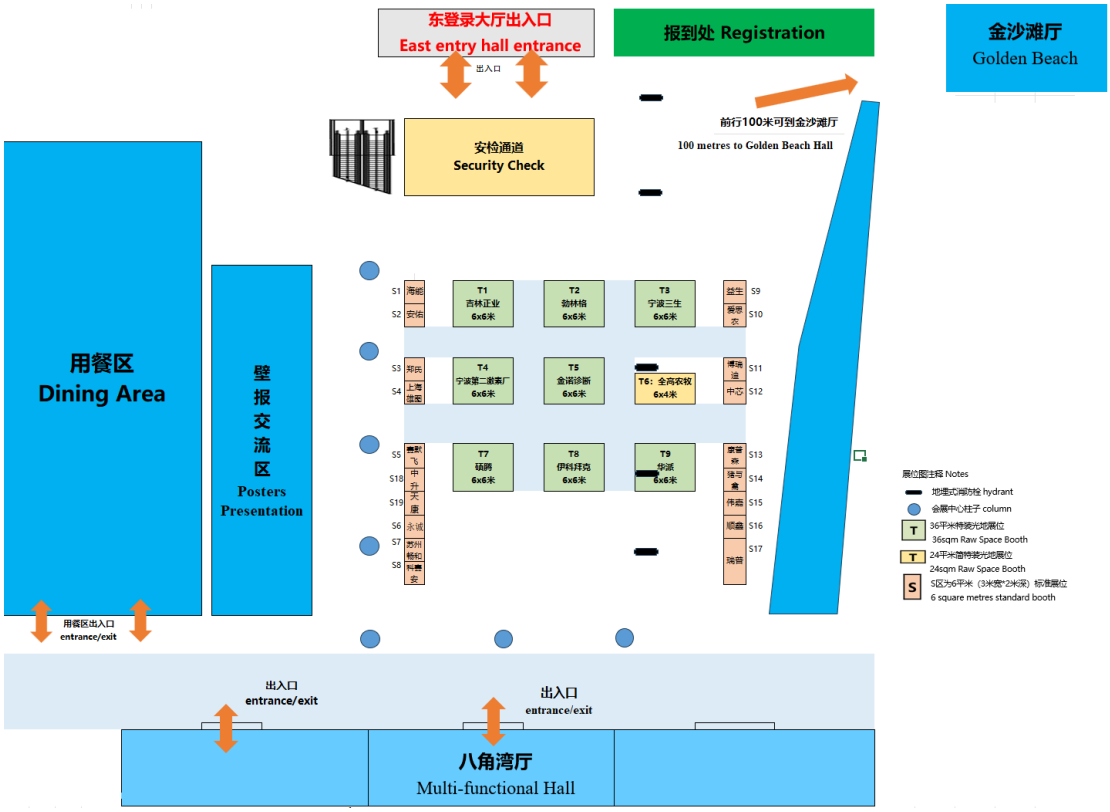
壁报交流名单
Posters Presentation

ID	Topic	Name	Afiliation	Title
6951	Pathogenesis	Xindong Wang	College of Animal Science and Technology, Guangxi University	Recombination pattern and pathogenicity of the epidemic dominant NADC30-like strains in southern China
7098	Pathogenesis	Jing Wang	North West Agriculture and Forestry University	PRRSV non-structural protein 5 inhibits antiviral innate immunity by degrading multiple proteins of RLR signaling pathway through FAM134B-mediated ER-phagy
7106	Pathogenesis	Dianning Duan	Longyan University	Hypoxia inducible factor-1 alpha regulates porcine reproductive and respiratory syndrome virus induced inflammation of porcine alveolar macrophages
7124	Pathogenesis	Changyan Li	Tianjin University	Research on iron metabolism regulation and ferroptosis mechanism during PRRSV infection
7129	Pathogenesis	Zhenbang Zhu	Yangzhou University	LGP2 regulates PRRSV infection via enhancing MDA5-mediated signaling
7224	Pathogenesis	Ermin Xie	South China Agricultural University	Research on the molecular mechanism of PRRSV-induced lipid droplet accumulation
7296	Pathogenesis	Xiaoyu LU	Guizhou University	Mechanism of Action of APOBEC3F in PRRSV Infection
7298	Pathogenesis	Qianxiong Xiong	Guizhou University	Mechanism of action of helicase DDX6 in PRRSV infection
6937	Diagnostics-Epidemiology	Jingneng Wang	China Agricultural University	Development of a quadruplex TaqMan real-time PCR system for simultaneous differentiation of HP-PRRSV-like, PRRSV NADC30-like, NADC34-like and PRRSV-1
6947	Diagnostics-Epidemiology	Heng Zhang	North West Agriculture and Forestry University	Differential Diagnosis and Control of PRRS Complicated by Haemophilus parasuis and Streptococcus suis Infection
6969	Diagnostics-Epidemiology	Julia Baker	University of Minnesota	Repeat Offenders: understanding PRRSV clinical re-breaks
7015	Diagnostics-Epidemiology	Pu Sun	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Molecular epidemiological characteristics of porcine reproductive and respiratory syndrome virus circulated in Gansu, China
7059	Diagnostics-Epidemiology	Yufu An	PIG PEACE (Hangzhou) Technical Service Co. Ltd	A preliminary study on the the relationships between the severity of pulmonary gross lesions, histological changes and and viral quantities in PRRS
7077	Diagnostics-Epidemiology	Yue Li	South China Agricultural University	Metatranscriptomic sequencing analysis and identification of an example of co-infection with European-type and North American-type porcine reproductive and respiratory syndrome
7111	Diagnostics-Epidemiology	Zhaochun Li	IDEXX Laboratories, Inc	Evaluation of commercial qPCR kits for detection of PRRSV-1 and PRRSV-2 strains in China
7140	Diagnostics-Epidemiology	Chaosi Li	Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd	Prevalence, Time of Infection, and Diversity of Porcine Reproductive and Respiratory Syndrome Virus in China
7141	Diagnostics-Epidemiology	Chaosi Li	Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd	Tail blood swabs can be utilized for precise localization of PRRSV-positive litters during the perinatal period in order to enhance the effectiveness of PRRS elimination programs.
7200	Diagnostics-Epidemiology	Jinhao Li	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Genomic Characterization of HLJDZD55: The First L1B PRRSV in China
7203	Diagnostics-Epidemiology	Shangshang You	Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Pathogenicity studies of Two Recombinant Viruses of the Porcine Reproductive and Respiratory Syndrome Virus in China

ID	Topic	Name	Afiliation	Title
7223	Diagnostics-Epidemiology	Wansheng Li	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Genomic characteristics of a novel emerging PRRSV branch in sublineage 8.7 in China
7225	Diagnostics-Epidemiology	Chao Li	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Prevalence and genetic evolution of porcine reproductive and respiratory syndrome virus in commercial fattening pig farms in China
6935	Vaccines	Simon Paul Graham	The Pirbright Institute	Evaluation of live attenuated porcine reproductive and respiratory syndrome virus vaccines genetically engineered to express peptide-based immune checkpoint inhibitors
7018	Vaccines	Yueyi Gao	China Institute of Veterinary Drug Control	Immune efficacy evaluation of recombinant protein skeleton LigB chimeras with PRRSV neutralizing epitope
7139	Vaccines	Chaosi Li	Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd	Ingelvac PRRS® MLV efficacy against high pathogenicity of a Chinese NADC34-like PRRSV challenge in Pigs
7142	Vaccines	Chaosi Li	Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd	The efficacy of Ingelvac PRRS® MLV against heterologous strains of porcine reproductive and respiratory syndrome virus: A meta-analysis
7153	Vaccines	Shuang Li	Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Study on the Impact of the PRRSV NSP4 Coding Region on Regulating Antigen Presentation Function of DC Cells in Vaccine Strains
7163	Vaccines	Jiakai Zhao	North West Agriculture and Forestry University	Design of a marker vaccine candidate for porcine reproductive and respiratory syndrome
7175	Vaccines	Jie Zhang	Nanjing Agricultural University	Attenuation by passage and immunprotection efficacy of NADC30-Like PRRSV FJ1402
7188	Vaccines	Hao Yang	Huazhong Agricultural University	Preliminary development of NADC34-Like PRRSV inactivated vaccine
7305	Vaccines	Xinnuo Lei	Jiangsu Agri-Animal Husbandry Vocational College	Boosting PRRSV-Specific Cellular Immunity: Development of an Fc-fused Multi-CTL Epitope Vaccine
7131	Emerging Viruses	Hu Xu	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Novel characteristics of Chinese NADC34-like PRRSV during 2020-2021
7238	Emerging Viruses	Hu Xu	Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Novel characterization of NADC30-like and NADC34-like PRRSV strains in China epidemiological status and pathogenicity analysis of L1A variants
6880	Field Disease Control	Jing Wu	Institute of Animal Science, Guangdong Academy of Agricultural Sciences	The inhibitory effects of baicalein on PRRSV infection in weaned piglets
6954	Field Disease Control	Kai Wang	Jilin Agricultural University	Prevention and control measures of positive NADC-34-like porcine reproductive and respiratory syndrome virusin pig farms
7039	Field Disease Control	Jing Hou	Institute of Animal Science, Guangdong Academy of Agricultural Sciences	Effects of glucuronolactone on the growth performance, antioxidant and immunity of PRRSV-DON co-infected piglets
7112	Field Disease Control	Zhaochun Li	IDEXX Laboratories, Inc	Gilt serum acclimation monitoring for PRRSV
7228	Field Disease Control	Shuangcheng Zhao	Boehringer Ingelheim Animal Health (Shanghai) Co. Ltd	A successful case of PRRS control within frequent and multiple gilt introduction farm
7268	Field Disease Control	Yang Li	Lanzhou University	Effect of different antimicrobial use in the perinatal period on the performance of sows and piglets in PRRSV-positive farms
7270	Field Disease Control	Xiao Liu	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences	The natural compound Sanggenon C resists PRRSV infection

ID	Topic	Name	Afiliation	Title
7280	Field Disease Control	Lei Zhou	China Agricultural University	A case study on rapid stabilization and eradication of a 2400-head GP farm after an PRRSV outbreak caused by a NADC30-like Strain
7303	Field Disease Control	Dan Li	China Agricultural University	GP5 and M proteins play a major role in the immune protection function of PRRSV structural proteins
6936	Virus Replication	Shaojie Han	Ghent University	Better understanding of PRRSV/ASFV entry in macrophages by the use of macrophage-specific mAbs
7035	Virus Replication	Xu Chen	North West Agriculture and Forestry University	A nanobody inhibiting porcine reproductive and respiratory syndrome virus replication via blocking self-interaction of viral nucleocapsid protein
7122	Virus Replication	Yuchao Yan	Tianjin University	The mechanism of host protein NUDT7 participating in PRRSV infection
7143	Virus Replication	Xinnuo Lei	Hunan Agricultural University	The N-terminal Intermolecular Disulfide Bond of PRRSV GP5 and M Facilitates VLPs Secretion and Cell binding
7148	Virus Replication	Dihua Zhu	South China Agricultural University	Tripartite motif 56 Inhibits Porcine Reproductive and Respiratory Syndrome Virus by Promoting Generation of NLRP3 Inflammasome
7174	Virus Replication	Dandan Jiang	Institute of Animal Science and Veterinary Medicine, Shandong Academy of Agricultural Sciences	PRRSV NSP1α degrades TRIM25 through proteasome system to inhibit host antiviral immune response
7229	Virus Replication	Yuanqi Yang	Nanjing Agricultural University	Ursonic acid from medicinal herbs inhibits PRRSV replication through activation of the innate immune response by targeting the phosphatase PTPN1
7248	Virus Replication	Ruirui Ye	South China Agricultural University	PRRSV GP4 targets PINK1 to induce mitochondrial autophagy for self-replication
7263	Virus Replication	Lechen Lu	South China Agricultural University	PRRSV Nsp2 affects cell autophagy to regulate PRRSV replication
7264	Virus Replication	Zifang Zheng	Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences	Host cells reprogram lipid droplet synthesis through YY1 to resist PRRSV infection
7274	Virus Replication	Xueyan Liu	China Agricultural University	Molecular Mechanisms of PRRSV Replicase Membrane Proteins nsp2 and nsp3 in Recruiting Core Replicases for RTC Assembly

会场平面图
Floorplan



T1 吉林正业生物制品股份有限公司
JILIN ZHENGYE BIOLOGICAL PRODUCTS CO.,LTD
T2 勃林格殷格翰动物保健（上海）有限公司
Boehringer Ingelheim Animal Health （Shanghai） Ltd
T3 宁波三生生物科技股份有限公司
Ningbo Sansheng Biological Technology Co., Ltd.
T4 宁波第二激素厂
NSHF
T5 北京金诺百泰生物技术有限公司
Beijing Jinnuo Biotech Co.,Ltd.
T6 河南全高农牧科技有限公司
Henan Quangao Agriculture and Animal Husbandry Technology Co., Ltd
T7 硕腾（中国）
Zoetis
T8 中英合资 伊科拜克
ECO-BIOK （Sino-British JV）
T9 华派生物技术（集团）股份有限公司
Huapai Biotechnology (Group) Co., Ltd.
S1 山东海能生物工程有限公司
SHANDONG HAINENG BIOENGINEERING.,LTD
S2 安佑生物科技集团股份有限公司
Anyou Biotechnology Group Co.,Ltd
S3 福建郑氏榕馨农牧育种有限公司
Fujian Zheng's Rongxin Agro-Breeding Co., Ltd.
S4 上海雄图生物科技有限公司
Shanghai Xiongtu Biotechnology Co., Ltd.
S5 赛默飞世尔科技（中国）有限公司
Thermo Fisher Scientific., Ltd.
S6 福建永诚农牧科技集团有限公司
FUJIAN YONGCHENG BREEDING TECHNOLOGY GROUP CO., LTD
S7 苏州畅和生物科技有限公司
Changhe Biotech
S8 湖南科赛安生物科技有限公司
Hunan Coscian Biotechnology Co., Ltd
S9 山东益生种畜禽股份有限公司
Shandong Yisheng Livestock and Poultry Breeding Co.,Ltd
S10 法国爱思农银合
ISAGRI
S11 石家庄博瑞迪生物技术有限公司
MolBreeding Biotechnology Co., Ltd.
S12 广东中芯种业科技有限公司
GuangDong zhongxin breed ing swine Tech.Co.,LTD
S13 北京康普森农业科技有限公司
Beijing Compass Agritechnology Co., Ltd.
S14 北斗农科融媒体
Beidou Convergence Media of Agricultural Sciences
S15 沈阳伟嘉生物技术有限公司
Shenyang Vica Biotechnology Co.,Ltd.
S16 北京顺鑫农业股份有限公司小店种猪分公司
Beijing shunxin agriculture co.,Ltd, xiaodian breeding subsidary
S17 天津瑞普生物技术股份有限公司
Tianjin Ringpu Bio-technology Co., Ltd.
S18 天津中升挑战生物技术有限公司
Tianjin Speerise Challenge Biotechnology Co., Ltd.
S19 天康制药股份有限公司
TECON Pharmaceutical Co., Ltd.

参会须知

1. 会场

2024 国际猪繁殖与呼吸综合征学术会议将于 2024 年 8 月 7-9 日在烟台八角湾国际会展中心召开。

地址：山东省烟台市经济技术开发区北京中路 31 号

特别注意事项：

（1）请会议代表进入会议室前关闭手机或者调至静音（如需接电话请离开会议室）。

（2）请尊重知识产权，不要在会场拍摄 PPT 或录制演讲。

（3）留意会场逃生出口，如遇特殊情况，请听从现场指挥人员安排，谢谢合作！

2. 注册报到

注册报到地点：烟台八角湾国际会展中心东登录厅

注册报到时间：8 月 7 日，09:00-18:00；

发票领取时间：8 月 8 日，09:00-18:00；

注意：

1）会前微信在线支付、现场缴费统一开具普通电子发票，请参会代表于会议结束后 3 个工作日后登录大会官网个人中心领取并下载发票；

2）银行汇款交费申请增值税专用发票请于会议现场直接领取；

3. 代表证

会议报到时请提供参会人员姓名并凭身份证领取会议代表证及会议资料，代表证上附有条码，会议期间仅凭代表证扫码进入会场及用餐，请妥善保管。

4. 大会用餐

8 月 8 日、9 日

早餐：请到入住酒店用餐。

午餐：烟台八角湾国际会展中心 A1 展厅（临近八角湾厅）用餐。

晚餐：大会不提供晚餐，请自行解决。

8 月 8 日，Banquet

时间：18:00-21:30，地点：烟台鑫广万豪酒店户外草坪，地址：烟台市经济技术开发区黄河路 365 号。

提示：17:40 学术会议结束后，请代表务必携带 Banquent 门票（提前购买）到八角湾国际会展中心动登录厅外马路边乘坐免费巴士到达活动地点。

5. 壁报展示

壁报展示时间：8 月 8 日、9 日 8:30-18:00

壁报交流时间：8 月 8 日、9 日上午、下午休息时段

壁报展示地点：烟台八角湾国际会展中心东登录厅

6. 同传设备领取

8 月 8 日 -9 日会议期间，大会主会场语言为英文，为方便广大参会人员参会交流，组委会将于会议期间提供同传翻译服务，有需要的参会代表请到会场门口（八角湾厅 2）凭身份证或护照领取同传耳机，用完后请及时将同传耳机还给工作人员。使用期间请妥善保管好同传耳机，如有遗失，需赔偿 2000 元 / 副耳机。

7. 学术交流

（1）学术交流日程

A. 发言代表：学术交流完全按大会最终日程安排进行，请以会议公布的发言日期和时间为准。发言代表需在演讲时间前 15 分钟到所在会场前排就坐。

B. 主持人：请担任大会主持人的专家以会议公布的主持日期和时间为准，提前做好准备。

（2）演讲课件提交

发言的代表 8 月 7 日到大会报到处提交课件，比例为 16:9，文件格式为 PPT 或 PDF。

为尊重知识产权并确保会议电脑的使用安全，保障会议正常进行，会议用电脑一律不允许拷出文件，会务组也不提供拷贝 PPT 的服务，敬请各位代表谅解！请有需要的代表直接与发言代表本人联系。

8. 安全防护

由于参会人员较多，请各位代表注意会议期间的个人安全，保护好个人的财物。祝各位在烟台会议愉快！

Notice to Participants

1.The venue

The International Porcine Reproductive and Respiratory Syndrome Symposium (IPRRSS 2024) will be held from August 7th to August 9th in Yantai Bajiaowan International Convention & Exhibition Center.

Address: 31 Beijing Middle Road, Economic and Technological Development Zone, Yantai, Shandong Province, China.

Special notice:

(1) Please turn off or mute the cell phone before entering the conference room (Please leave the conference room if you need to take a phone call).

(2) Please respect intellectual property rights and do not take photo of PowerPoint slide or record presentations.

(3) Please pay attention to the exit of the conference room. In case of special circumstances, please follow the arrangement of the on-site conductor, thank you for your cooperation!

2.Onsite registration

Location: East Entry Hall of Yantai Bajiaowan International Convention & Exhibition Center

registration time: August 7th, 09:00-18:00

Invoice collection: August 8th, 09:00-18:00.

Please notice: PayPal online payment before the conference and on-site payment will be issued with an ordinary electronic invoice, we will send the invoice to you by e-mail 3 working days after close of the conference.

3.Participant Badge

Please provide name of participant when registering and present ID card (or passport) to collect Participant Badge and conference material. Participant Badge is attached with QR code, it will be the official certificate to enter conference room and take lunch, and the QR code will be scanned accordingly, please keep it properly during the whole conference.

4.Conference meals

August 8th and August 9th

·Breakfast: Please have breakfast at your hotel.

·Lunch: Hall A1 , Yantai Bajiaowan International Convention& Exhibition (Near Multi-functional Hall)

·Dinner: The conference does not provide dinner, please arrange it yourself.

August 8th, Banquet

Time: 18:00-21:30

Location: Yantai Marriott Hotel Outdoor Lawn

Address: No. 365, Huanghe Road, Yantai Economic and Technological Development Zone, Yantai, China

Note for Banquet: After the conference, delegates are kindly requested to bring their banquet ticket (purchased in advance) to the outside of Multi-functional Hall of Yantai Bajiaowan International Convention & Exhibition Center and take free bus to banquet venue.

5.Posters presentation

Posters presentation time: August 8th and 9th, 08:30-18:00.

Posters exchange time: August 8th and 9th, tea break time of morning and afternoon.

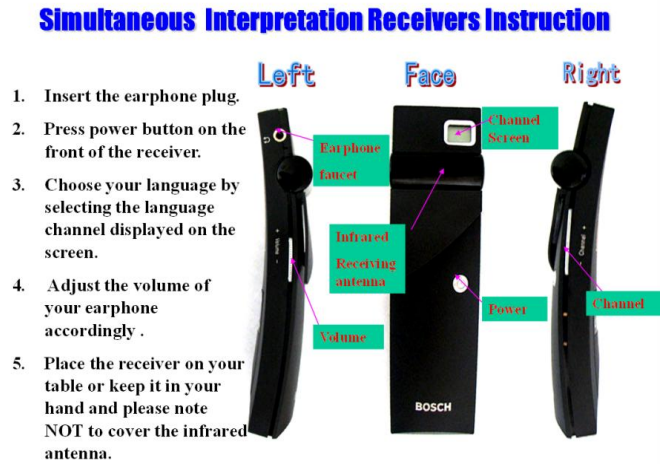
Posters presentation location: East Entry Hall of Yantai Bajiaowan International Convention & Exhibition Center

6.Simultaneous interpretation

Simultaneous interpretation Receiver Specification

The language of the conference is English. In order to facilitate the exchange of participants, the organizing committee will provide simultaneous translation services during the conference. Please go to the entrance

of the venue (Multi-fuctional Hall) to get the receiver with your ID card or passport. Please return the receiver to the staff in time after use. In case of loss, it is necessary to compensate 2000 RMB/per piece.



Guidance:

1. Insert the earphone into the earpieces of the receiver (the receiver can only be turned on when earphone has been inserted). Press the frontal argent button to turn on the receiver and then you can see number displayed on the screen.

2. Choose the language by pressing Channel button on the right side. "1"— Chinese; "2"—English

3. Adjust the Volume by pressing the button on the left side.

4. Do not block the receiver module under the screen since it is infrared receiver.

5. Two ways to turn off the receiver — touch and hold the power-off button or pull out the earphone directly.

6. Exchange passport as collateral for borrowing simultaneous interpreting receiver. Return the simultaneous interpreting receiver and collect your passport at the same time.

7.Academic Exchange

(1) Academic Exchange Schedule

A. Speakers: Academic exchanges will be conducted in full accordance with the final program of the conference. Please refer to the date and time of presentation as announced by the conference. Speakers are required to be seated in the front row of the room 15 minutes before the presentation time.

B. Moderator: The expert serving as the conference moderator should prepare in advance according to the session (date and time) announced by the conference.

(2) Presentation submission

Speakers should submit the PowerPoint slide on August 7th to the register desk. The Presentation PowerPoint (PPT) ratio should be 16:9, and the file format should be PPT or PDF.

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8.Safety and Security

Due to the large number of participants, please pay attention to your personal safety and belongings during the conference. We wish you a pleasant conference in Yantai!

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Keynote Speakers

The Art of PRRSV Replication: Novel Proteins and Unprecedented Mechanisms

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PRRSV is an enveloped, positive-sense, single-stranded RNA virus. The viral genome is about 15 kb in length. The 3′ end of the viral genome encodes envelope proteins and also nucleocapsid protein that encapsulates the genomic RNA. The 5′ two-thirds of the viral genome encodes two large replicase polyproteins, pp1a and pp1ab, which are proteolytically processed into at least 14 functional nonstructural proteins. In our previous studies, two novel proteins, nsp2TF and nsp2N, were found to be expressed in the nsp2-coding region through a −2/−1 programmed ribosomal frameshifting (PRF) mechanism that is unprecedent in the eukaryotic system. Our recent study revealed that the frameshifting rate at nsp2 PRF site is temporally regulated, in which the increasing of -2 PRF efficiency is likely facilitated by accumulation of the PRF-stimulatory viral protein, nsp1β. Remarkably, PRF efficiency at the canonical ORF1ab frameshift site is also temporally regulated, which challenges the traditional assumption of a fixed efficiency for the numerous other viruses with canonical PRF sites. The identification of nsp2TF and nsp2N proteins adds to the functional complexity of the nsp2 region of the viral replicase. Our studies showed that nsp2TF and nsp2N had the ability to modulate the host innate immune responses against infection with PRRSV. On the other hand, nsp2TF was found to target the exocytic pathway to reduce proteasome-driven turnover of GP5/M proteins, which promotes the formation of GP5-M dimers that are critical for virus assembly. Recombinant viruses with impaired expression of these frameshifting products are attenuated upon infection of animals, suggesting that manipulation of the nsp2TF/nsp2N expression may provide a rational basis for developing improved PRRSV vaccines in the future.

The multifaceted functions of PRRSV nsp2: a mysterious replicase with divergent genetic evolution

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Porcine reproductive and respiratory syndrome virus (PRRSV) has remained a prominent threat to the worldwide swine industry. The past 30 years have seen a staggering expansion of PRRSV genetic diversity as a result of rapid genetic variation and frequent recombination. This has led to emergence of many milestone strains, exemplified by ATP, JA-142, MN184, the Chinese highly pathogenic PRRSV (HP-PRRSV), Lena, NADC30, NADC34, and so on. Phylogenetically, these variants and their subsequent derivatives in the globe can be categorized into two species, namely PRRSV-1 and PRRSV-2, with a genetic difference of more than 40% at nucleotide level. The PRRSV-1 strains (traditionally European type) can be further classified into 3 subtypes, whereas the PRRSV-2 strains (North American type) have evolved into at least 9 lineages. The most divergent and fastest evolving genomic area has been attributed to the region coding for viral nonstructural protein 2 (nsp2), a multi-domain replicase protein that is essential for PRRSV replication. This replicase contains an N-terminal papain-like cysteine protease domain (PLP2), a functionally unknown large middle region with a size of 300-500 amino acids, a C-terminal transmembrane domain (TMD), and a cytoplasmic tail (CT). Also, there exist isoforms for nsp2, such as nsp2N and nsp2TF, that differ in their C-termini and are expressed via a novel frameshift mechanism. PRRSV nsp2 is responsible for the size and genetic variations among different strains. Insertions and most notably deletions, recombination, as well as extensive amino acid substitutions, are most often seen within nsp2, and at least 20 patterns have been discovered. In this talk, I will discuss the role of PRRSV nsp2 in viral replication, antibody responses, and host immune modulation. Our results show that PRRSV nsp2 is a key component of viral replication and transcription complex (RTC) and its interactions with viral polymerase and helicase are subject to conformational regulation. It also plays a critical role in recruiting host factors such as stress response protein ATF4 to RTC to promote viral negative-stranded RNA synthesis via induction of a stressed environment. Dissection of functional domain suggests that the N-terminal PLP2 domain is a target of neutralizing antibodies and a key region showing antigenic differences between HP-PRRSV and NADC30 strains, whereas the hypervariable region is associated with viral cellular tropism of primary porcine alveolar macrophages. Our most recent studies demonstrate that the nsp2 hypervariable region also plays a key role in modulation of cellular inflammation and that the Chinese HP-PRRSV nsp2 is a virulence factor and a much stronger inducer of host immune responses (e.g., inflammation) than its genetically distant counterpart from currently epidemic NADC30-like strains. These findings have important implications in understanding PRRSV replication, evolution, inter-lineage recombination, and persistence in the field.

Tissue differences and environmental changes give rise to 'chameleon-like' adaptation of macrophages, which drives PRRSV-1 evolution

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Most tissue macrophages come from blood monocytes, which in turn originate from myeloid progenitor cells in red bone marrow. Blood monocytes circulate in the body for months, till the moment there is a call from certain tissues to leave the blood circulation. This call is mediated by chemokines and adhesion molecules and is a part of the normal physiological homeostasis. It can locally be activated by certain pathological circumstances, such as inflammation. In our laboratory, we have demonstrated that, at the moment that the monocyte migrates through the endothelial cell wall of a vessel, it uses filopodia to sense 'the environment' before leaving the blood vessel. This leads to the proper expression of a certain subset of genes/proteins in the macrophages and prepares the monocyte to fulfill its job in the tissue where it arrives. Every tissue and every circumstance will drive the differentiation in a certain direction. This leads to many final phenotypes of macrophages. The vision that there is 'one macrophage' is totally wrong. It concerns a 'chameleon-like' cell type that expresses a broad and variable number of proteins in order to optimally function in a certain area and under certain circumstances. It may even express certain epithelial, endothelial and fibrocyte markers.

PRRSV has a strict tropism for certain subsets of macrophages. In Europe, PRRSV1 showed a special evolution. Before the emergence of PRRSV1 in Western Europe, it already circulated behind the iron curtain in Eastern Europe. As there were no clinical signs recognized, the virus had to replicate at low levels in a restricted number of macrophages that did not have an important function. Siglec-$10^{+}$$CD163^{+}$ macrophages, which are mainly located in lymphoid tissues such as tonsils, spleen and lymph nodes, fit in this context. The sialic acid binding molecule, Siglec-10, is a binding and internalization molecule for PRRSV-1 and CD163 mediates the disassembly. Siglec$10^{+}$$CD163^{-}$ B-lymphocytes may have helped the virus to hide and form a reservoir. Indeed, the virus enters the B-lymphocyte but cannot infect them. In the beginning of the 90s, upon the disappearance of the iron curtain, Lelystad virus-like viruses emerged, which additionally infected Siglec$1^{+}$$CD163^{+}$ macrophages, resulting in mainly reproductive failure (late abortion, early farrowing, weak- and stillborn piglets) and, during co-infections, also in respiratory problems. This PRRSV-1 was genetically and phenotypically strongly different from the strains that were circulating at that time in the US (PRRSV-2). Lelystad virus-like viruses were slow-spreading viruses; infections started in the nursery and it took a whole fattening period before all animals became infected. This was correlated with a restricted replication level in the nasal mucosa. Tonsils and lungs were main primary targets. The viremia, which is the mirror of the PRRSV-1 replication remained at a low level (10^{2}-10^{3} TCID50/ml). From the moment that PRRSV-1 vaccines became available, PRRS was effectively controlled. This situation changed with the appearance of more heterologous strains which primarily emerged in Italy. Later on, heterologous strains appeared all over Europe. Within a period of ten years, PRRSV-1 found its way to escape from immunity upon vaccination and infection. Several immune evasive mechanisms have been described, such as absence of envelope proteins in the outer plasma membrane, transmission through apoptotic cell bodies, hampered induction of interferon, restricted cell lysis by NK cells and $CD3^{+}$$CD8^{+}$ cytotoxic T-lymphocytes, …. In addition, between

2005 and 2015, the virus succeeded in replicating in additional subsets of macrophages, such as those residing in the superficial layers of the nasal mucosa, giving more power to the virus to spread between pigs and resulting in a one log increase of virus titers in the blood (10^3-10^4 $TCID_{50}$/ml). These macrophages are Siglec-1 and Siglec-10 negative but CD163 positive. Up to date, the binding and internalization molecule has not yet been identified in these superficial nasal macrophages. In 2006, highly virulent PRRSV-1 strains were circulating in Eastern Europe, at the same moment as the Chinese high fever PRRSV-2 strains in Asia. They (subtypes 2 and 3) were genetically quite different from the strains present in Western Europe (subtype 1). Lena (prototype PRRSV-1 subtype 3) has been extensively studied. Single infections with this virus resulted in an extremely high replication in all organs, a top viremia (10^4-10^6 $TCID_{50}$/ml), extreme exudate formation in body cavities, high fever for weeks, listlessness, anorexia and respiratory problems in young pigs. Infections with these highly virulent/pathogenic strains even killed affected pigs. This virus replicated in cell types that were never infected before, such as microglia cells in the brain. This type of virus luckily never spread to Western Europe. More recently, more virulent and pathogenic PRRSV-1 subtype 1 strains were popping up in Italy (PR40/2014; 2014) and Spain (Rosalia (2020)). This evolution stimulated all Western European countries to activate surveillance by whole genome sequencing. More pathogenesis work is necessary to better understand this evolutionary move of PRRSV-1. Based on this evolution, we believe that even more virulent strains will emergence in Western Europe in the coming years.

Elucidating the immune evasion mechanisms of PRRSV through viral protein-host protein interactions

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Porcine reproductive and respiratory syndrome (PRRS), has caused significant losses to the global pig industry. The interaction between viral structural proteins and host receptors facilitates virus entry, while interactions between host and viral non-structural proteins influence virus genome replication and transcription. Virus invasion triggers a cascade of immune responses, and virus particles evade the host's immune system through interactions, promoting their replication. In this study, Yeast Two-Hybrid (Y2H) and Immunoprecipitation-Mass Spectrometry (IP-MS) were used to identify PRRSV proteins' binding partners. The interactions between PRRSV proteins and host proteins were confirmed by Co-Immunoprecipitation (Co-IP) and Pulldown assays. The functions of NSP4, NSP10, and NSP11 in innate immune responses, including interferon (IFN) production and inflammatory responses, were investigated. It was found that PRRSV NSP4 interacted with sIGLL5 and the components of the IKK complex, which negatively regulated the activation of NF-κB and IFN production. Additionally, NSP10 was identified as a novel viral and bacterial RNA sensor. NSP10 interacted with NLRP3 and impaired viral and bacterial RNA-mediated NLRP3 inflammasome activation. PRRSV infection can cause mitochondrial swelling and content disappearance, while PRRSV infection up-regulates IL-1β mRNA and protein levels. Mitochondrial double-stranded RNA (mtdsRNA) was labeled using RNAscope technology, revealing that PRRSV infection can release mtdsRNA from mitochondria to initiate NLRP3 inflammasome activation. Interestingly, NSP11 was found to inhibit the activation of the NLRP3 inflammasome by inhibiting the oligomerization of ASC, thus reducing the inflammatory reaction.

Present and future of PRRSV vaccines

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Since the launch of the first modified live vaccines (MLV) for PRRSV, this type of vaccines have become the standard, although the effectiveness of MLVs is far from ideal. Certainly, vaccination has helped alleviate the clinical and productive impact of the infection, particularly in sows; however, vaccinated animals can still become infected and transmit the infection. For piglets, the popularity of vaccination is lower. The development of effective immunity is relatively slow and, under conditions of high infection pressure, animals can be infected before becoming solidly immune. Moreover, maternally-derived antibodies can interfere with vaccination if they have neutralizing capacity against the vaccine virus. Furthermore, with the current MLVs, shedding and possible transmission of the vaccine virus is possible, which can led to the establishment of clades derived from the vaccines that, eventually, can acquire virulence. Recombination phenomena can be relatively common and cause some concern.

With the emergence of higher virulence strains, there is an urgent need for new vaccines. Unfortunately, up to now all strategies aimed at developing more effective vaccines against PRRSV have not been successful enough and have not not surpassed the efficacy of MLVs. The reasons for this collective failure are diverse and include the ever-expanding genetic diversity of the virus, insufficient knowledge about critical T and B epitopes, lack of clear identification of virulence determinants, and lack of identification of subpopulations of T cells involved in effective memory responses.

With SARS-CoV-2, RNA technologies have revolutionized vaccinology. However, it seems unlikely that these technologies will produce more effective PRRSV vaccines in the short term. However, there is room for some improvements. Research on adjuvants and routes of administration, combinations of MLV and improved autogenous vaccines, the use of strains with greater capacity to produce broadly neutralizing antibodies, etc., can help improve currently available vaccination products and schedules.

Keywords: PRRS, ARC, Control

Three decades of marching towards a better PRRS vaccine

Shishan Yuan
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It has been three decades since the first MLV against Porcine reproductive and respiratory syndrome (PRRS) launched in the U.S.. Coincidentally, it has been 30 years since PRRSV appeared in the Chinese swine herds in 1995. Many kinds of PRRS vaccines have gained marketing authorizations across the world, yet PRRS remain one of the biggest threats to the global swine industry. Many puzzles remain and become obstacle to rationally design the next-generation of vaccine. In this presentation, I will summarize current situations of PRRS vaccine development and application as well as the missing virological and vaccinological links towards a better PRRS vaccine.

Arteriviruses are enveloped positive-strand RNA viruses for which the attachment proteins and cellular receptors have remained largely controversial. In particular, it is debatable about the mechanism of membrane fusion resulting in the virus entering the target cells via receptor-mediated endocytosis. Current evidences point towards that minor glycoproteins GP4 or GP2 interact with CD163, but conclusive data is needed to elucidate the exact fusion process.

Arterivirus particles contain at least eight envelope proteins, an unusually large number among RNA viruses. It has been proposed that the N-linked glycan addition at certain sites in glycoproteins (GP2-GP5) of PRRSV is important for their immunigencity or shielding thereof and virus infectivity. We conducted systematic ablation of those glycoslation site mutation, and concluded that not a single individual N-linked glycoslation is critical for virus infectivity. However, a combination of multiple site ablation indeed affecting virus growth, especially in infected pigs. Further stdies is warranted to decipher the exact role of such glycoproteins and their domains in the process of enveloped viral entry and immunogen design.

The current PRRS MLVs are not ideal for heterologus protection, and lack of broad-spectrum neutralizing antibodies. How to find and rationally design universal immunogens relys on further protein structure and function studies. The notorious drawback of modified live vaccines lies in is propensity for virulence reversion after prolong exposure in the farm. It is surprising that how quickly an “attenuated” virus can revert back to wild type and pathogenic form. Care should be taken to balance out the safety and efficacy features during vaccine R&D.

PRRSV variant emergence: examples and considerations for prospective monitoring

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As with any RNA virus, a high substitution rate is expected to generate great diversity from evolutionary changes, and PRRSV2 substitution rates are considered high, even when compared to other RNA viruses. Thus, new variants are constantly emerging in the population and monitoring them is crucial to quickly identify if there is potential for rapid spread and high impact due to disease severity, especially when, like SARS-CoV-2, not all variants might be of concern or of high consequence.

We highlight the need to use a phylogenetic-based approach when identifying PRRSV variants. Lineage/sub-lineage or RFLP pattern on their own are insufficient to correctly identify cases belonging to these genetically similar clades, leading to low positive predictive values (34% to 42% for the L1C.5 variant, and 3% to 41% for the L1C.2 variant). Although the combination of both classifications substantially improves case ascertainment, accuracy is still not 100%. Another example of the importance of phylogenetic-based case ascertainment is variant L1H.18. Initially, sequences belonging to this group were classified as a somewhat rare RFLP pattern (1-12-2) and were assigned to sub-lineages L1C or L1H, depending on the classification method employed (PRRSView or distance to the nearest reference). When constructing a phylogenetic tree, this clade consists of sequences positioned between sub-lineages L1H and L1C.

Sequence counts generated from secondary data from veterinary diagnostic labs per region or state need to account for the total submissions within each unit of analysis, as surveillance is not equal throughout space. Epidemiological curves can look very distinct if plotting sequence counts or sites affected because multiple sequences are often generated by the same site over time. For example, while the epidemiologic curve of L1C.2 looks similar over the first few months of its emergence, as most sequences represent new cases, the difference between sequence counts and sites affected becomes more pronounced as unstable sites continue to generate new sequence submissions.

Because not all variants emerging will be of high consequence, it is important to critically assess clinical presentations. Case descriptions, although informative, might generate a bias in perceived virulence because comparisons with more typical cases are lacking. A case-control study comparing L1C.5 cases to PRRS outbreaks due to other variants showed increased finisher mortality within the first four weeks of PRRS detection. Time to stability in breeding herds affected by L1C.2 was longer than average, and heavily influenced by the presence of other co-circulating PRRS variants. However, measuring virulence in the field is not straightforward, as factors influencing clinical presentation, such as co-infections and management practices, can be difficult to obtain. Altogether, we highlight that an in-depth rigorous investigation is crucial before raising concerns about a new emerging variant.

Keywords: PRRS, Epidemiological Monitoring, Viral Variants, Virus Emergence

The practice and enlightenment of PRRS elimination in large scale pig farm of China

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Introduction

This case study showed how pig producers can make decisions whether their farm is eligible for the Porcine Reproductive and Respiratory Syndrome (PRRS) elimination. How to do the elimination process and evaluate the results. Taking a 5,000-sow basic sow farm as an example to adopt a closed-herd and feedback strategy for PRRS elimination.

Materials and Methods

Review of the outbreak of PRRS and feasibility study of PPRS elimination were conducted. All the gilts for the next 6 months' replacement were prepared. All the breeders were acclimatized by its own wild virus. The clinical signs of the sow herd after the acclimatization were observed. ELISA and PCR tests were done to confirmed that all the breeders were infected by wild PRRS virus. The McREBEL program was implemented in farrowing rooms. All the weaners were raised in offsite WF farms. Collected the samples to evaluate PRRSv status.

Result

In the 7th week after the PRRSv acclimatization, the serums of 253 sows showed PPRS antibodies 100% positive. According to the clinical signs and test results, we confirmed that the entire sow herd was successfully exposed to PRRSv. In the 19th week, all the samples from abnormal sows were PRRSv negative. In the 24th week, all the samples from weaned piglets were PRRSv negative. Since the 28th week, all the samples from piglets began to show PCR negative. After getting the negative results from all the samples for 8 consecutive weeks, we confirmed that the PRRS elimination was successful.

Conclusions and Discussion

It is crucial for pig producers to recognize the value of PRRS-negative pig herds, but not all farms are suitable for PRRS elimination. There were three key points for the elimination, enough gilts for the closure of sow herd, all the breeders infected with the wild PRRSv at the same time, all the weaners should be offsite before we confirmed the herd was negative. It took 32 weeks to finish the PRRS elimination.

Keywords: Porcine Reproductive and Respiratory Syndrome, Domestication, Purification

PRRSV-1: the meaning of genetic subtypes

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of one of the most economically important diseases in the swine industry. The genome of PRRSV is a ss(+)RNA and consists of ~15,000 nucleotides organized into 11 ORFs. The analysis of ORF5, ORF7 and whole genome sequences of currently known PRRSV-1 and PRRSV-2 strains suggest that the former may be more genetically diverse, and multiple genetic subtypes were proposed: namely globally spread subtype 1, and subtypes 2, 3 and tentative 4, that were identified only in the east European countries formerly being members of the Soviet Union, such as Russian Federation, Ukraine, Belarus, Lithuania and Latvia. ORF5 targeted sequence analysis is often used for molecular epidemiological studies in PRRSV-2 and PRRSV-1. However, due to frequent recombination events the analysis of a single ORF is often insufficient to show the identity of a given strain. Also, the incongruent results of ORF5 and ORF7 clustering of some of the east European PRRSV-1 sequences, resulting in classification in different genetic subtypes, indicated that the recombination can also occur between the viruses of distant relationship. Therefore, the classification of the viruses into the proposed genetic subtypes of PRRSV-1, and lineages, needs verification employing larger fragments of PRRSV-1 genomes. Unfortunately, unlike genomes of globally spread subtype 1 viruses, which are well represented in public data bases, currently only five, complete or nearly complete, genomes of east European viruses are available, two closely related from Belarus, two distinct ones from Russian Federation, and one from Lithuania. Recently, near complete sequences of four Belarusian and one Russian PRRSV-1 isolates from 2009-2010 were obtained. The comparison of clustering of individual ORFs, as well as the recombination analysis, indicated that most of the studied east European viruses emerged from inter-subtype recombination, but not involving the globally spread subtype 1. These findings underline the complex evolutionary history of PRRSV-1 and demonstrate that the classification scheme of its strains requires revision.

Overview of PRRSV diagnostics and molecular epidemiology in the USA

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Traditionally, individual serum and tissue samples have been used for PRRSV testing. However, over the past decade, population-based specimens such as processing fluid, family oral fluid, regular oral fluid, and tongue tips fluid have become increasingly popular for PRRSV surveillance. Despite this, the success rate of PRRSV virus isolation (VI) from these population-based specimens is generally low. Serum and lung samples with CT<30 are preferred for PRRSV VI. The ZMAC cell line is superior to MARC-145 cell line, offering a better success rate for isolating PRRSV from clinical samples and more effectively isolating the predominant virus strain when a clinical sample contains ≥2 PRRSV-2 strains.

PRRSV molecular assays, including the screening real-time RT-PCR, vaccine-like PCRs, ORF5 Sanger sequencing without or with CLAMP technology, and next-generation sequencing (NGS) technology, will be discussed for their applications in different combinations.

In the USA from 2014-2023, the yearly PRRSV screening PCR-positive rates ranged 22.5–26.3% for all age of pigs, 15.7–23.9% for sow farms, and 35.2–44.2% for wean-to-finish pigs. An analysis of 106,392 PRRSV screening PCR-positive cases in the USA from 2007−2018 revealed that the percentages of PRRSV-2, PRRSV-1, PRRSV-2 & PRRSV-1 positive cases were 95%, 2%, and 3%, respectively.

Our recent study of 82,237 global PRRSV-2 ORF5 sequences during 1989–2021 led to the classification of PRRSV-2 into 11 genetic lineages (L1−L11) and 21 sublineages (L1A−L1F, L1H−L1J, L5A−L5B, L8A−L8E, and L9A−L9E). Additionally, fine-scale classification below the sublineage level is beneficial. As a proof-of-concept, the sublineage L1C was further divided into L1C.1−L1C.5 and L1C-others at the “variant” level. Further classification at the variant level for L1C and other sublineages is in progress. A web-based tool using Nextclade classifier (available at https://prrsv.vdl.iastate.edu/) can facilitate the PRRSV-2 ORF5 genetic classification. The dynamic changes of PRRSV-2 ORF5 sequences collected in the USA during 1989–2024 will be presented. A proposed refinement of PRRSV-1 ORF5-based classification system will also be discussed. Efforts to achieve global standardization of PRRSV-1 and PRRSV-2 genetic classification are necessary.

Creation and breeding of gene edited pigs resistant to porcine reproductive and respiratory syndrome virus

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CD163 has been confirmed to be a key role in proliferation of porcine reproductive and respiratory syndrome virus (PRRSV). By preparing gene edited pigs with base deletions in the fifth domain of the seventh exon of the CD163 gene, breeding materials and core breeding groups for pigs resistant to PRRSV were obtained, which has attracted attention from various countries around the world to alleviate the harm of PRRSV from the breeding level. Until now, more than 10 organizations have successfully obtained gene edited pigs resistant to PRRSV internationally.

Our research team obtained somatic cloned pigs with CD163 gene editing in 2016 firstly, as well as double gene edited pigs with CD163 and pAPN. These two pig breeding materials were approved for intermediate trials in 2017. Subsequently, our research team successfully obtained multiple CD163 single gene edited pigs, CD163 and pAPN double gene edited pigs, as well as CD163, pAPN and MSTN triple gene edited pigs. So far, our research team has obtained approval to conduct biosafety evaluations on 7 pig breeding materials involving CD163 gene editing. Our research team has systematically carried out molecular characterization, off target detection, genetic stability, major production performance measurement, environmental safety, and food safety evaluation for genetically edited pigs resistant to PRRSV. Significant progress has been made in the construction of breeding core groups. In response to the target trait of anti-PRRSV, our research and development team has conducted in vivo antiviral experiments three times separately using three different strains of PRRSV.

As for CD163 single gene edited pigs, the highly pathogenic PRRSV challenge test confirmed these CD163 gene-edited pigs were completely resistant to PRRSV infection, in contrast, the WT were high susceptibility. At present, these gene-edited pigs have been bred to the F6 generation with more than 200 pigs. The enviromental release phase has been completed in terms of safety assessment. Notably, the important economic traits of the homozygotes such as growth, development and litter size are normal. Taken CD163 and pAPN double-gene edited pigs as an example. The in vivo challenge assay of CD163 and pAPN double-gene edited pigs shown they are completely resistant to PRRSV and TGEV infection, and partly resistant to PDCoV. Moreover, the slaughtering testing and performance testing confirmed the main economic traits of CD163 and pAPN double-gene edited pigs are normal. Altogether, the research results of our research team indicate that CD163 gene edited pigs can effectively resist the PRRSV infection, and are genetically stable. There is no significant difference in growth, development, and main production performance compared to the wild type, and there are no environmental or food safety issues. In summary, the CD163 gene edited pigs have shown good development and application prospects.

Genetics of Disease Resilience in Grow-Finish Pigs

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Infectious disease represents one of the largest costs of pork production. The impact of infectious disease can be reduced using biosecurity, vaccination, and medications. Susceptibility to most infectious diseases is in part determined by the genetics of the animal. Thus, genetic selection is another strategy that can be used to reduce disease and/or the impact of disease. Unfortunately, the ability to make animals completely resistant is limited to only a few pathogens (e.g. F18 E.coli) or only possible by gene editing (e.g. the PRRS-resistant pig created by editing the CD163 gene). Host response to most other infectious diseases is affected by many genes and complete resistance is not possible. Given these limitations, commercial pigs will continue to be exposed to, and infected by, pathogens for the foreseeable future. Disease resilience is the ability of an animal to be minimally affected by disease or to rapidly return to normal performance after infection. Combined with the fact that pathogens evolve, that new (foreign) diseases can emerge (e.g. African Swine Fever) and that complete resistance is not possible for most pathogenic diseases, disease resilience is a promising target for inclusion in breeding programs. Disease resilience can be evaluated by exposing animals to disease and recording disease incidence, mortality, and performance. The purpose of this presentation is to describe results of a large-scale study on the genetics of disease resilience in a polymicrobial natural disease challenge of grow-finish pigs, including identification of major genes and of indicator traits that can be measured on young healthy pigs to select for improved disease resilience without exposure to disease.

Novel Vaccine Candidates against Porcine Epidemic Diarrhea Virus

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Porcine Epidemic Diarrhea Virus (PEDV) is an economically-important pathogen in the global swine industry, and its control remains challenging as the available vaccines do not confer adequate protection against PEDV infection. In collaborations with the lab of Steven Zeichner of the University of Virginia School of Medicine, and the lab of Chenming (Mike) Zhang of Virginia Tech Department of Biological System Engineering, we recently developed novel PEDV vaccine candidates with the goals of enhancing both cell-mediated and humoral immune responses. **(1).** By utilizing dendritic cell (DC)-targeting vaccine approach, we delivered PEDV S antigen directly to the porcine DCs through a single-chain antibody specific to porcine Langerin, a C-type lectin receptor expressed on DCs. The DC-targeting PEDV vaccine candidate greatly improved PEDV S antigen-specific T cell IFN-γ responses in the $CD4^{pos}CD8^{pos}$ T cell compartment and induced higher serum IgG and IgA responses in pigs. Immunization of sows with DC-targeted PEDV S vaccine significantly reduced the amount of PEDV antigen in intestine tissues of piglets after PEDV challenge. Piglets born to sows vaccinated with DC-targeted PEDV S vaccine had a decreased intestinal villous damage after virus challenge. **(2).** By utilizing the hepatitis B virus core capsid antigen (HBcAg) that can self-assemble into virus-like particle (VLP), we expressed PEDV S-specific B-cell epitopes in the backbone of the HBcAg that subsequently assembles into VLPs for use as a vaccine candidate. A PEDV-HBcAg VLP vaccine candidate induced higher virus neutralization response in gilt milk, and provided alleviation of clinical signs for piglets challenged with PEDV. **(3).** By employing a synthetic biology-based vaccine platform that expresses the conserved PEDV fusion peptide (FP) on the surface of genome-reduced bacteria, we evaluated the use of killed whole-bacterial cells expressing PEDV FP as vaccine candidate to enhance the interaction of vaccine antigen with the immune system. We showed that the genome-reduced bacteria vaccine candidate expressing PEDV FP induced potent anamnestic responses upon virus challenge, reduced viral RNA loads in jejunum tissue, and alleviated PEDV-induced diarrhea and body condition. These novel PEDV vaccine candidates are warranted for further development.

Unveiling Porcine Antiviral Immunity: Transcriptomic Profiling of PRRSV Infection for Targeted Strategies

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Porcine reproductive and respiratory syndrome virus (PRRSV) devastates the swine industry, with production costs to pig producers in the United States estimated at $560 million annually, highlighting the need for improved control measures. Our studies leverage evolving technologies - microarrays, serial analysis of gene expression (SAGE), digital gene expression tag profiling expression (DGETP), RNA-Seq, small non-coding RNA-Seq (sncRNA), spatial transcriptomics, prediction matrices, machine learning, cell lines and organoids - to investigate virus-host interactions in the porcine respiratory tract. We hypothesize that transcriptomic profiling will reveal cell-specific immunogenic responses with viral pathology and antiviral protection during PRRSV infection. Using our established porcine-PRRSV infection models and validated RNAseq pipeline we can decipher the cellular and molecular mechanisms underlying PRRSV pathogenesis. By characterizing the porcine antiviral immunome, we aim to develop cell-targeting antiviral regulation for early protection and immune potentiation.

Developing recombination-resistant attenuated vaccines to fight porcine epidemic diarrhea virus (PEDV)

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Porcine epidemic diarrhea virus (PEDV) is an enteric alphacoronavirus. It causes acute watery diarrhea, vomiting, and dehydration, leading to high mortality rates (50-100%) in neonatal piglets and slow growing in older pigs. It emerged in the US in 2013, quickly spread to 39 states and killed about 10% pig population during 2013-2014 outbreaks, resulting in huge economic losses. Currently, although epidemic outbreaks have been controlled mainly by herd immunity and well-established biosecurity in the United States, endemic outbreaks occur, and some swine farms suffer from persistent PEDV infections. Safe and effective PEDV vaccines are urgently needed but are not available. Due to the ability to induce broad and prolonged protective immunity and the convenient administration routes, live attenuated vaccines (LAVs) are promising arms for controlling the deadly coronavirus infections. However, the safety of LAVs is a concern because conventional LAVs, generated by continuous passaging the virus in tissue culture to obtain attenuated strains, can revert to virulent strains or recombine with wildtype virus to generate new virus variants when they are applied on farms. We aim to develop safe LAVs that are unable to revert to virulence or recombine with field strains.

In the past decade, we generated a reverse genetics system for PEDV, identified attenuating mutations in PEDV nonstructural protein 1 (nsp1), spike (S) protein, accessory protein ORF3, and nsp16, and engineered a recombination-resistant PEDV platform by recoding the viral transcriptional regulatory sequences (TRSs) for LAV development.

These discoveries have a significant impact for pig health and the sustainability of swine industry. Also, PEDV infection of pigs is a good model to test our universal strategies for the generation of recombination-resistant LAVs for coronaviruse. The success of this project will aid in innovative vaccine design against PEDV and newly emerging animal and human coronaviruses.

Keywords: Porcine epidemic diarrhea virus, PEDV, vaccine, recombination-resistant, reverse genetics

Enteric coronavirus PEDV non-structural protein 2 acts as a novel virulence determinant by linking innate antiviral immunity and autophagy

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Coronaviruses (CoVs) are a challenge for both public health and a veterinary and economic concern, whose infection usually causes subclinical or respiratory (exemplified by SARS-CoV-2) and gastrointestinal diseases. Non-structural protein 2 (nsp2) exists in all CoVs; however, the main biological role and underlying mechanism of how nsp2 regulates the infectious life cycle and pathogenesis of CoVs have not been elucidated to date. In this study, using an enteric CoV, porcine epidemic diarrhea virus (PEDV; causes high mortality in neonatal piglets worldwide), and a corresponding nsp2 deletion mutant (rPEDV-Δnsp2) as virus models, and utilized their host animals (neonatal piglets) as clinical infection models, we demonstrate the role of nsp2 in CoV infection, pathogenicity, and host cell signaling associated with autophagy for the first time.

Firstly, we generated a PEDV mutant containing a complete nsp2 deletion (rPEDV-Δnsp2) from a highly pathogenic strain by reverse genetics, showing that nsp2 was dispensable for PEDV infection, while its deficiency reduced viral replication in vitro. Next, intriguingly, rPEDV-Δnsp2 was entirely avirulent in vivo, with significantly increased productions of interferon (IFN)-β and IFN-stimulated genes (ISGs) in various intestinal tissues of challenged newborn piglets. Notably, nsp2 targets and degrades TANK-binding kinase 1 (TBK1), the critical kinase in the innate immune response. Mechanistically, nsp2 induces the autophagy process directly, and recruits a selective autophagic receptor neighbor of BRCA1 (NBR1). NBR1 and nsp2 subsequently facilitated the K48-linked ubiquitination of TBK1 by formation of NBR1-nsp2-TBK1 complex, and delivered TBK1 for autophagosome degradation. Accordingly, the replication of rPEDV-Δnsp2 CoV was restrained by excess productions of type I IFNs and ISGs.

Our data collectively define enteric CoV nsp2 as a novel virulence determinant, propose a crucial role of nsp2 in diminishing innate antiviral immunity by targeting TBK1 for NBR1-mediated selective autophagy, and pave the way to develop a new type of nsp2-based attenuated PEDV vaccine. The study also provides new insights into the prevention and treatment of other pathogenic CoVs such as SARS-CoV-2.

Virus Replication

Oral

Glycoprotein 2b (E) of porcine reproductive and respiratory syndrome virus (PRRSV) enhances the expression of the GP2/3/4 complex on the plasma membrane.

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-stranded RNA virus categorized within the family *Arteriviridae* of the order *Nidovirales*. One of its notable components is the PRRSV E protein, a nonglycosylated structural protein encoded by the ORF2b gene, spanning approximately 70-73 amino acids. Despite being an important component of virus particles, the specific role of the 2b protein within the PRRSV heteromultimeric complex remains to be elucidated.

Materials and Methods

The protein sequences of GP2b, GP2, GP3, and GP4 were amplified from the PRRSV European Lelystad virus (LV) strain. To enable the co-expression of these three proteins within a single vector, we employed the 2A peptides strategy in our construction system. Specifically, P2A and T2A peptides were inserted between GP4 and GP3, and GP3 and GP2, respectively. HEK-T cells were transiently co-transfected with the GP2/3/4 vector and GP2b or without GP2b. After 48 hours, the cells were harvested for immunofluorescence staining under various treatment conditions. The expression of minor structural proteins on the membrane was evaluated under non-permeabilized conditions. The percentage of cells expressing proteins on the membrane was quantified and compared to the permeabilized groups (cytoplasmic expression).

Results

The vector for co-expression of PRRSV minor structural proteins (GP2, GP3, GP4) was successfully constructed. Approximately 36.01 ± 5.98% of HEK-T cells exhibited expression of all three proteins in the cytoplasm. Immunofluorescence analysis revealed colocalization of GP2, GP3, and GP4 glycoproteins when transfected cells were permeabilized. However, only a very low proportion (0.29 ± 0.03%) of transfected cells expressed the glycoproteins on the plasma membrane under non-permeabilized conditions. When the cells were co-transfected with the GP2-GP3-GP4 vector and GP2b, a higher fraction (4.05 ± 0.06%) of the transfected cells expressed GP2, GP3, and GP4 proteins on the plasma membrane.

Conclusions and Discussion

In our current experiment, PRRSV GP2, GP3, and GP4 were observed to be exclusively expressed in the cytoplasm when transfected individually or in combination. However, upon co-transfection with GP2b, the minor structural GP2/3/4 complex translocated to the membrane, suggesting a significant role for GP2b in directing the viral glycoproteins during virus replication.

Keywords: PRRS, GP2, GP3, GP4, GP2b, membrane expression

Porcine reproductive and respiratory syndrome virus nonstructural protein 2 promotes the autophagic degradation of adaptor protein SH3KBP1 to antagonize host innate immune responses by enhancing K63-linked polyubiquitination of RIG-I

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Introduction To better understand NSP2's role during PRRSV infection, we identified the NSP2-interacting host protein SH3KBP1 using mass spectrometry. SH3KBP1 is involved in apoptosis, cell migration, B cell activation, and cell shape regulation. The RIG-I signaling pathway is crucial for host resistance to RNA virus infections, requiring K63-linked ubiquitination of RIG-I for activation.

Material and Methods We transfected 293T cells with Flag-NSP2 and performed co-immunoprecipitation. The immunoprecipitates were identified with LC-MS/MS. We examined the effects of SH3KBP1 overexpression and knockdown/knockout on PRRSV replication in Marc-145 and iPAM cells. Sh3kbp1-/- mice were challenged with VSV via tail intravenous injection. We measured protein levels of RIG-I, TBK1, MAVS, and IRF3 in cells overexpressing or lacking SH3KBP1. In vitro ubiquitination assays explored whether SH3KBP1 affects RIG-I polyubiquitination. We constructed a PRRSV GSWW15-NSP2Δ435-438 mutant strain to investigate the role of the NSP2 435-438 amino acid sequence.

Results Exogenous SH3KBP1 significantly inhibited PRRSV replication by enhancing IFN-I and related ISGs, while its knockdown promoted virus replication by downregulating IFN-I and ISGs. Sh3kbp1-/- mice were more sensitive to VSV infection with reduced serum IFN-β levels. SH3KBP1 indirectly increased K63-linked polyubiquitination of RIG-I to enhance signal transduction through interaction with the E3 ubiquitin ligase TRIM25. PRRSV infection and NSP2 overexpression led to autophagic degradation of SH3KBP1, indicating an antagonistic effect on the host immune response. The third proline-rich motif in NSP2 (453PVPAPR458) was the key interactive site with SH3KBP1, as recombinant PRRSV lacking this motif showed attenuated virulence and decreased SH3KBP1 degradation.

Conclusions and Discussion This study revealed a novel role for SH3KBP1 in regulating IFN-I signaling. SH3KBP1 promoted RIG-I expression and inhibited PRRSV replication in vitro. PRRSV NSP2 degraded SH3KBP1 through the autophagic pathway, antagonizing its antiviral effect. This study illustrates SH3KBP1's regulatory role in innate immunity and provides a novel mechanism by which PRRSV antagonizes the host immune response.

Key words: PRRSV, NSP2, SH3KBP1, RIG-I, autophagy

The dimerization of porcine reproductive and respiratory syndrome virus nsp1α is important for viral subgenomic RNA synthesis

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PRRSV nsp1α is the first viral protein translated in virus-infected cells and released from viral polyprotein 1a via auto-cleavage. It plays important roles in viral replication, suppression of host innate immune response and cell-mediated immune responses. Nsp1α was demonstrated to form a homodimer *in vitro* based on its crystal structure. In this study, we aimed to characterize the role of dimerization in the functions of nsp1α. Initially, a recombinant HP-PRRSV expressing an additional nsp1α with Flag-tag was generated to confirm the homodimer confirmation of nsp1α in PRRSV-infected cells. Through alaine scanning, the valine^{132} and proline^{134} were identified as key residues for the dimerization of nsp1α. using the recombinant viruses expressing an additional Flag-nsp1α mutant of V132A or P134A, we confirmed that V132A and P134A disrupted the dimerization of nsp1α in PRRSV-infected cells. The critical role of valine^{132} and proline^{134} in the dimerization of nsp1α was also confirmed in a PRRSV-1 strain. When ectopically expressed, the mutants containing a substitution of V132A or P134A have no obvious effect on the function of nsp1α in antagonism host type I IFN production and degrading SLA-I. The mutations of V132A and P134A introduced in a replicon system of HP-PRRSV significantly attenuated the expression of the Gaussia luciferase reporter, suggesting the dimerization of nsp1α is critical for viral replication. To confirm this, the mutations of V132A and P134A were respectively introduced into the PRRSV genome. A recombinant virus (vV132A) carrying V132A mutation was rescued, while the P134A mutant was lethal. In comparison with the WT virus, the growth of vV132A in MARC-145 cells was significantly attenuated. To further assess the mechanism, viral RNA synthesis in BHK-21 cells transfected with the cDNA clones was evaluated by RT-PCR and RT-qPCR. The substitution of P134A downregulated the subgenomic RNA 6/7 and completely disrupted the transcription of other subgenomic RNA, while V132A mutation also downregulated the transcription of subgenomic RNAs. By contrast, the synthesis of viral genomic RNA was not affected by these two mutations. Taken together, our results proved that the dimerization of nsp1α is important for viral subgenomic RNA synthesis and the expression of structural proteins.

Posters

Better understanding of PRRSV/ASFV entry in macrophages by the use of macrophage-specific mAbs

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Porcine reproductive and respiratory syndrome virus (PRRSV) and African swine fever virus (ASFV) cause serious economic losses to the swine industry worldwide, and there are no highly effective strategies for prevention and treatment. Both PRRSV and ASFV show a tropism for monocytes/macrophages, based on the use of specific entry mediators (e.g. Siglec1, Siglec10, CD163). A better understanding of viral entry (identification of receptors and viral ligands) is helpful in the development of antivirals and vaccines. Therefore, it is important to continue the search for additional mediators of viral entry. Monoclonal antibodies may be helpful tools. This study aimed to identify and characterize monoclonal antibodies (mAbs) directed against porcine alveolar macrophages (PAMs) that may block the early stages of infection (binding, internalization, fusion). We used a library of 166 monoclonal antibodies (mAbs) against macrophages that were previously developed in our laboratory. By immunofluorescence staining of PAMs, 74 mAbs showed cytoplasmic staining in permeabilized cells and 70 mAbs showed a membrane staining in non-permeabilized cells. Fifteen of these mAbs reacted against blood monocytes by flow cytometry analysis; 62 did not. A mAb blocking assay was performed at 4 ℃ and 37 ℃ to analyse if the mAbs were able to block PRRSV and ASFV infection in PAMs. Two mAbs (28C10 and 28G10B6) partially blocked PRRSV and ASFV infection; an additional mAb partially blocked only PRRSV infection (26B8F5-I). By Western blotting, the size of the targeted proteins were determined: 28C10 -> 210 kDa; 28G10B6 -> 280 kDa and 26B8F5-I -> 37 kDa. The identification by mass spectrometry is ongoing.

The role of these macrophage proteins in the early stages of PRRSV/ASFV infection will be further elucidated in the future.

Keywords: PRRSV, ASFV, macrophage, monoclonal antibody, receptor

A nanobody inhibiting porcine reproductive and respiratory syndrome virus replication via blocking self-interaction of viral nucleocapsid protein

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ABSTRACT Porcine reproductive and respiratory syndrome (PRRS) is a serious global pig industry disease. Understanding the mechanism of viral replication and develop- ing efficient antiviral strategies are necessary for combating with PRRS virus (PRRSV) infection. Recently, nanobody is considered to be a promising antiviral drug, espe- cially for respiratory viruses. The present study evaluated two nanobodies against PRRSV nucleocapsid (N) protein (PRRSV-N-Nb1 and -Nb2) for their anti-PRRSV activity *in vitro* and *in vivo*. The results showed that intracellularly expressed PRRSV-N-Nb1 significantly inhibited PRRSV-2 replication in MARC-145 cells (approximately 100%). Then, the PRRSV-N-Nb1 fused with porcine IgG Fc (Nb1-pFc) as a delivering tag was produced and used to determine its effect on PRRSV-2 replication in porcine alveolar macrophages (PAMs) and pigs. The inhibition rate of Nb1-pFc against PRRSV-2 in PAMs could reach >90%, and it can also inhibit viral replication *in vivo*. Epitope mapping showed that the motif Serine 105 (S105) in PRRSV-2 N protein was the key amino acid binding to PRRSV-N-Nb1, which is also pivotal for the self-interaction of N protein via binding to Arginine 97. Moreover, viral particles were not successfully rescued when the S105 motif was mutated to Alanine (S105A). Attachment, entry, genome replication, release, docking model analysis, and blocking enzyme-linked immunosorbent assay (ELISA) indicated that the binding of PRRSV-N-Nb1 to N protein could block its self-bind- ing, which prevents the viral replication of PRRSV. PRRSV-N-Nb1 may be a promising drug to counter PRRSV-2 infection. We also provided some new insights into the molecular basis of PRRSV N protein self-binding and assembly of viral particles.
IMPORTANCE Porcine reproductive and respiratory syndrome virus (PRRSV) causes serious economic losses to the swine industry worldwide, and there are no highly effective strategies for prevention. Nanobodies are considered a promising novel approach for treating diseases because of their ease of production and low costing. Here, we showed that PRRSV-N-Nb1 against PRRSV-N protein significantly inhibited PRRSV-2 replication *in vitro* and *in vivo*. Furthermore, we demonstrated that the motif Serine 105 (S105) in PRRSV-N protein was the key amino acid to interact with PRRSV-N-Nb1 and bond to its motif R97, which is important for the self-binding of N protein. The PRRSV-N- Nb1 could block the self-interaction of N protein following viral assembly. These findings not only provide insights into the molecular basis of PRRSV N protein self-binding as a key factor for viral replication for the first time but also highlight a novel target for the development of anti-PRRSV replication drugs.
KEYWORDS porcine reproductive and respiratory syndrome virus, nanobody, nucleocapsid protein, antiviral drug, viral replication

The mechanism of host protein NUDT7 participating in PRRSV infection

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRSV) is a typical immunosuppressive virus that poses a serious threat to the global pig farming industry. Peroxisomes are organelles that play important roles in lipid and reactive oxygen species metabolism, as well as signaling platforms that mediate innate immune mechanisms. Peroxisome matrix protein NUDT7 is a coenzyme A (CoA) diphosphatase that mediates CoA cleavage, participates in beta oxidation of long-chain fatty acids, ether lipid synthesis, and bile acid metabolism [3]. Therefore, this study analyzed how NUDT7 regulates PRRSV replication, aiming to explore the interaction between PRRSV and the host, and provide new insights for the prevention and control of PRRSV.

Materials and Methods

This study used porcine alveolar macrophages (3D4/21) as a model and combined transcriptome sequencing to determine NUDT7 as the subsequent research object. QRT PCR, Western blot, and immunofluorescence methods were used to investigate the effect of NUDT7 on PRRSV proliferation.

Results and Discussion

This study found that PRRSV infection leads to an increase in the expression level of NUDT7 in 3D4/21 cells. Overexpression of NUDT7 promotes PRRSV proliferation. Mechanism studies have found that NUDT7 significantly inhibits VSV induced activation of IFN-β and IFN stimulus response element (ISRE) reporter genes. Overexpression of NUDT7 significantly inhibited IRF3 phosphorylation and transcription of antiviral genes, including IFN - β, ISG15, and ISG56. Meanwhile, similar phenomena were observed in Marc-145 cells infected with PRRSV. Therefore, NUDT7 inhibits the production of IFN - β and ISGs, negatively regulating the type I interferon pathway. In addition, BODIPY lipid droplet staining experiments showed that overexpression of NUDT7 promoted the number of lipid droplets. The results of qRT PCR and Western blot detection of lipid metabolism related genes showed that NUDT7 significantly promoted the expression of PGC1A and PPAR γ. Therefore, NUDT7 promotes the replication of PRRSV by promoting lipid droplet formation. This study reveals the mechanism by which NUDT7 regulates PRRSV replication, providing a theoretical basis for the prevention and control strategies of PRRS.

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The N-terminal Intermolecular Disulfide Bond of PRRSV GP5 and M Facilitates VLPs Secretion and Cell binding

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to significantly impact on the global swine industry for over three decades. GP5 and M are the two primary structural proteins of PRRSV, playing pivotal roles in the processes of virus attachment, entry, assembly and budding. However, the underlying mechanism has yet to be fully elucidated. This study employs virus-like particles (VLPs) as a tool to reveal the critical role of the interaction between GP5 and M in the secretion and cell binding of PRRSV virions.

Materials and Methods

The baculovirus expression vector system (BEVS) was utilized to express GP5 and M. The VLPs were purified by density gradient centrifugation and observed via transmission electron microscopy. Subsequently, the secretion characteristics of VLPs were investigated and the interaction between GP5 and M was confirmed by BirA technology. Additionally, the N-terminal intermolecular disulfide bond was disrupted to investigate the impact on the assembly and secretion of VLPs. Finally, the binding of VLPs to MARC-145 cells was analyzed using indirect immunofluorescence.

Results

GP5 and M were co-expressed in Sf9 cells and successfully assembled into VLPs. The secretion of VLPs was found to be regulated by the expression levels of GP5 and M, with the highest yield observed at equimolar expression. The BirA assay confirmed the interaction between GP5 and M in Sf9 cells. Disruption of the N-terminal intermolecular disulfide bond between the two proteins weakened their interaction and consequently reduced VLP secretion. Importantly, the absence of N-terminal intermolecular disulfide bond resulted in the loss of VLP binding capability to MARC-145 cells.

Conclusions and Discussion

This study has revealed that the N-terminal intermolecular disulfide bond is a crucial interaction site for GP5 and M, playing a key role in facilitating the secretion and cell binding of VLPs. In addition, the results strongly suggest the existence of potential interaction sites between GP5 and M.

Keywords: PRRSV, virus-like particles, protein interaction, disulfide bond

Tripartite motif 56 Inhibits Porcine Reproductive and Respiratory Syndrome Virus by Promoting Generation of NLRP3 Inflammasome

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Abstract
The tripartite motif 56 (TRIM56) protein is the part of the innate immune system. TRIM56 has antiviral activity. However, whether TRIM56 is important in the inhibition of Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is unknown. Here, we demonstrated that PRRSV induced the up-regulation of TRIM56 expression. PRRSV Nsp11 significantly up-regulated TRIM56 mRNA, implicating PRRSV Nsp11 as a key protein affecting TRIM56 in cells. TRIM56 overexpression inhibited PRRSV replication. Conversely, silenced expression of TRIM56 resulted in increased PRRSV replication. Potential intracellular proteins interacting with TRIM56 were screened by co-immunoprecipitation-mass spectrometry. Bioinformatics analysis identified differentially expressed proteins. Differential proteins VDAC2, ITPR3, DNM1L, and Hsp90ab1 focused on the upstream regulatory region of the NLRP3 inflammasome pathway. We confirmed that TRIM56 promoted the mRNA expression of NLRP3 inflammasome components and NLRP3 protein. and induced the generation of mRNA level of IL-1β, suggesting that TRIM56 is a positive regulatory protein of NLRP3 inflammasome. The observed interaction of TRIM56 with Hsp90ab1 indicated that Hsp90ab1 protein was a potential key regulatory molecule in NLRP3 inflammasome regulation by TRIM56. The collective findings revealed that TRIM56 was a critical antiviral immune effector that exerted anti-PRRSV activity by promoting the generation of NLRP3 inflammasome. These findings provided new insights into the antiviral mechanism of PRRSV infections.

Keywords: PRRSV, TRIM56, NLRP3 inflammasome, CoIP-MS, innate immunity

PRRSV NSP1α degrades TRIM25 through proteasome system to inhibit host antiviral immune response

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Introduction/purpose: TRIM25 is an important E3 ubiquitin ligase in cells which can regulate interferon signaling pathway to increase host antiviral function. In addition, TRIM25 significantly inhibits PRRSV infection. As an critical protease of PRRSV, whether NSP1α antagonizes the activation of TRIM25 has not been reported. Our study reveals the new mechanism that PRRSV NSP1α inhibits the function of TRIM25 to promote viral infection and provides new theoretical basis for the prevention and control of PRRSV.

Materials and methods: Coimmunoprecipitation (Co-IP) assay detected that NSP1α regulated ISGylation of TRIM25. Real-time PCR detected that antiviral function and regulation of interferon signaling pathway of TRIM25 was influenced by NSP1α. Luciferase reporter gene assay revealed that TRIM25-mediated IFN-β promoter activity regulated by NSP1α. Then, we constructed the key active sites inactivation mutant NSP1α (C76S, H146Y). Western blotting and real-time PCR assay detected that NSP1α and NSP1α (C76S, H146Y) influenced the protein and transcription levels of TRIM25. Western blotting detected that degradation pathway of TRIM25 regulated by NSP1α after treated with proteasome inhibitor MG132, and the autophagy inhibitors CQ and Baf A1.

Results: Our study suggests that PRRSV NSP1α decreased ISGylation level of TRIM25 and significantly inhibited TRIM25-medited IFN-β expression to promote PRRSV infection. PRRSV NSP1α. PRRSV NSP1α degraded TRIM25 in proteasome system and did not rely on its papain-like cysteine protease activity.

Discussion: PRRSV is an enveloped positive-sense single-stranded RNA virus which has led to huge economic losses to the global swine industry. PRRSV NSP1α，as a papain-like cysteine protease, inhibits interferon signaling pathway activity to promote PRRSV infection. As an important E3 ubiquitin ligase, TRIM25 catalyzes K63-linked polyubiquitination of retinoic acid-induced gene I (RIG-I) and activates innate immunity response. However, whether PRRSV NSP1α regulates function of TRIM25 is still unknown. Our study suggests that NSP1α degraded TRIM25 to inhibit ISGylation of TRIM25 and interferon signaling pathway. Our study reveals a new function of PRRSV NSP1α in antagonizing host innate immunity, providing a new approach for the prevention and treatment of PRRSV.

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Ursonic acid from medicinal herbs inhibits PRRSV replication through activation of the innate immune response by targeting the phosphatase PTPN1

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Abstract: Porcine reproductive and respiratory syndrome (PRRS), caused by the PRRS virus (PRRSV), has caused substantial economic losses to the global swine industry due to the lack of effective commercial vaccines and drugs. There is an urgent need to develop alternative strategies for PRRS prevention and control, such as antiviral drugs. In this study, we identified ursonic acid (UNA), a natural pentacyclic triterpenoid from medicinal herbs, as a novel drug with anti-PRRSV activity in vitro. Mechanistically, a time-of-addition assay revealed that UNA inhibited PRRSV replication when it was added before, at the same time as, and after PRRSV infection was induced. Compound target prediction and molecular docking analysis suggested that UNA interacts with the active pocket of PTPN1, which was further confirmed by a target protein interference assay and phosphatase activity assay. Furthermore, UNA inhibited PRRSV replication by targeting PTPN1, which inhibited IFN-β production. In addition, UNA displayed antiviral activity against porcine epidemic diarrhoea virus (PEDV) and Seneca virus A (SVA) replication in vitro. These findings will be helpful for developing novel prophylactic and therapeutic agents against PRRS and other swine virus infections.

Keywords: Ursonic acid (UNA), PRRSV, protein tyrosine phosphatase nonreceptor type 1 (PTPN1), antivirals

PRRSV GP4 targets PINK1 to induce mitochondrial autophagy for self-replication

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the pathogens that seriously jeopardize the development of the world pig industry. PRRSV structural proteins are still unknown for the molecular mechanism of autophagy induced by PRRSV. This thesis explores the effects of structural protein GP4 and its interacting host proteins on the mitochondrial autophagy mechanism to provide a basis for understanding and elucidating the autophagy and immune escape mechanisms of PRRSV.

Materials and Methods

The effect of PRRSV infection on cellular autophagy was verified by WB, laser confocal and transmission electron microscopy. The viral protein GP4 was screened by WB, laser confocal and Co-IP assays, and the mitochondrial autophagy protein PINK1, which interacts with the viral protein GP4, was identified by transmission electron microscopy, WB, laser confocal and Co-IP assays, and the interaction region of GP4 protein with PINK1 was identified by laser confocal and Co-IP assays. Subsequently, the effect of PINK1 on the replication capacity of PRRSV after silencing PINK1 on PRRSV-infected Marc-145 cells was examined by WB, qPCR, IFA and viral titer.

Results

The results showed that PRRSV infection induced autophagy in Marc-145 and Pam cells. Transmission electron microscopy showed that GP4 formed autophagic vesicle formation wrapped around mitochondria. Overexpression of GP4 resulted in the reduction of mitochondria-associated proteins TOMM20 and TIM23, and the subcellular localization of GP4 to mitochondria and lysosomes, suggesting that GP4 induces mitochondrial autophagy. The interaction of GP4 with PINK1 was also demonstrated. Silencing of PINK1 on Marc-145 cells significantly reduced GP4-induced mitochondrial autophagy, suggesting that PINK1 plays an important role in GP4-induced mitochondrial autophagy.

Conclusions and Discussion

In this study, we confirmed that the PRRSV structural protein GP4 was able to target PINK1 to induce mitochondrial autophagy, thereby promoting PRRSV replication. Silencing PINK1 inhibits PRRSV replication. This study reveals the mechanism of action of PRRSV GP4 targeting PINK1 to induce mitochondrial autophagy to regulate PRRSV replication, and reveals the molecular mechanism of PRRSV GP4-induced autophagy.

Keywords: Porcine reproductive and respiratory syndrome virus; Structural protein GP4;Mitophagy

PRRSV Nsp2 affects cell autophagy to regulate PRRSV replication

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Introduction

Nsp2 interacts with various host proteins to regulate the transcription and replication of the PRRSV genome, as well as multiple host antiviral responses. This study found that Nsp2 induces cell autophagy and identified host protein, STX17 that interact with Nsp2. The role of these interactions during viral infection was then investigated to explore how Nsp2 influences viral replication by regulating autophagy. This research provides new theoretical insights into the mechanisms of PRRSV replication.

Materials and Methods

Laser confocal microscopy and Western Blot (WB) were used to validate Nsp2-induced autophagy. Laser confocal microscopy and Co-IP were employed to screen for autophagy-related proteins STX17 that interact with Nsp2, and their interaction domains were identified. Additionally, SiRNA was synthesized to knock down the expression of the STX17 gene. MARC-145 cells were then overexpressed or silenced for STX17 and infected with PRRSV. Virus supernatant and cellular proteins were collected at different time points, and WB, qPCR, and virus titration were performed to detect the impact of STX17 on viral replication. Subsequently, the effects of Nsp2 overexpression or PRRSV infection on STX17 expression levels and cellular localization were investigated to elucidate the specific mechanism by which Nsp2 downregulates STX17.

Results

The results showed that Nsp2 induces incomplete cell autophagy and interacts with STX17. The 989-1166 amino acids (aa) region of Nsp2 interacts with the 124-166 aa region of STX17. Overexpression of STX17 in cells infected with PRRSV resulted in significantly reduced expression levels of the N protein and lower progeny virus titers compared to the control group. PRRSV infection and Nsp2 overexpression decrease in its protein expression levels and hindered the interaction between STX17 and SNAP29.

Discussion

This study demonstrated that Nsp2 interacts with STX17 and downregulates its expression, while also hindering the interaction between STX17 and SNAP29. This leads to incomplete autophagy in cells, which in turn impacts virus replication. The research reveals the mechanism by which Nsp2 influences PRRSV replication through the regulation of autophagy, providing new theoretical insights into the replication mechanism of PRRSV.

Keywords: Porcine Reproductive and Respiratory Syndrome Virus; Viral Protein; Autophagy; SNARE Complex

Host cells reprogram lipid droplet synthesis through YY1 to resist PRRSV infection

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Introduction

PRRSV has complex biological characteristics such as high variability, strain diversity and immunosuppression, which makes it unable to be effectively controlled by existing vaccines. Understanding the molecular mechanisms of host resistance to PRRSV infection is necessary to develop safe and effective strategies to control PRRSV. We found that host transcription factor YY1 was up-regulated after PRRSV infection and inhibited PRRSV replication, suggesting that YY1 is a limiting factor during PRRSV infection, but the regulatory mechanism is unknown. This study aims to explore the mechanism of YY1 inhibiting PRRSV replication, and provide theories for exploring new and effective PRRSV prevention and control targets.

Materials and Methods

The antiviral function of YY1 was further demonstrated by in vitro and in vivo. The effect of YY1 on intracellular lipid droplet (LD) synthesis induced by PRRSV infection was investigated by oil red O staining. The lipid metabolism genes regulated by YY1 were identified and the mechanism of YY1 inhibiting PRRSV replication by reprogramming LD synthesis through this gene was clarified.

Results

YY1 reprograms the synthesis of intracellular LDs after PRRSV infection, exerts anti-PRRSV function by negatively regulating FASN-mediated fatty acid synthesis and positively regulating PPARγ-mediated LD synthesis pathway.

Conclusions and Discussion

We elucidated the mechanism by which YY1 inhibits PRRSV replication through reprogrammed LD synthesis. Previous studies have found that PRRSV induces lipophagy and releases fatty acids for viral replication. The puzzling phenomenon is the increase of intracellular LDs after PRRSV infection, and Our study provides a possible explanation. Suppose the increased of LDs is the result of active induction by PRRSV, it can directly hijack the fatty acids synthesized by LDs for its replication rather than inducing synthesis and subsequent lipolysis. Increasing LD synthesis may be a measure for the host to resist viral infection, but the virus will evolve corresponding measures to induce LD lipolysis into fatty acids to supply energy for viral replication, which may be the coexistence of resistance and utilization between LD and virus.

Keywords: PRRSV, YY1; reprogram; lipid droplet; antiviral

Molecular Mechanisms of PRRSV Replicase Membrane Proteins nsp2 and nsp3 in Recruiting Core Replicases for RTC Assembly

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded positive-sense RNA virus that primarily induces reproductive disorders in sows and respiratory diseases in piglets and fattening pigs. Positive-sense RNA viruses interact with the host to form viral transcription and replication complexes (RTCs) in the cytoplasm of host cells, which are essential for viral RNA synthesis. PRRSV non-structural proteins are the principal components of RTC assembly; however, the molecular mechanisms by which these non-structural proteins interact to form RTCs remain unclear. This study investigates the interactions between PRRSV replicase membrane proteins nsp2 and nsp3 and core replicases nsp9 and nsp10, providing a theoretical foundation for the assembly mechanism of PRRSV RTCs.

Materials and Methods

Passage cell lines such as BHK-21 and MARC-145, the highly pathogenic PRRSV strain JXwn06, and expression plasmids for relevant non-structural proteins are all maintained in our laboratory. Techniques such as laser confocal microscopy, co-immunoprecipitation, site-directed mutagenesis, and reverse genetics were employed to investigate the interactions between non-structural proteins and elucidate the molecular basis of replicase membrane proteins recruiting core replicases.

Results

When transfected individually, PRRSV nsp2 and nsp3 exhibit a diffuse distribution in the cytoplasm. However, when co-transfected, nsp2 and nsp3 form distinct punctate co-localizations in the cytoplasm. The full-length nsp9 does not interact with nsp2 and nsp3, but the truncated expression of the nsp9 RdRp domain can be recruited by nsp2 and nsp3. Furthermore, co-transfection of nsp2 and nsp3 significantly enhances the efficiency of recruiting the nsp9 RdRp domain, indicating that the interaction between nsp2 and nsp3 is a critical step for recruiting the core replicase. Co-transfection experiments to investigate the interaction mechanisms among the three proteins revealed the following: 1. Nsp2 and nsp3 primarily interact through their transmembrane domains (TMD). 2. Both nsp2 and nsp3 recruit nsp9 through their C-terminal extracellular domains. 3. Overexpression of the C-terminal extracellular domain of nsp2 significantly inhibits PRRSV replication, suggesting that the presence of this peptide may interfere with the proper assembly of the viral RTC. 4. The R210 site of nsp3 is critical for its interaction with nsp9, and mutation of this site significantly inhibits the interaction between nsp3 and the nsp9 RdRp region. Further investigation into the interaction between polymerase nsp9 and helicase nsp10 revealed that deletion of the linker regions of nsp9 and nsp10 results in their translocation from the nucleus to the cytoplasm and co-localization, indicating that their interaction involves conformational regulation.

Conclusions and Discussion

PRRSV non-structural proteins nsp2 and nsp3 are critical membrane proteins responsible for remodeling the host cytoplasmic membrane and recruiting other viral proteins and host factors to form the RTC. This study found that the interaction between PRRSV nsp2 and nsp3 is a crucial step in recruiting the core viral replicase to the RTC. The process by which nsp2 and nsp3 recruit the core replicase, as well as the interactions among other non-structural proteins, is regulated. Our research reveals the key molecular mechanisms involved in the assembly of the PRRSV RTC, providing a theoretical foundation for developing effective antiviral drugs. However, the precise assembly steps require further investigation.

Keywords: PRRSV, Viral Transcription and Replication Complex (RTC), Membrane Proteins, Conformational Regulation

Proceedings

Minor envelope proteins from GP2a to GP4 contribute to the spread pattern and yield of type 2 PRRSV in MARC-145 cells

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Introduction

In China, porcine reproductive and respiratory syndrome virus (PRRSV) vaccines are widely used. These vaccines, which contain inactivated and live attenuated vaccines (LAVs), are mainly produced by MARC-145 cells derived from the monkey kidney cell line. However, some PRRSV strains in MARC-145 cells have a low yield. Here, we used two type 2 PRRSV strains (CH-1R and HuN4) to identify the genes responsible for virus yield in MARC-145 cells.

Materials and Methods

In this study, we evaluated the virus spreads and replicates of wo different strains of type 2 PRRSV in MARC-145 cells using growth kinetics assay, immunofluorescence assay, transwell coculture system, and drug inhibition assay. Then, using a viral reverse genetics platform, we created a series of new chimeric strains and evaluated their spreading patterns and yields in MARC-145 cells.

Results

The results showed that the yield of CH-1R in MARC-145 was significantly higher than that of HuN4. In addition, HuN4 and CH-1R strains showed different diffusion patterns on MARC-145 cells. To further identify the key genes that influence PRRSV spreading patterns in MARC-145 cells using a viral reverse genetics platform. We found that secondary envelope proteins GP2a to GP4 were able to determine viral production and spreading patterns in MARC-145 cells.

Conclusions and Discussion

In this study, we found significant differences in the replication levels of CH-1R and HuN4 in marc-145 cells due to their different modes of propagation in marc-145 cells. By replacing the viral envelope genes with a reverse genetics system, we discovered that the minor envelope proteins, from GP2a to GP4, play a crucial role in determining the spread pattern and yield of type 2 PRRSV in MARC-145 cells. Overall, our findings provide new insight into the lifecycle of PRRSV and will be helpful for PRRSV vaccine development.

Keywords: PRRSV, spread pattern, yield

PRRSV Infection Induces Polarization of M1 Porcine Alveolar Macrophages Promoting Virus Replication by Caspase-1 mediated pyroptosis pathway

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an economically important pathogen that affects the global swine industry. Our preliminary research found that porcine alveolar macrophages (PAMs) targeted cells for PRRSV infection can be induced into M1 polarization and promote virus proliferation in the early infection stages, but the underlying mechanism have not been well clarified. The aim of this paper was to study the effects and mechanisms of PRRSV infection on macrophage polarization and virus proliferation,which may be conducive to providing treatment methods for PRRSV infection.

Materials and Methods

PRRSV infected PAMs with different multiplicity of infection (MOI), and the viral replication and polarization types at different time points were detected. Additionally, the indicators related to Caspase-1 mediated cell apoptosis have also been tested. Furtherly, the key markers of cell apoptosis, caspase-1 and GSDMD were inhibited using special inhibitors and siRNA respectively, the viral titers and polarization markers in different treatment groups were also detected through qPCR, ELISA and Western Blottingting assay, to explore the effects of cell apoptosis on macrophage polarization and PRRSV virus proliferation.

Results

PRRSV infection could cause PAMs to produce CPE with cell shrinkage or swelling, lysis, and increased cell debris, resulting in M0 to M1polarization. The results of LDH and PI staining showed that PRRSV infection could also induces pyroptosis of PAMs, exhibiting time-dependent and dose-dependent phenomena.Further studies confirmed that cell pyroptosis was activated by caspase-1 mediated classical pathway. VX-765 and siRNA could downregulate the expression level of caspase-1 protein in PAMs cells after infection and inhibit the proliferation of PRRSV.

Conclusions and Discussion

PRRSV infection can induces polarization of M1 PAM and cell pyroptosis, which can affect the proliferation of PRRSV. The viral titer in cell culture supernatant is positively correlated with the expression of caspase-1 protein.

Keywords: PRRSV; M1 Polarization; cell pyroptosis; Replication

miR-451-targeted PSMB8 promotes PRRSV infection by degrading IRF3

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Abstract: Porcine respiratory and reproductive syndrome (PRRS) is one of the most devastating infectious diseases of pigs, causing reproductive failures in sows and severe respiratory symptoms in piglets and growing pigs. MicroRNAs (miRNAs) are reported to play an essential role in virus-host interaction. Here, we demonstrated that miR-451 enhanced type I interferon (IFN-I) production through targeting proteasome subunit β8 (PSMB8), therefore restricting PRRS virus (PRRSV) replication. We showed that the expression of PSMB8 was up-regulated by PRRSV infection, and knockdown of PSMB8 inhibited PRRSV replication by promoting IFN-I production. Moreover, we demonstrated that PSMB8 interacted with the regulatory domain of IRF3 to mediate K48-linked polyubiquitination and degradation of IRF3. Our findings reveal that miR-451 is a negative regulator of PRRSV replication. And importantly, we show that PSMB8, as a target gene of miR-451, negatively regulates IFN-I production by promoting IRF3 degradation, which is a previously unknown mechanism for PSMB8 to modulate innate immune responses.

Keywords: PRRSV; miR-451; PSMB8; type I interferon; IRF3

Pathogenesis

Oral

PDCD4 restricts PRRSV replication in an eIF4A-dependent manner and is antagonized by the viral nonstructural protein 9

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Abstract: As obligate parasites, viruses have evolved multiple strategies to evade the host immune defense. Manipulation of the host proteasome system to degrade specific detrimental factors is a common viral countermeasure. To identify host proteins targeted for proteasomal degradation by porcine reproductive and respiratory syndrome virus (PRRSV), we conducted a quantitative proteomics screen of PRRSV-infected Marc-145 cells under the treatment with proteasome inhibitor MG132. The data revealed that the expression levels of programmed cell death 4 (PDCD4) were strongly downregulated by PRRSV and significantly rescued by MG132. Further investigation confirmed that PRRSV infection induced the translocation of PDCD4 from the nucleus to the cytoplasm, and the viral nonstructural protein 9 (Nsp9) promoted PDCD4 proteasomal degradation in the cytoplasm by activating the Akt-mTOR-S6K1 pathway. The C-terminal domain of Nsp9 was responsible for PDCD4 degradation. As for the role of PDCD4 during PRRSV infection, we demonstrated that PDCD4 knockdown favored viral replication, while its overexpression significantly attenuated replication, suggesting that PDCD4 acts as a restriction factor for PRRSV. Mechanistically, we discovered eukaryotic translation initiation factor 4A (eIF4A) was required for PRRSV. PDCD4 interacted with eIF4A through four sites (E249, D253, D414, and D418) within its two MA3 domains, disrupting eIF4A-mediated translation initiation in the 5'-untranslated region of PRRSV, thereby inhibiting PRRSV infection. Together, our study reveals the antiviral function of PDCD4 and the viral strategy to antagonize PDCD4. These results will contribute to our understanding of the immune evasion strategies employed by PRRSV and offer valuable insights for developing new antiviral targets.

Keywords: Nsp9; PDCD4; PRRSV; eIF4A

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Pathogenicity of Chinese NADC34-like PRRSV and commercial PRRSV vaccine efficacy against its infection

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NADC34 PRRSV was initially reported in the United States in 2018. Subsequently, this virus strain spread to other countries, including Peru, South Korea, and China. The virus was first found circulating in Northeast China and then spread to more than 10 provinces in China. Our nationwide porcine reproductive and respiratory syndrome virus (PRRSV) molecular surveillance results indicated that NADC34-like PRRSV has been prevalent in China since 2018 and became one of the main epidemic strains in some areas of China. One NADC34-like PRRSV strain designated JS2021NADC34 PRRSV, which shares the highest similarity with IA/2014/NADC34, a PRRSV strain originated from Iowa State in 2014, has been successfully isolated and further characterized. Genetically, JS2021NADC34 strain had a continuous 100 aa depletion in NSP2, as compared to VR-2332 strain, and had no recombination with other domestic strains. The pathogenicity of JS2021NADC34 PRRSV has been evaluated on pigs, and clinical symptoms, gross pathology and histopathological results confirmed the high pathogenicity of this virus strain with more than 40% mortality to different ages of pigs. Pigs infected with this virus had lasting fever and reduced body weight with high morbidity and mortality. Histopathological changes, including interstitial pneumonia, lymphocyte depletion, acute hemorrhage, and infiltration of neutrophils in the lymphoid tissues, were observed with the viral proteins detected by immunohistochemistry staining using PRRSV-specific antibody.

The commercial PRRSV vaccines were next tested for the protection to JS2021NADC34 PRRSV infection. After viral challenge, the vaccinated pigs had low level of viremia but suffered pathological lesions in lungs and lymphoid tissues. The viral antigens were also detected in the above tissues of the vaccinated pigs by immunohistochemistry staining. One out of five pigs in vaccinated group died at 13 days post-challenge. The above results suggested that the commercial PRRSV vaccine could not provide complete protection to the NADC34-like PRRSV infection. Therefore, the geographical spread of NADC34-like PRRSV and economic losses caused by this PRRSV strain to Chinese pig industry warrants high attention.

Key Words: NADC34-like PRRSV, Pathogenicity, Vaccine efficacy

The 5'UTR of porcine reproductive and respiratory syndrome virus strain JXwn06 harbors an uORF that regulates cellular inflammation

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Introduction

The porcine reproductive and respiratory syndrome virus (PRRSV) stands as a significant threat to the pig-raising industry worldwide, including China. Currently, there is an urgent lack of a definitive and effective vaccine or antiviral medication to combat this virus. The 5'UTR region of positive-strand RNA viruses harbors functional regulatory elements that are crucial in regulating host gene expression and protein synthesis. Nevertheless, the specific role these elements play in modulating viral translation and virulence remains unclear. To address this gap, our study leverages advanced bioinformatics techniques to identify and characterize these functional regulatory elements. We further combine in vitro reporter gene systems and reverse genetics approaches to comprehensively examine the potential role of these elements in regulating viral translation and virulence, both in vitro and in vivo. This innovative approach offers profound insights into the intricate interplay between the virus and its host, ultimately advancing our understanding of PRRSV's biology and pathogenicity.

Materials and methods

This study employed HP-PRRSV JXwn06 as a model pathogen to investigate the conservation of uORFs within the Arteriviridae family through rigorous bioinformatics analysis. Subsequently, the functionality of these uORFs was identified utilizing an in vitro reporter gene system. Finally, to evaluate the pathogenicity of the PRRSV uORF mutant viruses, a reverse genetics system was implemented, and one-month-old SPF piglets served as the animal infection model for comprehensive analysis.

Results

In the current study, we have conclusively shown the existence of uORFs within the 5'UTR of PRRSV, emphasizing their notable conservation within the Arteriviridae family. Leveraging in vitro reporter gene analysis and reverse genetics techniques, we discovered that the uORF in PRRSV JXwn06, particularly uORF2, functions not only to repress the expression of the downstream primary open reading frame but also exhibits coding potential, serving as a novel viral modulator that regulates cellular inflammation and viral pathogenicity.

Discussion

Viruses hijack the host's translational machinery and adjust their gene expression patterns based on intracellular and extracellular stimuli, relying on precise regulation of gene expression. Such regulatory mechanisms are actually controlled by specific elements in the 5'UTR of certain viral transcripts. This study not only reveals the conservation and function of the uORF in the 5'UTR region of PRRSV but also emphasizes the importance of uORF2 encoding a trans-acting factor. Through cellular experiments and validation in animal models, we have gradually deepened our understanding of the key role of uORF2 in regulating the host's immune response and viral pathogenicity. These findings are of utmost significance for further analyzing the biological characteristics and pathogenic mechanisms of PRRSV, as well as for vaccine development.

Posters

Recombination pattern and pathogenicity of the epidemic dominant NADC30-like strains in southern China

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Introduction

Whole-genome sequencing of viruses is crucial for tracking mutation trends and fostering vaccine development. This study seeks to illuminate the genetic diversity and pathogenicity of clinically recombinant PRRSV isolates, unravel the intricate patterns of recombination, and provide insights for effective PRRSV management and control strategies.

Materials and Methods

Clinical samples testing positive for PRRSV underwent routine virus isolation using Marc-145 cells culture. Viral RNA was extracted, reverse transcribed, and subjected to whole genome sequencing. Comprehensive genetic analysis included sequencing, phylogenetic studies, and recombination detection. Recombinant strains were identified by comparing recent NCBI database entries with isolates from this study, facilitating a detailed analysis of recombination characteristics. Epidemiologically significant NADC30-like recombinant strains were then used to infect piglets to assess pathogenicity.

Results

We successfully isolated 15 PRRSV strains with various recombinant profiles. Our analysis revealed that 11 strains predominantly emerged from recombination events between lineage 1 and lineage 8, while four involved three to four lineages. We mapped recombination breakpoints, noting that most occurred within the nsp coding regions, especially nsp2 and nsp9, and additional sites in ORF2 and ORF5. Two dominant NADC30-like recombinants, GXNN202004a and GXGG20210301, elicited distinct clinical symptoms and weight loss in piglets, though neither proved fatal. Compared to historical data, both strains exhibited moderate pathogenicity.

Conclusions and Discussion

Our research highlights intricate recombination patterns within PRRSV-2 strains isolated in South China, with NADC30-like recombinants showing significant epidemiological impact and maintaining moderate virulence. Analysis confirmed frequent recombination within nsp1-nsp4, nsp9, and ORF2, aligning with prior discoveries of these regions as recombination hotspots. These findings enhance our comprehension of PRRSV epidemiology, virus replication, and recombination mechanisms, emphasizing the imperative for ongoing surveillance and focused research in these domains.

Keywords: PRRSV isolation, recombination, pathogenicity

PRRSV non-structural protein 5 inhibits antiviral innate immunity by degrading multiple proteins of RLR signaling pathway through FAM134B-mediated ER-phagy

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Introduction

Innate immunity is the first line of host defense against viral infections. Therefore, viruses developed numerous mechanisms to evade the host innate immune responses for its own benefit. Porcine reproductive and respiratory syndrome virus (PRRSV) is a significant pathogen in swine industry, characterized by reproductive failures in sows and respiratory distress in pigs of all ages, leading to substantial economic losses globally. PRRSV has been reported to be adept at exploiting both its structural proteins and non-structural proteins (Nsps) to antagonize various facets of the host antiviral response. Nevertheless, the precise mechanism still needs to be fully elucidated. Here, the role of Nsp5 in modulating innate immune responses, and the precise mechanisms underlying its actions are investigated.

Materials and Methods

The conventional experimental methods, involving in molecular biology, virology and immunology methods, including molecular cloning, Quantitative PCR, luciferase reporter assays, ELISA, confocal, western blot (WB), co-immunoprecipitation (Co-IP), transmission electron microscope (TEM) were used to explore the host cell proteins targeted by Nsp5, the signaling pathways, effector molecules, and the modes of action involved.

Results

In this study, we found that PRRSV strain HN07-1 Nsp5 suppresses IFN-I and IFN-stimulated genes (ISGs) by degrading multiple proteins in the RLR signaling pathway (RIG-I, MDA5, MAVS, TBK1, IRF3, IRF7) via the autophagy-lysosome pathway. Further research indicated that Nsp5 located in the endoplasmic reticulum (ER), promotes the accumulation and degradation of RLR signaling pathway proteins via ER-phagy. Additionally, one of the six identified ER-phagy receptors, FAM134B, was found to be responsible for Nsp5-induced ER-phagy. Nsp5 interacts with FAM134B to promote FAM134B oligomerization, which is essential for ER fragmentation and subsequent ER-phagy activation.

Conclusions

This study elucidates a novel function of Nsp5 and a newly discovered strategy by which PRRSV utilizes FAM134B-mediated ER-phagy to counteract host antiviral immunity, deepening our understanding of evasion mechanisms of viruses and provide further insights into the prevention and control of PRRSV.

Keywords

PRRSV; ER-phagy; IFN-I; FAM134B; Nsp5

Hypoxia inducible factor-1 alpha regulates porcine reproductive and respiratory syndrome virus induced inflammation of porcine alveolar macrophages

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Introduction

PRRSV infection leads to the pulmonary inflammatory injury characterized by the massive release of pro-inflammatory factors. The objective of this abstract is to explore the roles and mechanism of HIF-1α in PRRSV-induced inflammation responses in porcine alveolar macrophages.

Materials and Methods

Established a model of PRRSV-infected porcine alveolar macrophages, western blotting and real-time, fluorescence-based quantitative PCR were used to evaluate the effects of PRRSV on the mRNA and protein expression of HIF-1α in the cells and to determine the changes of TNF-α and IL-6 after HIF-1α inhibition. After cells were treated with mTOR or HIF-1α activator, the levels of cytokines and mTOR signaling in the cells were determined. The regulatory effect of mTOR on HIF-1α and the effect of mTOR complex 1 (mTORC1) on PRRSV-induced HIF-1α regulation were also detected by western blotting.

Results

The results showed that PRRSV infection elevated HIF-1α expression. HIF-1α inhibitor BAY87-2243 was shown to inhibit PRRSV-induced mRNA expression of TNF-α and IL-6, and BAY87-2243 didn't affected PRRSV replication. mTOR inhibitor rapamycin was shown to inhibit PRRSV-induced mRNA expression of tumor necrosis factor-α and interleukin 6, mTOR phosphorylation levels and HIF-1α elicitation. Anti-inflammatory effect of rapamycin was reversed by HIF-1α activator DMOG. We demonstrated that constitutive mTORC1 activation via TSC2 loss leads to increased activity of HIF-1α. The results showed that mTORC1 overexpression could enhance HIF-1α expression induced by PRRSV. Similarly, knockdown of mTORC1 significantly decreased HIF-1α expression induced by PRRSV.

Conclusions and Discussion

These results indicate that HIF-1α facilitates PRRSV-induced inflammation and that mTORC1 plays a vital role in enhancing the expression and stabilization of HIF-1α.

Keywords: PRRSV, HIF-1α, inflammation

Research on iron metabolism regulation and ferroptosis mechanism during PRRSV infection

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly variable immunosuppressive virus. Pathogens can induce ferroptosis in host cells, impacting the host's immune response levels, and promoting their proliferation or evasion from host immune surveillance. This study investigates the novel mechanism by which PRRSV infection disrupts host cell iron metabolism and leads to ferroptosis, providing a new perspective for the prevention and treatment of PRRSV.

Materials and Methods

Transcriptome sequencing analysis of PRRSV-infected cells revealed a significant upregulation of the iron metabolism gene STEAP3. Fluorescence imaging of Fe^{2+} in live cells was performed using FerroOrange, while the fluorescent probes DCFH-DA was used to detect host cell ROS and lipid peroxidation, characterizing the level of ferroptosis in host cells. The role and pathways of STEAP3 in ferroptosis were further explored through experiments such as STEAP3 overexpression, knockdown, mass spectrometry, and immunofluorescence.

Results

Transcriptome data analysis revealed a significant upregulation of the iron reductase STEAP3 after PRRSV infection, accompanied by an increase in intracellular Fe^{2+} levels, leading to ferroptosis in cells and inhibition of PRRSV proliferation. Mass spectrometry data analysis revealed an interaction between STEAP3 and the STING protein. After PRRSV infection, the co-localization of STING and STEAP3 was enhanced, accompanied by an increase in the endoplasmic reticulum localization of STEAP3. The STING protein modulated the oligomerization of STEAP3, leading to increased iron reductase activity of STEAP3 protein and elevation of host ferroptosis levels.

Conclusions and Discussion

This study elucidates the significant role and pathways of STEAP3 in the disruption of host cell iron metabolism and ferroptosis following PRRSV infection, providing new insights for the defense and treatment of PRRSV from the perspective of ferroptosis.

Keywords: PRRSV, Ferroptosis, STEAP3

LGP2 regulates PRRSV infection via enhancing MDA5-mediated signaling

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Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important pathogens in the global pig industry, which modulates the host's innate antiviral immunity to achieve immune evasion. RIG-I-like receptors (RLRs) sense viral RNA and activate the interferon signaling pathway. LGP2, a member of the RLR family, plays an important role in regulating innate immunity. However, the role of LGP2 in virus infection is controversial. Whether LGP2 has a role during infection with PRRSV remains unclear. Here, we found that LGP2 overexpression restrained the replication of PRRSV, while LGP2 silencing facilitated PRRSV replication. LGP2 was prone to interact with MDA5 and enhanced viral RNA enrichment and recognition by MDA5, thus promoting the activation of RIG-I/IRF3 and NF-κB signaling pathways and reinforcing the expression of proinflammatory cytokines and type I interferon during PRRSV infection. Meanwhile, there was a decreased protein expression of LGP2 upon PRRSV infection *in vitro*. PRRSV Nsp1 and Nsp2 interacted with LGP2 and promoted ubiquitination of LGP2, resulting in degradation of LGP2. Furthermore, Nsp1 and Nsp2 attenuated the interaction between LGP2 and MDA5 following PRRSV infection, and even decreased the recognition and recruitment of viral RNA by MDA5, ultimately leading to a block of antiviral immunity mediated by MDA5 and LGP2. These findings indicate that LGP2 plays a role in regulating PRRSV replication through synergistic interaction with MDA5. Moreover, targeting LGP2 is responsible for PRRSV immune evasion. Our work describes a novel mechanism of virus-host interaction and provides the basis for preventing and controlling PRRSV.

Research on the molecular mechanism of PRRSV-induced lipid droplet accumulation

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease in pigs caused by the PRRS virus (PRRSV). Lipid metabolism and lipid droplets (LDs) are important in the infection of various viruses, and there have been few reports of PRRSV infection inducing LDs accumulation. This study selected the PRRSV Nsp4 and the host protein ATGL that it regulates, and further studied the functions of this interaction in the virus infection process and explored the way in which PRRSV Nsp4 regulates ATGL.

Materials and Methods

Marc-145 cells were infected with EGFP-PRRSV, and the effect of PRRSV on LDs accumulation was observed by laser confocal microscopy. The changes in lipid metabolic proteins induced by PRRSV infection and overexpression of various PRRSV proteins were detected by western blotting, and it was observed that PRRSV Nsp4 downregulated the expression of ATGL. According to the pig PNPLA2 gene sequence, an ATGL target fragment was synthesized, we constructed a eukaryotic expression plasmid . The interaction between PRRSV Nsp4 and ATGL was further confirmed by western blotting, laser confocal microscopy, and Co-IP. Finally, the specific mechanism by which PRRSV Nsp4 regulates ATGL was explored.

Results

The research results showed that PRRSV infection could induce lipid droplets accumulation and downregulate the expression of ATGL. Overexpression of various PRRSV proteins showed that PRRSV Nsp2, Nsp4, Nsp5, and GP5 could downregulate the expression of ATGL. Further, laser confocal microscopy, western blotting, and Co-IP revealed that PRRSV Nsp4 had a significant downregulatory effect on the expression of ATGL. Finally, the autophagy inhibitor 3-MA could reduce the downregulation of ATGL expression by PRRSV Nsp4. Additionally, the qPCR results showed that the mRNA level of ATGL was downregulated after PRRSV infection.

Conclusions and Discussion

This study confirmed that PRRSV infection caused lipid droplets accumulation, further confirming that PRRSV Nsp4 significantly downregulated the expression of ATGL and explored the specific mechanism by which it did so. This study reveals the specific mechanism by which PRRSV causes lipid droplets accumulation, which is beneficial for the discovery of new antiviral drugs and therapeutic targets.

Keywords: Swine reproductive and respiratory syndrome virus, lipid droplets accumulation, downregulation

Mechanism of Action of APOBEC3F in PRRSV Infection

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Introduction

PRRSV causes significant economic losses to the global pig industry. APOBEC3F, a member of the APOBEC family, is a crucial component of P-body. Its interaction with PRRSV and the underlying mechanism remain unclear.

This study investigates the interaction between APOBEC3F and PRRSV, analyzing APOBEC3F expression in various pig tissues. It explores the interaction in PAM CD163 and Marc145 cells to clarify APOBEC3F's functional structure against PRRSV. The aim is to establish the interaction mechanism between APOBEC3F and PRRSV virus proteins.

Materials and Methods

Test materials include African green monkey embryonic kidney cells (Marc145 cells), PAM CD163 cell line extension, PRRSV BJ3 strains, DH5 alpha cells, and eukaryotic expression vectors pCMV-Myc and pcDNA3.1-5'flag-pAPOBEC3F. qPCR was used to detect APOBEC3F mRNA expression in pig tissues. Plasmids for APOBEC3F domains and PRRSV proteins were constructed. qPCR, Western blot, IFA, overexpression, and knockdown were used to analyze APOBEC3F transcription and translation in PRRSV-infected cells. The role of APOBEC3F in inhibiting PRRSV proliferation and its functional structure domain were explored. IP-MS and confocal laser techniques were used to analyze APOBEC3F-PRRSV protein interactions.

Results and Discussion

Experimental research results showed APOBEC3F widely expressed in pigs, highest in lymph nodes and spleen. Successfully constructed 11 PRRSV nonstructural protein eukaryotic expression vectors. Tested APOBEC3F function domain structure in PAM-CD163 and Marc-145 cells, infected with PRRSV, analyzed APOBEC3F's effect on PRRSV replication. Full-length APOBEC3F and its CD2 domain downregulated PRRSV nucleocapsid N protein transcription and translation. NSP1 alpha, beta, NSP5, NSP1, NSP7, GP4, GP5, and N protein in PAM-CD163 and Marc-145 cells reduced intracellular APOBEC3F mRNA levels. Viral proteins interacted with APOBEC3F in the cytoplasm. Mass spectrometry analysis revealed direct interaction between APOBEC3F and DDX6, MOV10, COX2, ATP5P0, suggesting APOBEC3F may cooperate with these molecules to resist PRRSV. CD2 domain at APOBEC3F's C-terminal was the main functional domain for inhibiting PRRSV proliferation. Results provide new insights into APOBEC3F's mechanism against PRRSV.

Keywords: PRRSV,APOBEC3F,Control

Mechanism of action of helicase DDX6 in PRRSV infection

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) can cause serious health issues and economic losses in pregnant sows. DEAD-box proteins regulate antiviral immunity. However, research on DDX6's role in PRRSV replication is limited. This study aims to investigate the interaction between DDX6 and PRRSV during replication.

Materials and Methods

Experimental materials included African green monkey embryonic kidney cells (Marc145), PAM-CD163 cell line, PRRSV-BJ3 strain, DH5α cells, and eukaryotic expression vectors pCMV-Myc and pCMV-HA.

T clone technology created eukaryotic expression vectors targeting the DDX6 genetic encoding region from congjiang pigs and Marc145 cells.

Real-time PCR, Western blot, and knockdown techniques were used to assess DDX6 transcription and translation levels in congjiang pig organs after PRRSV infection and the impact of DDX6 on PRRSV N gene transcription and translation.

The effect of overexpressing PRRSV viral proteins on DDX6 mRNA levels was also studied.

Laser confocal microscopy and co-immunoprecipitation-mass spectrometry identified the interaction between viral proteins and DDX6.

Conclusions and Discussion

In this study, we obtained conserved DDX6 coding region sequences from Congjiang pigs and Marc145 cells. DDX6 was expressed in various tissues of pigs, with higher mRNA levels in lymph nodes and lower in skeletal muscle.

After PRRSV infection in Marc145 and PAM-CD163 cells, DDX6 transcription and translation levels decreased. Overexpression of DDX6 inhibited PRRSV proliferation, while knocking down DDX6 up-regulated PRRSV capsid protein N gene expression. This relationship was dose-dependent.

Using Co-IP and LC-MS/MS, we found PRRSV nucleocapsid protein N might interact with DDX6. Direct interaction was observed through Co-IP and confocal fluorescence tests in HEK293 cells.

This study demonstrates PRRSV interacts with DDX6 in infected cells, and PRRSV nucleocapsid protein has a direct relationship with DDX6. These findings provide a new perspective for exploring PRRSV pathogenesis and immune escape mechanisms.

Keywords: PRRSV, DDX6, N-protein, Innate immunity, Protein interaction

Proceedings

Pathogenesis, clinical symptoms and prevention of porcine reproductive and respiratory syndrome

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Abstract: Porcine reproductive and respiratory syndrome (PRRS), a highly contagious viral disease, has rapidly spread to pig-raising countries around the world , causing significant economic losses to the global pig industry. The porcine reproductive and respiratory syndrome virus (PRRSV) is an important virus in the global pig industry that often establishes long-term infections through a variety of complex mechanisms, which is one of the biggest obstacles to controlling the related disease PRRS. This disease is characterized by reproductive disorders and respiratory symptoms, posing a serious threat to the health and production performance of pig herds. In recent years, with the rapid development of the pig industry and the increase in the degree of scale, porcine reproductive and respiratory syndrome has shown a clear trend of high incidence in China, which has become one of the important challenges facing the pig industry in China. Porcine reproductive and respiratory syndrome is one of the diseases that plague the global pig industry, characterized by reproductive failure in breeding pigs and respiratory distress in pigs of all ages. Each type of PRRS virus, PRRSV, is the pathogen that rapidly spreads on their respective continents and ultimately widely spreads to countries with the largest pork production. PRRSV is an enveloped, positive-sense, single-stranded RNA virus belonging to the Arteriviridae family. PRRSV infection is characterized by poor induction of innate immune responses in the host. The pathogenesis of PRRS involves the invasion of lung macrophages by the virus, causing lung damage and interstitial pneumonia. Virus proliferation leads to macrophage collapse, weakened immune recognition, and susceptibility to comorbidities with other diseases, increasing treatment difficulty and mortality. This makes PRRS difficult to prevent, control, and eliminate. In order to deeply study and explore the pathogenesis of porcine reproductive and respiratory syndrome, this article synthesizes recent research results and practical experience to sort out and analyze the pathogenic characteristics, transmission methods, clinical symptoms, and prevention of porcine reproductive and respiratory syndrome. Suggestions are also proposed to provide theoretical references for the pig industry.

Keywords: PRRS; PRRSV; pathogenesis; clinical symptoms

Symptoms and preventive measures of porcine reproductive and respiratory syndrome

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Abstract： Porcine Reproductive and Respiratory Syndrome (PRRS), also known as porcine blue ear disease, can be transmitted through respiratory tract, digestive tract, blood, placenta, mating, mucous membrane contact and other ways. It can cause reproductive disorders in sows as well as respiratory difficulties in piglets and other symptoms, with a very high lethality rate, which is extremely harmful to the pig breeding industry.PRRS is mainly sensitive to pigs, regardless of any age or breed of pigs can be susceptible, piglets infected with high fever, decreased food intake, abdominal respiration, visible mucous membrane cyanosis, and ultimately died due to asphyxiation; sows infected with the virus mainly manifested as mental instability, shortness of breath, appetite decreased significantly, while the body temperature rose to 40 ℃ and other conditions, if it is the infection of sows at the late stage of gestation, then the occurrence of miscarriages, production of stillborn fetuses, Weak fetus, mummified fetus, etc., mortality rate of up to 80% or more; breeding boars infected with the virus mainly infected testes, resulting in increased sperm mortality, difficult to conceive, or even lose breeding ability; fattening pigs infected with a rapid rise in body temperature, up to 41 ℃ or more, depressed, insensitive to the cycle of stimulation, dorsal, edge of the ear cyanosis and purplish, impaired growth and development.In the process of pig breeding, it is necessary to focus on all-round comprehensive disinfection, and do a good job of environmental hygiene management in the farm, maintain the appropriate temperature and humidity inside the enclosure, and reduce the impact of organic matter in the environment on the disinfectant solution. It is best for farms to do a good job of self-breeding and strictly control the quality of introduced pigs. Farms need to conduct regular tests and isolate and eliminate sick pigs in a timely manner. Farms need to be based on the local epidemic and the actual situation in the farm, can develop a suitable vaccine immunization program, in the vaccination should pay attention to biosafety protection, to prevent the occurrence of cross-infection.

Key words： Porcine Reproductive and Respiratory Syndrome;Mechanisms of infection; Protective measure

Pathological observation of respiratory tract tissue damage in the early stage of HP-PRRSV infection

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Introduction

The infection of highly pathogenic PRRSV can lead to respiratory diseases in piglets. PRRSV can spread through various pathways, with respiratory infection being its primary mode of transmission. Here, we observe mucosal damage and viral distribution in the nasal cavity, pharynx, trachea, and extrapulmonary bronchi of piglets in the early stage of HP-PRRSV infection.

Materials and Methods

SPF piglets, 4-6 weeks of age, were intranasally infected with HP-PRRSV JXwn06 strain. Three days post-infection, the piglets were euthanized and dissected. Paraffin-embedded sections with H&E staining, immunohistochemistry, transmission electron microscopy (TEM) and RT-qPCR were used for morphological and virus detection.

Results

(1) Infected piglets showed elevated body temperature, interstitial pneumonia pathological changes in lung tissues. PRRSV was detected in both lung tissues and blood. (2) H.E. staining results showed serious histopathological lesion. The mucosal lamina propria of the nasal cavity, pharynx, trachea, and extrapulmonary bronchi showed vascular dilation and congestion. The epithelial cells of the mucosa were swollen, necrotic, and sloughed off. The lamina propria of the mucosa exhibited infiltration of inflammatory cells predominantly composed of mononuclear cells. (3) Immunohistochemistry staining results showed that PRRSV positive signals can be seen in epithelial cells, macrophages in the mucosal lamina propria, also in areas near blood vessels and glands. (4) TEM results showed tight junctions damaged in nasal mucosal epithelial cells, swelling and sloughing of endothelial cells in blood vessels. Virus particles were observed in the nasal mucosal epithelium and endothelial cells of blood vessels. (5) RT-qPCR results show that the copy number of PRRSV ORF5 in nasal mucosa is significantly higher than in lung tissue.

Conclusions and Discussion

The findings reveal that in early-stage HP-PRRSV infection, inflammatory pathological damage occurs in nasal, pharyngeal, and tracheal mucosa, all these tissues supporting PRRSV replication. Particularly, nasal mucosa and pharynx may play a crucial role in the process of HP-PRRSV invasion and subsequent respiratory dysfunction.

Keywords: HP-PRRSV, Respiratory tract tissues, Pathological injury

Vaccines

Oral

PRRSV-specific IgM as a novel adjuvant for inactive PRRSV vaccine

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Current strategies for PRRS control are inadequate and mainly restricted to immunization using different PRRSV vaccines. Although the first modified live virus (MLV) vaccine has been commercially available in market for over two decades, the prevalence of PRRSV infection in swine herds remains high. It is also notable that weeks-long viremia of vaccine virus persisted in MLV immunized piglets can lead to transmission of vaccine virus to naive animals, thereby arising the concern that PRRSV-MLVs might revert to virulence or recombine with wild-type fields strains. Conversely, although no safety concern, the poor performance of inactivated PRRSV vaccines (KIVs) has restrict its practical application. PRRSV-KIVs have been only licensed in China and used to be licensed in the United States. However, since 2005, KIV is no longer marketed in the US due to it poor performance. It is believed that the poor protection of PRRSV-KIVs is caused by failure to elicit detectable PRRSV-specific neutralizing antibodies (NA) and cell-mediated immunity (CMI) after KIVs immunization. Therefore, attempts focusing on improvement of PRRSV-KIV performance are required for practical application, such as such as intranasally delivered nanoparticle-entrapped KIV along with poly (lactic-co-glycolic) acid or whole cell lysate of *Mycobacterium tuberculosis* as adjuvant elicits broadly cross-protective anti-PRRSV immunity against heterogeneous PRRSV strain. These reports suggested that special formulation (nanoparticles) combined with novel adjuvants poly (lactic-co-glycolic) acid or lysate of *M. tuberculosis* may enhance immune response evoked by PRRSV-KIVs during immunization. In our previous report, we identified a novel IgM type monoclonal antibody (Mab)-PR5nf1 which is capable to neutralize all tested PRRSV isolates of both *PRRSV-1* and *PRRSV-2 in vitro*. Although IgM is not suitable to apply as a treatment *in vivo* compared with IgG, immunization of mice with a cocktail of inactivated PRRSV virus and Mab-PR5nf1 at a ratio of 1: 10 for twice significantly enhanced cell-mediated immunity, as determined by IFN-γ ELIspot after using recombinant PRRSV-N protein as recalling antigen to stimulate the $CD8^{+}$T cell isolated from immunized mice, suggesting that co-immunization with antigen-specific IgM might be employed as a novel method for PRRSV-KIV immunization. To examine this speculation, the PRRSV-specific IgM Mab-PR5nf1 was produced in large scale and purified for using as vaccine adjuvant, and a novel cocktail formulation composed by inactivated PRRSV virus (VR2332 strain) and Mab-PR5nf1 along with normal adjuvant (Montanide™ ISA 206 water-in-oil) was developed to enhance PRRSV-KIV vaccine protection and further compared it with normal KIV vaccine (VR2332 strain) and commercial modified live virus vaccine (MLV, Ingelvac PRRSV-MLV) of PRRSV. After challenging all immunized piglets with HP-PRRSV-JXA1 strain, our result suggested that the overall-survival rate (OSR) and cell-mediated immunity (CMI) as determined by serum IFN-γ level and IFN-γ ELISpot, were significantly improved by added PRRSV-specific IgM to PRRSV KIV vaccine. Meanwhile, it was also notable that both OSR rate and CMI in Mab-PR5nf1 adjuvanted KIV group were even higher than MLV group, whereas CMI response is normally poorly evoked by KIV vaccine or subunit vaccine. Conversely, compared with piglets immunized with normal KIV vaccine, viral shedding and serum neutralizing antibodies were improved as well, and reduced viral shedding appeared to be a result of enhanced CMI caused by using PRRSV-specific IgM as an novel adjuvant. Based on these observations, our further analysis suggested that the enhancement of PRRSV-KIV by its specific IgM may be a consequence by targeting IgM immune complex formed by IgM and PRRSV-KIV to antigen presenting cells (APCs). Currently, a recombinant IgM based on this Mab by further swapping the mice originated IgM-Fc region to porcine originated IgM-Fc is currently under development. By employing the newly developed porcine PRRSV-specific IgM for PRRSV-KIV vaccine, a new formula for development of an effective PRRSV-KIV vaccine for practical use will be developed with improved safety and efficiency.

Dissecting the neutralizing antibody response to porcine reproductive and respiratory syndrome virus to identify novel vaccine targets

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to cause economic losses to the global swine industry. The causative PRRS viruses (PRRSV) exist as two species, PRRSV-1 and -2, causing recurrent outbreaks which cannot be adequately controlled by existing live attenuated vaccines. The high degree of variability between circulating PRRSV strains, enabled by an inherent propensity for rapid mutation, poses a significant challenge to the development of effective vaccines. Characterising the targets of broadly neutralising antibodies is of great interest and should guide next-generation vaccine design. However, the conserved neutralising epitopes on PRRSV glycoproteins are largely unknown, and our understanding of the mechanisms behind antibody-mediated neutralisation of PRRSV is incomplete.

Materials and Methods

Recombinant soluble forms of the PRRSV-1 minor envelope complex glycoproteins, sGP2, sGP3, and sGP4, were produced using a mammalian cell expression system. Serum from hyperimmunised pigs were screened for binding to these recombinant proteins and sGP3 was identified as the most highly recognised by antibodies in these sera. Fluorescently tagged sGP3-tetramers were therefore constructed and used to isolate single B cells from a hyperimmune pig by flow cytometry. mRNA encoding immunoglobulin VH and VL regions was amplified using a semi-nested RT-PCR workflow, sequenced, and complementarity-determining regions annotated using the IgMAT tool. Unique heavy and light chain pairs were cloned and expressed as recombinant monoclonal antibodies (mAbs). mAbs were evaluated for their specificity, cross-reactivity and neutralising potency by ELISA, immunostaining of PRRSV-infected cells, and virus-neutralisation tests. The immunogenicity of GP3 was additionally assessed using both protein and self-amplifying RNA formulations. As an alternative strategy, commercial sows repeatedly vaccinated with PRRSV-1 and naturally exposed to PRRSV-1 field strains were screened to identify animals possessing antibodies capable of broadly neutralising PRRSV-1 and -2. Ongoing experiments are being performed to assess the effect of depletion of PRRSV glycoprotein-specific antibodies from the serum on virus neutralisation in vitro to prioritise antigens for single-cell sorting and mAb isolation and characterization. Purified PRRSV- 1 and -2 virions have been fluorescently labelled to offer a second complimentary unbiased approach.

Results

Since recombinant PRRSV-1 sGP3 was well recognised by hyperimmune sera, sGP3-based tetramers were

constructed to sort single antigen-specific B cells and produce recombinant mAbs. The binding of selected mAbs was mapped to a conserved sequence, however, mAbs failed to neutralise PRRSV-1 or -2 *in vitro*. A parallel evaluation of GP3 immunogenicity, including recombinant sGP3 glycoprotein delivered on MPLA and QS-21-adjuvanted liposomes (displayed on the bilayer surface via CoPoP anchoring) or in an oil adjuvant, or delivery of membrane displayed sGP3 via a self-amplifying RNA vector, failed to induce a measurable neutralising antibody response, despite high sGP3-binding antibody titres. For the sow experiment, three sows were selected based on their serum neutralisation titres against PRRSV-1 and -2. Evaluation of antibody responses to recombinant PRRSV-1 and -2 glycoprotein ectodomains revealed an increase in reactivity against PRRSV-1 GP2, 3, and 4 after MLV immunisation. Cross-reactive binding was observed against all recombinant PRRSV-2 glycoprotein ectodomains. However, no correlations were found between antibody reactivity to recombinant glycoproteins and neutralisation titres.

Conclusions and Discussion

A workflow has been established for the isolation of porcine mAbs that could be used in future work to further dissect the immune response to PRRSV infection and vaccination. Regarding the sows, the next step will be to prioritize the recombinant glycoproteins for use as baits for single B cell sorting. To do this, we are depleting antibodies that bind recombinant glycoproteins and assessing its impact on PRRSV neutralization. It is hoped that this will facilitate the isolation of broadly neutralizing mAbs, which could be used as tools to define conserved epitopes and support a structural vaccinology approach to the development of broadly protective PRRSV vaccines.

Keywords: Porcine reproductive and respiratory syndrome virus, glycoprotein, neutralising antibody, vaccine

Porcine antibody response to epitope A on porcine reproductive and respiratory syndrome virus glycoprotein 5 and its role in virus neutralization

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Porcine reproductive and respiratory syndrome virus (PRRSV) represents a significant economic burden in swine production and hampers vaccine effectiveness due to its high mutability. Viral glycoprotein 5 (GP5) harbors a linear, immunodominant, and non-neutralizing epitope, designated as epitope A. Closely adjacent to epitope A lies a conserved neutralizing epitope, epitope B. It is hypothesized that antibody binding to epitope A hinders neutralizing antibody binding to epitope B through steric hindrance. The retention of epitope A on mature viral particles is linked to an alternative signal peptide cleavage site on GP5. This study aims to explore the presence of epitope A in selected vaccine and field strains and whether epitope A-specific antibodies (Abs) impede virus neutralization (VN). Analysis of historical GP5 sequences indicates high variability in epitope A sequences, with no discernible lineage/sub-lineage-specific distribution. Enzyme- linked immunosorbent assay revealed the presence of epitope A-specific Abs in pigs vaccinated with modified live virus (MLV) vaccines, while such Abs were absent in pigs infected with various field isolates. Vaccinated pigs challenged with a field strain identical in epitope A to the vaccine strain failed to develop memory antibody responses to epitope A, suggesting absence of epitope A on the challenge virus. Western blotting with affinity-purified Abs to epitope A confirmed the absence of epitope A in the field strain 46/2020, whereas the lab-adapted FL12 retained it. *In- silico* analysis of signal peptide cleavage sites showed that all five commercial MLV strains retained epitope A, while the three selected field strains lacked it due to cleavage at a secondary site. However, depletion of Abs to either epitope A or epitope B had no effect on the overall VN by PRRSV immune serum from pigs. This finding suggests that Abs to other neutralizing epitopes may play a compensatory role in the total polyclonal virus neutralizing response in pigs. Further investigation into the relationship between epitope A and B specific antibodies on VN are ongoing. Together, these findings will offer insights into the selection of candidate strains for future vaccine design and underscore the significance of antigenic specific pressure on PRRSV evolution in an immune population.

Lineage 1 Porcine Reproductive and Respiratory Syndrome Virus Attenuated Live Vaccine Provides Broad Cross-Protection against Homologous and Heterologous NADC30-Like Virus Challenge in Piglets

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that endangers the swine industry worldwide. Recently, lineage 1 PRRSVs, especially NADC30-like PRRSVs, have become the major endemic strains in many pig-breeding countries. Since 2016, NADC30-like PRRSV has become the predominant strain in China. Unfortunately, current commercial vaccines provide only partial protection against NADC30-like PRRSV.

Materials and Methods

In this study, an attenuated lineage 1 PRRSV strain, named SD-R, was obtained by passaging an NADC30-like PRRSV strain SD in Marc-145 cells for 125 passages. Four-week-old PRRSV-free piglets were vaccinated intramuscularly with $10^{5.0}TCID_{50}$ SD-R and then challenged intramuscularly (2 mL) and intranasally (2 mL) with homologous NADC30-like PRRSV SD ($1\times10^{5.0}TCID_{50}$/mL) and heterologous NADC30-like PRRSV HLJWK108-1711 ($1\times10^{5.0}TCID_{50}$/mL).

Results

The results showed that antibodies against specific PRRSVs in all immunized piglets were positive after a 14-day post-vaccination. When challenged with homologous and heterologous strains of NADC30-like PRRSV, the challenge control piglets exhibited varying degrees of clinical symptoms such as fever, reduced appetite (lower daily weight gain), and coughing. Conversely, the immunized challenged group showed no abnormal clinical signs. The immunized group displayed significantly reduced viral loads in blood and various tissues compared to the challenged control group. Necropsy revealed typical pathological changes in the lungs of the challenged control group, including patchy consolidation and lymph node hemorrhage, while no significant pathological changes were observed in the immunized challenged group.

Conclusions and Discussion

In conclusion, we developed the first China's attenuated lineage 1 PRRSV candidate vaccine strain, SD-R. Furthermore, SD-R was sufficiently attenuated and antigenic enough to confer clinical protection against the homologous and heterologous NADC30-like PRRSV challenges. SD-R provide a new choice for the prevention and control of NADC30-like PRRSV in China.

Keywords: PRRSV; lineage 1; NADC30-like; SD-R; pathogenicity; cross-protection efficacy

Posters

Evaluation of live attenuated porcine reproductive and respiratory syndrome virus vaccines genetically engineered to express peptide-based immune checkpoint inhibitors

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Introduction

Porcine reproductive and respiratory syndrome viruses (PRRSV) cause significant economic losses in the global pig industry. Live attenuated PRRSV vaccines (MLV) are widely used, but are weakly immunogenic and provide limited protection, possibly due to the modulation of immunoregulatory pathways. This study tested the hypothesis that engineering PRRSV MLV to express immune checkpoint inhibitors (ICI) could enhance the induction of protective responses in pigs.

Materials and Methods

Three PRRSV-1 MLV were engineered by reverse genetics to express peptide-based ICIs: (i) Microtides LD01 and (ii) LD10, which are both PD-1 and CTLA-4 dual antagonists, and (iii) 4ZQK13_Pig20, a synthetic porcine PD-1 binder protein. MLV-ICI were rescued and characterized in vitro. The safety, immunogenicity and efficacy of the MLV-ICI were then compared against the parental recombinant MLV in piglets.

Results

All three PRRSV-1 MLV-ICI were successfully rescued and propagated in MARC-145 cells. Immunization of piglets with PRRSV MLV-ICI showed no adverse effects. Compared to the parental MLV and other MLVs expressing ICI, immunization with the MLV expressing Microtide LD10 showed a trend towards greater IFN-γ and virus-neutralizing antibody responses. Following challenge with a PRRSV field strain, pigs vaccinated with MLV expressing Microtide LD10 showed significantly reduced viral loads in the lungs compared to the other MLVs.

Conclusions and Discussion

Novel PRRSV-1 vaccine candidates have been constructed that express peptide-based ICI. The LD10 candidate exhibited the greatest potential and provides a basis for future research on optimizing peptide ICI-based adjuvant approaches for better control of PRRSV and other viral infections of swine.

Keywords: PRRSV, vaccination, PD-1, CTLA-4, immune checkpoint inhibitors, immunity.

Immune efficacy evaluation of recombinant protein skeleton LigB chimeras with PRRSV neutralizing epitope

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Introduction

LigB is a protein with extremely high molecular weight that exists in Leptospira, belonging to the bacterial immunoglobulin superfamily [1]. LigB contains 12 class Ig domains [2]. Hsieh[3] et al. used SAXS to analyze the advanced structure of LigB antigen, and further rearranged and combined various individual Ig like domains to form a new recombinant protein. The combination of structural domains LigB showed good thermal stability and antibody affinity, and demonstrated good protective effects in animal experiments. This study used LigB as the backbone protein and predicted its immunogenic regions using software. The neutralizing epitopes of PRRSV were used to replace the amino acid sequences of the immunogenic regions outside the three β-fold structures of LigB. A recombinant protein of the viral epitope LigB skeleton was constructed, and the stability and immunogenicity of the aforementioned recombinant proteins were studied.

Materials and Methods

Select pET28a-SUMO as the expression vector, use LigB as the backbone protein, select the neutralizing epitopes of PRRSV embed three neutralizing epitopes of a virus into each LigB backbone, and use software to predict the effect of different epitope arrangements on the structure of the recombinant protein. After inducing the expression of the above-mentioned proteins, the recombinant proteins were purified and concentrated using His tags, emulsified, and immunized with mice to prepare high immune serum.

Conclusions and Discussion

According to the software prediction results, a total of 9 recombinant plasmids were constructed in this study. Transfor the recombinant plasmid into Rosetta competent cells, using IPTG as an inducer, and induce expression at 20℃ for 24 hours. The protein was purified by gel affinity chromatography. Using purified recombinant protein as immunogen, Balb/c female mice were immunized separately. After about 10 days of secondary immunization, serum titers were measured using indirect ELISA and IFA, The ELISA titer is above 2.30×10^4, IFA potencies are all above 1:2000. This study successfully constructed 9 recombinant proteins embedded with virus neutralizing epitopes using LigB as the backbone, providing important information and new research ideas for epitope vaccines.

Keywords: PRRSV, viral neutralizing epitope, Immune efficacy

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Ingelvac PRRS® MLV efficacy against high pathogenicity of a Chinese NADC34-like PRRSV challenge in Pigs

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Introduction

Porcine reproductive and respiratory syndrome (PRRS), caused by PRRS virus (PRRSV) infection, has invaded swine farms of China for more than 20 years from the first reported in 1996. Currently, because NADC30/34 is very easy to recombine with clinical strains in swine herds, those NADC30/34-like strains become the most dominant strain in swine herds of China. This study evaluated the cross-protective efficacy of PRRS modified-live virus (MLV) vaccine against challenge with a Chinese highly pathogenic NADC34-like strain. The results showed that MLV vaccines presented partial protection against NADC34-like challenge. Vaccinated piglets showed milder pathological lesions in the lungs, lower viral level in aerosol, as well as better average daily weight gain and lower mortality compared to the positive control group.

Materials and Methods

A total of 45 PRRSV-free pigs were randomly divided into five groups: 1) unvaccinated-unchallenged (SC); 2) unvaccinated-challenged (PC); 3) Ingelvac PRRS® MLV vaccine challenged (Ingelvac PRRS® MLV); 4) R98-1 vaccine challenged group (R98-1); and 5) R98-2 vaccine challenged group (R98-2). piglets in the Ingelvac PRRS® MLV, R98-1 and R98-2 groups were intramuscularly immunized at 4 weeks of age, according to label. After four weeks, piglets in SC group were given MEM medium, whereas the other groups were IM and IN infected with 2 mL 3×10^5TCID$_{50}$ of NADC34-like PRRSV. All pigs were euthanized and necropsied at 15 days post-challenge (DPC). The animal experiment protocols were approved by the Animal Ethics Committee of Institute of Animal Health, Guangdong Academy of Agricultural Sciences. The Animal Ethics Committee approval number was YC-PT2023009.

Results

On average, pigs vaccinated with Ingelvac PRRS® MLV increased by 414, 281 and 326 gram daily weight gain compared with PC, R98-1 and R98-2 groups. In addition, the groups of PC, R98-1 and R98-2 experienced a mortality of 30%, 23% and 20% respectively, but no piglets dead till the end of the study in Ingelvac PRRS® MLV group. Furthermore, Ingelvac PRRS® MLV reduced an average reduction of maximum MLL, virus load in serum, clinical presentations compared with other challenged groups.

Conclusions and Discussion

The data in this study demonstrate that Ingelvac PRRS® MLV provides protection against NADC34 like strains. Even though R98 and Ingelvac PRRS® MLV similarities in ORF5 significant differences in protection performance on NADC34-like were observed, which demonstrates that the vaccine efficacy cannot be predicted by genomic similarities in ORF5 sequences. Pig challenge model is still the gold standard for verifying vaccine effectiveness.

Keywords: PRRSV; NADC34, efficacy evaluation, heterologous protection

Reference:

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The efficacy of Ingelvac PRRS® MLV against heterologous strains of porcine reproductive and respiratory syndrome virus: A meta-analysis

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a significant viral pathogen that affects the global swine industry. Modified live vaccine (MLV) plays a crucial role in controlling porcine reproductive and respiratory syndrome (PRRS). The potential heterologous protection performance of MLV has been a constant concern for the hog rearing industry due to the rapid mutation and complex recombination of PRRSV. The aim of this study was to evaluate the overall efficacy of Ingelvac PRRS® MLV compared to no vaccination as reported in published studies mainly in China, using meta-analytic techniques.

Materials and Methods

Following an extensive literature search and review conducted by NCBI, a meta-analysis was performed to evaluate the efficacy of Ingelvac PRRS® MLV, considering predefined outcome parameters and co-variates. A total of eighteen eligible efficacy evaluation studies from China and the Asia-Pacific region were included in this analysis. Three protection-related parameters, namely average daily weight gain (ADWG), mortality, and macroscopic Lung Lesion (MLL), were extracted for meta-analysis using Review Manager 5.4 software developed by Cochrane Collaboration. The overall analysis combined all available comparisons for each parameter in a random-effects model. The Z-statistics were used to estimate the P-value for testing the null hypothesis. The ORF5 sequence similarity between challenged strains in these studies and Ingelvac PRRS® MLV ranged from 61.1% to 99.3%, encompassing both PRRSV-1 and Lineage 1, Lineage 5, and Lineage 8 of PRRSV-2.

Results

On average, pigs vaccinated with Ingelvac PRRS® MLV exhibited a higher daily weight gain of 116.8 g/d from the day of challenge until sacrifice (7-14 days) compared to non-vaccinated and challenged groups; additionally, they experienced a significant reduction in mortality by 64.0% (relative risk = 0.36). Furthermore, Ingelvac PRRS® MLV reduced an average reduction of maximum MLL score by 19.1% points.

Conclusions and Discussion

The results indicate that the use of Ingelvac PRRS® MLV vaccine effectively reduces MLL scores as well as improving key productivity indicators such as ADWG and mortality rates regardless of the strain used for challenge (PRRSV-1 or Lineage 1.5/Lineage 1.8/Lineage 5/Lineage8 of PRRSV-2).

Keywords: PRRSV; meta-analysis; efficacy evaluation; MLL; ADWG

Study on the Impact of the PRRSV NSP4 Coding Region on Regulating Antigen Presentation Function of DC Cells in Vaccine Strains

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Porcine reproductive and respiratory syndrome (PRRS) poses a significant threat to the swine industry in our country. Currently, the main prevention method is the use of live attenuated vaccines, but these vaccines produce slow immune protection. Studies indicate that following PRRS vaccination, both humoral and cellular immune responses are weak, and CD4+ T cells are reduced, suggesting potential antigen presentation blockage. It was also found that PRRSV infection of bone marrow-derived dendritic cells (DCs) significantly reduces CIITA transcription. This research focuses on the PRRSV NSP4 protein, exploring its regulation of the MHC II transactivator CIITA and its role in antigen presentation.

Using the PRRSV virulent strain HuN4 and attenuated strain HuN4-F112 to infect porcine alveolar macrophages (PAMs), we detected significant reductions in the transcription and expression of CIITA and its upstream regulatory factors. This indicates that PRRSV affects antigen presentation by regulating CIITA and its upstream factors. NSP4 and NSP11 significantly inhibit CIITA transcription and expression. NSP4 suppresses CIITA via the ubiquitin-proteasome pathway and directly interacts with CIITA. The inhibitory effect of attenuated NSP4 on CIITA is lower than that of the virulent NSP4.

The study shows that NSP4 affects antigen presentation in DC cells by inhibiting CIITA. Mutations in key sites of NSP4 can reduce its inhibition of CIITA, thereby enhancing vaccine immune efficacy. This finding provides new insights and theoretical support for the improvement of PRRS vaccines, with significant application and research value.

Design of a marker vaccine candidate for porcine reproductive and respiratory syndrome

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important infectious diseases impacting the swine industry worldwide and causes significant economic losses each year, which is caused by PRRS virus (PRRSV). For the disease control, the purification for PRRS is the ultimate goal of the prevention and control of this epidemic. But there is no commercial marker vaccine and supporting serological differential diagnosis, which makes it difficult to purify the disease in pig farms.

Materials and Methods

In this study, PRRSV-2 SX-HD infectious clone was constructed by reverse genetics technology, and then, the motif S105 in the N protein was mutated into M105 same as the N protein of PRRSV-1. The recombinant and mutated infection clones were transfected into MARC145 cells for viral rescue. Serial passage of mutated SX-HD virus on MARC-145 cells was used to analyze its reproduction and genetic stability. The titer of the progeny virus was determined and the protection of the vaccine candidate was evaluated.

Results and Discussion

IFA and Western blot showed that the PRRSV-2 SX-HD infectious clone was successfully constructed and rescued. And the mutated SX-HD was also successfully rescued in MARC-145 cells. The progeny of P120 generation of mutated SX-HD virus showed higher virus titers than the parental virus. Animal experiments showed that the marker vaccine candidate has high safety. All the pigs in the immune challenge group survived, and no pathological changes were found in the autopsy and lung tissue, indicating that the vaccine candidate could provide 100% protection against HP-PRRSV challenge. The previously developed cELISA assay did not detect antibodies produced by immunization with mutated SX-HD virus. In conclusion, mutated SX-HD virus is expected to be developed into a PRRSV marker vaccine. Combined with cELISA assay, the mutated SX-HD virus can be used for the differential diagnosis of vaccine immunity and natural infection, and provide a new strategy for the prevention and control of PRRSV.

Attenuation by passage and protection efficacy of NADC30-Like PRRSV FJ1402

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Abstract: In order to select live vaccine strains of predominant epidemic NADC30-Like PRRSV strain, the NADC30-like PRRSV FJ1402 was passed down to the F123 on Marc-145 cells. The titers of F7, F40, F60, F100, F113 and F123 were 105.5TCID50/ml, 106.3TCID50/ml, 106.7TCID50/ml, 107.5TCID50/ml, 108.0TCID50/ml and 108.5TCID50/ml, respectively. Plaque size of F100, F113 and F123 was stable. The whole genome analysis of F7, F40, F60, F100, F113 and F123 virus venom showed 25 and 29 amino acid mutations in non-structural proteins and structural proteins, respectively, compared with F7. The amino acid sequences derived from F100, F113 and F123 virus gene sequences were completely consistent. The results of pathogenicity test of piglets showed that the piglets infected with F100, F113 and F123 viruses did not show temperature rise, clinical symptoms and pathological changes, and the viral load in lung, lymph nodes and thymus tissues was significantly lower than that of F7. The results of immune protection test of piglets showed that F100 and F123 immunity induce humoral and cellular immunity of piglets effectively, and protect piglets against NADC30-Like FJ1402 strain attack effectively without obvious clinical symptoms and pathological changes after immunization. Viremia and viral load of lung tissue were significantly lower than those in challenge control group ($p < 0.0001$). F100, F113 and F123 presented no obvious pathogenicity, stable genetic characteristics and high immune protection, which can be used as attenuated vaccine candidates for NADC30-Like PRRSV.

key words: PPRRSV; NADC30; Attenuated vaccine

Preliminary development of NADC34-Like PRRSV inactivated vaccine

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Introduction

PRRSV is currently an important disease affecting sow reproduction and piglet survival in pig farms and seriously affects the global pig industry. The first NADC34 type of PRRSV was isolated in China in 2017 and has since spread to multiple provinces including Heilongjiang, Jilin, Liaoning, Hebei and Fujian, with no vaccine against this type of strain for the time being.

Materials and Method

Samples came from lungs and lymph nodes of pigs collected from a farm in China. PRRSV isolation and identification were performed using PAM cells and Marc-145 cells. Genetic evolution analysis and recombination analysis were performed using MEGA11 and SimPlot software, respectively.

Results

In this study, a NADC34-Like PRRSV HeB-05 strain was isolated, which could cause significant CPE in PAM cells and Marc-145 cells, while stable proliferation and passage could be performed. The genome of HeB-05 is 15110 bp in length, and genetic evolution results of Nsp2, ORF5, and the whole genome show that all of them are sublineage 1.5. The results of recombination analysis showed that there was no recombination in the full length of the genome, and all of them had the highest similarity with IA/2014/NADC34. Nsp2 has the same base and amino acid deletions as NADC34-like type PRRSV. In the virulence test, the body temperature of the challenged group increased, viremia, and the average daily gain decreased significantly, and HeB-05 had some pathogenicity to weaned piglets. In the immunization experiment, the body temperature of the immunized group was significantly lower than that of the positive control group, and the viremia in the serum disappeared faster, stimulating a higher antibody level. The inactivated vaccine prepared by HeB-05 provided some protection against challenge with the parental strain.

Conclusions and Discussion

At present, NADC34-Like PRRSV is detected in more and more provinces in China, and there is no vaccine against this type of strain for the time being. However, HeB-05 isolated in this study could be stably propagated and passaged in Marc-145 cells, while it could achieve a high virus titer, and the prepared inactivated vaccine played a certain protective role against challenge with the parental strain, providing a good vaccine candidate for the prevention and control of NADC34-Like PRRSV in China.

Keywords: PRRSV, Virus isolation，NADC34-Like, Inactivated vaccine

Boosting PRRSV-Specific Cellular Immunity: Development of an Fc-fused Multi-CTL Epitope Vaccine

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Introduction

The constantly evolving PRRSV has been plaguing pig farms worldwide for over 30 years, with conventional vaccines suffering from insufficient protection and biosecurity risks. To address these challenges, we aim to develop an Fc-fused multi-CTL epitope vaccine for specifically boosting cellular immunity against PRRSV.

Materials and Methods

Twenty-two reported PRRSV-specific CTL epitopes were predicted for their binding affinity to the SLA alleles, and then their immunostimulatory activity was evaluated via IFN-γ ELISPOT. A recombinant protein, pFc-PTE, was constructed based on the 10 screened CTL epitopes and fused with a modified porcine Fc molecule. The immunogenicity of pFc-PTE was evaluated by stimulating PRRSV-specific splenic lymphocytes and immunization in mice.

Results

10 screened CTL epitopes significantly stimulated PBMCs of PRRSV-challenged pigs to release IFN-γ. The pFc-PTE induced a robust cellular immune response, characterized by high levels of IFN-γ secretion. The fusion with Fc extended the duration of the immune response for at least 10 weeks post-immunization and induced a Th1-biased immune response.

Conclusions and Discussion

pFc-PTE holds promise as a novel, safe, and potent candidate vaccine in enhancing PRRSV-specific cellular immunity, and may also provide new perspectives for vaccine design against other viral diseases. Future studies will focus on *in vivo* experiments in pigs and assessing cross-protective capabilities against different PRRSV variants.

Keywords: PRRSV, CTL epitope, Fc fusion, cellular immunity, vaccine development

Proceedings

Genetic evolutionary analysis and prediction of conserved epitopes of PRRSV genome

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Abstract: Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically destructive pathogens threatening the pig industry. PRRSV genome has high variability and recombination diversity, and the development of a safe, stable and universal vaccine against PRRSV remains a major challenge. The traditional genotypes of PRRSV are PRRSV-1 and PRRSV-2, of which PRRSV-2 can be divided into lineages 1-9 according to the highly mutational ORF5, but it is not completely accurate. In this study, we compared and analyzed the whole genome sequences of 1617 PRRSV strains from different sources worldwide. The regional specificity and temporal correlation of these strains in the genetic evolutionary tree were comprehensively analyzed, and the conservated sequences were used for epitope predicted. The data showed that ORF alone was not a good genetic representative basis for strain classification. Based on comprehensive analysis of whole genome sequences, PRRSVs were divided into three genotypes: PRRSV-G1, PRRSV-G2 and PRRSV-G3. PRRSV-G3 can be subdivided into PRRSV-G3a, PRRSV-G3b, PRRSV-G3c and PRRSV-G3d subtypes. Among the 25 countries, PRRSV strains from China and the United States were independent branches in the genetic evolution tree, and the homology of PRRSV between the two countries was low, and the time correlation of isolates from each country was not significant. By comparing the PRRSV genome sequence with 90% conserved rate through conservative analysis, we further predicted 21 potential T cell epitopes and 18 B cell epitopes based on the conserved sequence. In conclusion, our results provide new insights into the rational classification of PRRSV and reveal multiple sets of potential conserved epitopes of PRRSV, which will contribute to the development of a vaccine with broad protective effect against PRRSV and have progressive significance for the prevention and treatment of PRRSV.

Research progress on vaccines against porcine reproductive and respiratory syndrome

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Abstract: Porcine reproductive and respiratory syndrome (PRRS), also known as porcine reproductive and respiratory syndrome (PRRSV), is a highly contagious disease caused by PRRSV. PRRSV is one of the most challenging viral pathogens in the global pork industry. PRRSV is classified as PRRSV-1 and PRRSV-2, PRRSV can infect macrophages.If the pathogen can infect and destroy, manipulate or maintain itself in macrophages or dendritic cells, it may regulate the immune response to a favorable state that is conducive to its own replication and survival.At present, PRRS is characterized by two different clinical manifestations. On the one hand, it is the reproductive failure of breeding pigs (reserve sows, sows and boars), and on the other hand, it is the respiratory disease of growing pigs.Vaccine is an effective strategy to prevent PRRSV infection. The development and application of PRRS vaccine has always been the focus of the industry. The research and development of PRRS vaccines show a trend of diversification and rapid development.At present, a variety of technical routes have been applied to the development of PRRS vaccines, including inactivated vaccines, modified vaccinia virus vaccines, live attenuated vaccines, subunit vaccines, DNA vaccines, and viral vector vaccines.These vaccines play an important role in the prevention and control of PRRS. In addition to the diversification of vaccine types, the development of PRRS vaccines also shows a specific trend for different strains. Although some achievements have been made in the development and application of PRRS vaccines, there are still some challenges and problems. First of all, the high variability of PRRSV makes the development of vaccines more difficult, and the protective effect of vaccines is difficult to stabilize in the long term. Secondly, different vaccine types have differences in immune effect, safety and other aspects, This paper mainly describes the advantages, disadvantages and applicability of the currently developed PRRSV vaccines, including modified live virus, inactivated virus, recombinant subunit, live vector, DNA, gene deletion, synthetic peptide and virus-like particle vaccine and other vaccines. In order to provide a theoretical basis for the development of new vaccines to prevent porcine reproductive and respiratory syndrome.

Keywords: PRRS; vaccine; pig; PRRSV

Advances in Porcine Reproductive and Respiratory Syndrome Vaccine

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Introduction

Porcine Reproductive and respiratory syndrome, commonly known as blue ear disease, is caused by porcine reproductive and respiratory syndrome virus (PRRS), which can cause reproductive disorders in sows and severe respiratory disease in piglets. The main infection route of porcine reproductive and respiratory syndrome is respiratory tract, air transmission, contact transmission, semen transmission and vertical transmission are the main transmission modes. Sick pigs, infected pigs and piglets of infected sows, as well as contaminated environment and utensils are important sources of infection. Vaccines are currently used to prevent and control PRRS infection in pigs.At present, PRRS vaccines include inactivated vaccines, attenuated vaccines, nucleic acid vaccines and so on.This article focuses on these three vaccines.

1 Inactivated Vaccine

An inactivated vaccine is a virus or bacterium that is cultured and then physically or chemically inactivated. The advantages of PRRS inactivated vaccine are good safety, non-toxic and easy to store and transport, but the long immunization time, large dose and poor immune effect of the vaccine on piglets have limited the application of PRRS inactivated vaccine in actual production to a certain extent, so the development of new vaccines has become the focus of the industry.

2 Attenuated Vaccine

Attenuated vaccine refers to a complete pathogen vaccine with weakened pathogenicity but still has vitality.After the attenuated vaccine is inoculated, its pathogenic microorganisms must replicate and proliferate in the body to stimulate the body to produce antibodies.PRRS attenuated vaccine has many advantages, such as strong phase immunity, good immune effect, low toxicity, small inoculation dose, can replicate in vivo and maintain long-term immunity, rapid antibody production, etc., but there are also shortcomings, such as strong virulence, atavism, and dispersed toxicity after immunization.

3 Nucleic Acid Vaccine

Nucleic acid vaccine belongs to a new type of vaccine, which directly introduces the foreign gene encoding a certain antigen protein into the somatic cells of animals, and synthesizes the antigen protein through the expression system of the host cells, inducing the host to produce an immune response to the antigen protein, so as to achieve the purpose of preventing and treating diseases.Nucleic acid vaccines are low-cost, simple to make, and can enable the body to produce a comprehensive immune response, but nucleic acid genes are foreign substances to the body and may cause damage to the body's own cells.

Result

With the rapid development of pig breeding industry, large-scale and intensive pig breeding mode has become inevitable, so the prevention and control of disease is the top priority for pig farms.At present, the prevention of PRRS mainly focuses on inactivated vaccines and attenuated vaccines. However, due to various shortcomings of the two vaccines, the shortcomings of inactivated vaccines and attenuated vaccines may become the focus of scientific research, and the development of new vaccines is also imminent.

Keywords: PRRS, Inactivated Vaccine, Attenuated Vaccine, Nucleic Acid Vaccine

Field Disease Control

Oral

Protective efficacy of PRRS MLV vaccine with reduced immunization dosage against either NADC30-like or NADC34-like strain prevalence in China

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Introduction

Since the widely prevalence of NADC30-like and NADC34-like strains in China and more field application of reduced immunization dosage of PRRS MLV vaccine in China, this study aimed to evaluate the efficacy of a popular commercially available attenuated porcine reproductive and respiratory syndrome virus (PRRSV) vaccine with different immunization dosage against the dominant NADC30-like and NADC34-like strains isolated in China.

Materials and Methods

For trial one, a total of 35 3-week-old PRRSV free piglets were randomly assigned to seven groups, each consisting of five piglets. They were intramuscularly (IM) inoculated with phosphate-buffered saline (PBS) and 1 dosage, 0.5 dosage, 0.1 dosage, 0.01 dosage of vaccine. Thirty-five days after vaccination, piglets were intranasally challenged (2mL total). One mock and each of the vaccinated groups was challenged with a 10^5TCID$_{50}$/mL dosage of the NADC30-like strain.

For trial two, a total of 36 27 days of age piglets, with 6 piglets in each group, were IM inoculated with PBS, 1 dosage, 0.1 dosage of vaccine. 42 days later, the negative control and vaccinated control with an equal amount of cell culture, other piglets were intranasally challenged with NADC34-like PRRSV (2mL total, $10^{5.5}$ TCID$_{50}$/mL).

The rectal body temperatures and clinical signs of the piglets were recorded once every two days throughout the experiment, and body weight was measured every week. During the experiment, the status of the piglets was recorded by scoring, including gross clinical scores (GCS), respiratory clinical scores (RCS), and nervous signs scores (NSS). Necropsy all the pigs 21 days after challenge and record gross and pathology lung lesion score of all pigs. Sera were collected at different time points and analyzed with ELISA and qRT-PCR.

Results

Comparison of the clinical score, average daily gain, gross and pathology lung lesion score of pigs vaccinated with different immunization dosage before and after challenge, 0.1 dosage ($10^{3.8}$ TCID$_{50}$) group of showed equal results to full dose of vaccination group and even better growth performance.

For the protective efficiency of reducing the shedding period after challenge, the commercial vaccine showed some effect for the NADC34 strain but little effect for the NADC30 strain used in this study. And 0.1 dosage($10^{3.8}$

$TCID_{50}$) group showed similar results to the full dose of vaccination group.

Conclusions and Discussion

NADC30-like and NADC34-like strain used in this study were the most virulent strains selected from more than 10 isolates prevalence in China but still showed low pathogenic when compared with the HP-PRRSV. In this study, based on the results of clinical score, average daily gain, shedding period and lung macroscopic and microscopic lesions, reduced immunization dosage (0.1 dosage, $10^{3.8}$ $TCID_{50}$) showed even better results than full dose vaccination. For pig producers, not only the etiology research data but also the economic benefits should be taken into consideration before making decisions for disease prevention and control, and our study provide evidence of reducing nine-tenth of the vaccination cost and may have better field performance during PRRSV prevention and control in China.

Keywords: efficacy evaluation, commerical PRRSV vaccine, NADC30-like PRRSV, NADC34 like PRRSV, challenge study

Nitazoxanide as a Potential Inhibitor of PRRSV Infection

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Introduction

Currently, there is no highly effective Modified Live Virus (MLV) vaccine that can efficiently combat viral mutations. Given the complex situation, relying on a single control measure—be it vaccination, public health measures, or gene-edited pigs—will not suffice to eradicate the disease nationwide. In this study, we report that Nitazoxanide (NTZ) may serve as a potential antiviral agent against PRRSV, capable of inhibiting PRRSV proliferation both *in vitro* and *in vivo*.

Materials and Methods

Forty-day-old Landrace pigs (weighing 11-13 kg) tested negative for PRRSV antigen and antibody. The animals were divided into an emergency prevention group, a treatment group, a negative control group, and a PBS-treated group receiving oral PBS. Both the emergency prevention and treatment groups were assigned three NTZ dosage. Rectal temperature, body weight, and oronasal and rectal swabs were collected daily, and clinical scores were recorded on days 0 and 7.

Results

Our study found that NTZ mitigates severe lung lesions caused by PRRSV. It also reduces viral shedding and viral load in tissues. Oral administration of NTZ at specific doses effectively reduced mortality in infected animals. NTZ inhibited PRRSV-induced viremia. In the emergency prevention group, a dose of 5 mg/kg NTZ significantly reduced the viral load in the blood, while a dose of 10 mg/kg significantly reduced oronasal viral shedding. In the treatment group, a 10 mg/kg dose significantly reduced both blood viral load and oronasal viral shedding. Notably, pharmacokinetic data indicated that NTZ distribution in the lungs was low, with higher concentrations in lymph nodes and a relatively short half-life.

Conclusion and Discussion

We confirmed that high doses of NTZ are associated with antiviral activity against PRRSV infection in animal experiments. The emergency prevention experiment demonstrated better antiviral efficacy, suggesting that NTZ may be more suitable for preventing potential infections. The role of NTZ in controlling the spread of PRRSV should be carefully considered if it is to be used as a major tool against PRRSV outbreaks. Finally, the broad-spectrum antiviral NTZ, proven effective in vivo, may become a crucial cornerstone in the fight against PRRSV pandemics in the future.

Keywords: PRRS, Nitazoxanide, Antiviral Drug

Implementing Unit Elimination Strategies for Rapid Elimination of Porcine Reproductive and Respiratory Syndrome in Swine Farms: A Case Study

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Background and Objectives

Porcine reproductive and respiratory Syndrome (PRRS), commonly referred to as "blue ear disease," is effectively managed globally through herd closure, a well-established yet lengthy process that significantly impacts sow farm productivity. With the advancing maturity of detection and elimination technologies for African swine fever, this study explores their applicability to PRRS elimination by developing a "unit elimination" scheme. This strategy, inspired by African swine fever management practices, utilizes laboratory testing combined with the assessment and removal of potentially infected swine to facilitate rapid on-site elimination of PRRS.

Materials and Methods

Constructed in 2020 and operational as a GP farm with 2500 sows, the facility is divided into two buildings equipped with a positive air filtration system. The west building houses 18 gestation units, while the east includes 10 farrowing rooms, 7 nursery units, and 2 GDU units, each with an emergency exit for rapid depopulation. The farm consistently tests negative for PRRS, PEDV, *Mycoplasma hyopneumoniae*, and APP antigens and antibodies. On February 22, 2023, sows in gestation unit PH7 tested positive for the PRRS antigen, marking confirmed infections. Leveraging techniques from early-stage African swine fever infections, a comprehensive plan was formulated involving "complete sampling, risk assessment, and small unit elimination" to expedite the elimination process. The strategy entailed isolating and monitoring affected pig, conducting tail blood swab tests on all sows for antigen detection, and additional pharyngeal and tail blood swab tests on symptomatic pigs. Additionally, tracing the movements of personnel, materials, and pigs within and between positive units facilitated targeted surveillance. Infected units were completely sealed and depopulated through emergency exits, followed by thorough disinfection through gas heating and ventilation pressure adjustments. Lastly, antibody results of relocated swine were meticulously evaluated to ensure effective disease control and prevention of recurrence.

Results

The initial detection of PRRS-positive pigs in unit PH7 (five positive, one suspicious, Ct_{min} 29) on February 22 led to immediate depopulation the following day. Subsequent testing identified positive pig in units PH2 on February 24 (one positive, two suspicious, Ct_{min} 32) and PH6 on February 25 (one positive, two suspicious, Ct_{min} 32), with similar rapid response measures implemented. An analysis of personnel, material, and pig movements pinpointed PH7 as the primary infection source, prompting temperature adjustments to 30°C and reductions in loft and internal room pressures to prevent viral escape. Follow-up antibody testing on March 1 and 8 confirmed 100% positivity with mean S/P values of 0.51 and 1.48, respectively. From the first detection to the resumption of normal operations, the outbreak was managed within 21 days with 440 sows removal, with the farm maintaining a dual-negative PRRS status for over a year.

Discussion and Conclusion

The rapid containment and elimination measures following the initial detection of PRRS antigen-positive pigs were pivotal. The farm's structured approach, including routine surveillance, unit-specific design, and a robust positive pressure ventilation system, played critical roles in minimizing airborne transmission and managing risks. Standardized production records detailing personnel roles, material usage, and pig transfers ensured thorough risk identification and management. The strategy of quick depopulation for positive swine, combined with early-stage partial elimination, proved highly effective in controlling and eradicating the outbreak, highlighting the importance of proactive and well-coordinated response strategies in disease management.

Posters

Baicalein inhibits PRRSV infection in weaned piglets by targeting EGFR

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Introduction:
Porcine Reproductive and Respiratory Syndrome (PRRS) is caused by the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), leading to a major economic burden for the global swine industry. Given the limited efficacy of current commercial vaccines and restrictions on the use of antiviral chemical drug, finding a safe and effective strategy to prevent and control PRRSV remains an urgent need in swine industry. The natural active ingredients of plant extracts have garnered significant interest because of their numerous benefits. Notably, the anti-viral role of baicalein has been reported increasingly. However, little information is available regarding the effects of baicalein on anti-PRRSV. This study aims to investigate the effects and mechanism of baicalein on anti-PRRSV infection in weaned piglets.

Materials and methods:
21-day-old piglets were received a basal diet in the control group while the piglets in the treatment group were given an extra 100 mg/kg of Scutellaria baicalensis flavonoid (SF) containing 2.4% baicalein for two weeks, then piglets were challenged with 10^5 $TCID_{50}$ PRRSV. Three weeks post infection, the growth performance, antioxidant and immunity were explored. The antiviral effect of baicalein on PRRSV were validated in Marc-145 cells. The targets of baicalein on anti-PRRSV were analyzed by network pharmacology analysis, molecular docking and dynamics simulations. Moreover, the expression of targets in PRRSV-infected lung tissues were also investigated.

Results:
This study revealed the enhancements in growth performance and immunity, attenuation of interstitial pneumonia and bronchopneumonia damage induced by PRRSV infection. Additionally, baicalein's ability to suppress PRRSV replication in a dose-dependent manner. Network pharmacology analysis identified 7 potential targets of baicalein against PRRSV, with the EGFR emerging as a core target. Molecular docking and dynamics simulations further confirmed the considerable binding affinity (-7.935 kcal/mol) of EGFR with baicalein, which also corroborated by the lower p-EGFR expression of PRRSV-infected piglets.

Conclusion:
This study elucidates the inhibition and targets of baicalein against PRRSV, suggesting baicalein's potential as a promising candidate for PRRS controlling.

Keywords: Weaned piglets, PRRSV, Baicalein, EGFR, MD simulations

Prevention and control measuresof positive NADC-34-like porcine reproductive and respiratory syndrome virusin pig farms

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This paper will analyze and summarize the cases and provide reference for pig farms. On December 20, 2023, 200 gilts were introduced in a pig farm in Jilin province. After seven days, abortions occurred among the sows and abortion rate continued to increase. The deaths and cullings of nursery pigs also increased. The main symptoms were wilting, huddling together, eyelid edema, increased secretions, emaciation, disheveled coat, obvious respiratory symptoms, increased lameness and posterior paralysis. The autopsy results mainly showed that there were diffuse bleeding points and widened interstitium in the lungs. The detected results showed that the positive rates of CSFV and PRV antigens were 0, the Ct value of PCV2 antigen was 26.83, and the Ct value of PRRSV antigen was 28.21. The GP5 gene of the PRRSV-positive sample was amplified and sequenced, the results showed that the infected strain was NADC-34-like PRRSV. The treatment method was that the sows and nursery pigs were fed with tylvalosin and astragalus polysaccharide powder for 14 days, and emergently vaccinated with 2 doses of PRRSV inactivated vaccines and 1 dose of PCV2 inactivated vaccine. One month later, the sows were generally vaccinated again with 2 doses PRRSV inactivated vaccines, 14-day-old piglets were immunized with 1 dose of PCV2 inactivated vaccine, and 2 doses of PRRS inactivated vaccine per head before weaning. When the nursery pigs were transferred, each pig was injected with 0.2 mL Draxxin. Two months after the whole pigs were immunized, the abortion rate of sows decreased from 5.4% to 0.3%, the survival rate of unweaned pigs increased from 86.6% to 95.7%, and the survival rate of nursery pigs increased from 86.5% to 95.6%. However, after continuing for 2 months, a small number of deaths of nursery pigs occurred again, and they were detected as PRRSV positive. Then all of nursery pigs were urgently vaccinated PRRS live vaccine. On the 10th day after vaccination, there was no death in the pig herd. Two months after the second vaccination, the serum of pigs aged 60-90 days were randomly collected. The test results showed that all PRRSV antigens were negative, the positive rate of PRRSV N protein antibody was 100%, the mean value of S/P was 1.98, and the degree of dispersion was 29.88%.

Effects of glucuronolactone on the growth performance, antioxidant and immunity of PRRSV-DON co-infected piglets

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Introduction:
Porcine reproductive and respiratory syndrome virus (PRRSV) causes acute highly pathogenic infectious diseases in pigs. DON, one of the most prevalent mycotoxins in grain-based feed, seriously aggravated PRRSV infection in piglets. Glucuronolactone plays an important role in detoxification. In this study, 21-day-old weaned piglets as the research object, the effects of glucuronolactone on the growth performance, antioxidant and immunity of PRRSV-DON co-infected piglets were investigated.

Materials and methods:
Thirty 21-day-old weaned piglets were selected and randomly assigned to three groups (n=10). The basic diet was designed according to the NRC and 200 mg/kg glucuronolactone was supplemented in treatment group. All piglets were fed the corresponding diet from the first day and injected with PRRSV(2.0 ml PRRSV10^5 $TCID_{50}$/mL) on the 22nd day. Three weeks post infection, all piglets were slaughtered for sampling.

Results:
The results showed that comparing with the PRRSV infected piglets, in PRRSV and DON co-infected weaned piglets, the average daily gain (ADG), mRNA expression of IFN-α and IFN-γ, as well as antioxidase level of GSH-PX in the liver and SOD in the lung were significantly reduced ($P < 0.05$), PRRSV loads in serum and lung of these piglets were significantly increased ($P < 0.05$). Conversely, in the PRRSV and DON co-infected piglets with dietary-supplemented glucuronolactone, the ADG and antibody level (IgA and IgG) were improved significantly ($P < 0.05$), the antiviral ability was enhanced with significantly mRNA increased expression of IFN-α, IFN-β, IFN-γ and PRRSV-N decreased expression ($P < 0.05$). Meanwhile, dietary-supplemented glucuronolactone could improve antioxidant capacity of PRRSV and DON co-infected piglets, such as the level of T-AOC in the lung and serum, SOD in the liver and lung, and GSH-PX in the live were significantly increased ($P < 0.05$).

Conclusion:
In conclusion, this study showed that dietary-supplemented glucuronolactone could improve the growth performance, antioxidant and immunity of PRRSV-DON co-infected weaned piglets, suggesting that glucuronolactone is a promising antidote for attenuation of DON toxicity in PRRSV-infected piglets.

Keywords: Glucuronolactone, PRRSV-DON, weaned piglets, growth performance, antioxidant, immunity

Gilt serum acclimation monitoring for PRRSV

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economically significant diseases affecting swine production. Avoiding the introduction of naïve gilts, susceptible to PRRSV infection, is one of the most important steps to control virus circulation. Gilt acclimation is therefore essential when using external sources for replacement. This study reports on the monitoring of PRRSV gilt serum acclimation in a Chinese farm.

Materials and Methods

A 2,500-sow farm located in the North of China was selected. In this farm, replacement gilts are externally sourced and inject serum with circulating PRRSV strains in this farm before introduction into the main herd. One batch of gilts was selected and monitored over time. For that, serum samples (n=581) were obtained before PRRSV infection and at 7, 14, 30, 60, 90, 120, 140, 150, 180 days post infection (dpi) [average 58 samples per sampling event]. Serum samples were individually tested by ELISA (PRRS 2XR, IDEXX Laboratories). In addition, serum samples obtained at 7 dpe were tested by PCR (RealPCR PRRSV Type 1 and Type 2 Multiplex RNA Test, IDEXX laboratories). Five months post infection, samples were taken from primiparous sows in the monitored batch (n=21 serum; n=22 throat swab) and their offspring (n=6 processing fluids; n=107 serum samples; n=109 throat swabs) and tested by PRRSV qPCR as described above.

Results

PRRS ELISA average S/P values showed during acclimation were: 1.46 before infection, 1.58 at 7 dpi, 1.76 at 14 dpi, 1.61 at 30dpi, 1.25 at 60 dpi, 0.96 at 90 dpi, 0.7 at 120 dpi, 0.5 at 140 dpi, 0.48 at 150 dpi and 0.39 at 180 dpi. PRRSV was detected by PCR at 7 dpi in 21% of serum samples. Five months post infection, 33% of processing samples (2 to 6)were positive to PRRSV-2 by qPCR. Other piglet and sow samples remained negative.

Conclusions and Discussion

Successful PRRSV infection was confirmed by PCR at 7 dpi. Serological monitoring showed rising average S/P values in the first weeks post exposure while slowly declining afterwards, indicating serological stability at the time of introduction. After farrowing, there was no evidence of active PRRSV infection in this group of animals.

Keywords: PRRS, PRRSV, ELISA, PCR, qPCR, RT-qPCR, acclimation

A successful case of PRRS control within frequent and multiple gilt introduction farm

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a highly contagious infectious disease that is endemic. PRRSV only infects pigs. PRRSV infection cause severe reproductive failure in sows. The movement of infected pigs is also an important mode of transmission of the disease. The objective of this study was to investigate the loss of PRRS caused by frequent and multiple source gilt introduction and develop comprehensive prevention and control measures.

Material & Methods

The farm is located in Qingyuan City, Guangdong Province. The farm studied introduced 750 gilts from different farms in six batches from 15th Dec. 2022 to 13th Jan. 2023, with age distribution ranging from 65 days to 100 days. 19 sows showed abortion within 1-2 weeks after introduction. Samples of aborted fetus and sick pigs in nurseries sent laboratory for PRRSV test. For samples with low levels of viral antigens, sequencing is performed after screening. The farm immediately adopted prevention and control measures as below:

Close the herd firstly, all animals were vaccinated against Ingelvac PRRS® MLV (Boehringer Ingelheim) 2 times at one-month intervals at beginning. All Sows was vaccination 4 times a year continuously after 2 times whole herd vaccination. Continuous PRRSV monitoring. Use Combat2.0 to assess biosecurity risks and to do improvement. Medicine treatment for sick pigs. The production data was analyzed using SPC (statistical process control).

Results

The pig herd performance was gradually increased within two months. The positive rate of wild PRRSV decreased from 10% to 1.5%. After sequencing, only QYYZ-like strain was detected. Biosecurity situation was improved, and COMBAT score increased from 310 to 550. The abortion rate of sow decreased to less than 1%. The average healthy and weak fetal numbers recovered to 7.46 and 0.6.

Discussion and conclusion

In conclusion, the data in this study demonstrate that frequent and multiple source gilt introductions are often one of the most important factors leading to PRRS outbreaks in farm. Comprehensive prevention and control measures are required to stabilize the herd.

Keywords: PRRS, Vaccination, Control

Effect of different antimicrobial use in the perinatal period on the performance of sows and piglets in PRRSV-positive farms

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Introduction
The purpose of this study was to evaluate and compare the effects of different antibiotics on the performance of sows and piglets in PRRSV-positive pig farms.

Materials and Methods
A total of 280 near-term pregnant sows were selected from a PRRSV-positive pig farm and divided into five groups randomly. The sows in each group were administered antibiotics such as tilmicosin, tiamulin, doxycycline, and tylvalosin, while the control group received no antibiotic treatment. The blood of weaned piglets was collected for PRRSV antigen and antibody detection to assess the effectiveness of the antibiotics in preventing PRRSV infection in piglets. The number and ratio of diarrhea cases were recorded for each group of weaned piglets . The weaning interval of weaned sows and the estrus mating rate within 7 and 10 days after weaning were counted in each group, and the production indexes such as the number of weaning per litter, weaning survival rate and weaning weight of piglets in each group were counted.

Results
The weaned piglets in the tylvalosin and tilmicosin groups showed a PRRSV pathogen positivity rate of 0%, and their PRRS antibody levels were significantly higher than those in the control group. The tylvalosin group exhibited a lower diarrhea rate in piglets compared to the other groups. What's more, compared to other groups, the weaning survival rate and weaning weight of piglets in the tylvalosin groups were the highest, the weaning interval of sows was the shortest, and the average litter loss was lower.

Conclusions and Discussion
It was found that using tylvalosin can effectively inhibit the vertical transmission of PRRSV, enhance piglet antibody levels, and significantly reduce the weaned piglet diarrhea rate. In addition, the use of tylvalosin significantly improved weaning survival rate and weaning weight of piglets, shortened the separation interval of sows, and reduced the loss of litter assets. The initiation of this study provides crucial experimental data and theoretical support for effective antimicrobial use in large-scale PRRSV-positive pig farms, and provided necessary supplementary measures for realizing cost reduction and efficiency increase in large-scale pig farms under the background of PRRSV pandemic.

Keywords: PRRSV; large-scale pig farms; perinatal; antibiotics; tylvalosin

The natural compound Sanggenon C resists PRRSV infection

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has caused serious economic losses to the pig industry worldwide. Due to the rapid mutation of the virus and the low cross-reaction between different strains, the protective effect of the vaccine is still not satisfactory. Currently, more and more antibiotics are widely used in the pig industry, which not only increase the resistance of pathogens, but pose a huge threat to the ecological environment and food safety. Therefore, the development of highly effective non-antibiotic anti-PRRSV drugs have important economic significance for the comprehensive prevention and control of PRRS.

Materials and Methods

In this study, 720 natural compounds of plant origin were screened, and the inhibitory effects of Sanggenon C on the replication of different PRRSV strains and different porcine viruses were detected in vitro by RT-qPCR, Western blot and $TCID_{50}$ assay. The effect of Sanggenon C on the replication of PRRSV in piglets was further verified in vivo, the inhibitory effect of Sanggenon C on PRRSV infection in piglets was evaluated by observing clinical signs, detecting virus replication level and pathological examination of piglets.

Results

In vitro experiment, the results showed that Sangenone C can significantly inhibit the replication of different PRRSV strains, has a good inhibitory effect on PRRSV MOI=30 infection. Sanggenon C could also significantly inhibit the replication of PEDV, PCV2 and CSFV. Mechanistically, Sanggenon C could inhibit the activation of NF-κB signaling pathway by promoting the expression of TRAF2, thereby inhibiting the replication of PRRSV. *In vivo* experiment, the results showed that Sanggenon C treatment could significantly alleviate the increase in body temperature, decrease in weight gain and clinical signs of PRRSV-challenged piglets, and decreased viral loads in the lungs, thymus and lymph nodes. It also alleviates viraemia and pathological damage of lung tissue of piglets, delays the excretion time of virus from mouth, nose secretions and feces of piglets.

Conclusions and Discussion

In conclusion, Sanggenon C is an effective natural compound against PRRSV infection, which has the potential to be a candidate drug for the prevention and control of PRRS in clinical practice.

Keywords: PRRS, Sanggenon C, piglets, treatment

A case study on rapid stabilization and eradication of a 2400-head GP farm after an PRRSV outbreak caused by a NADC30-like Strain

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Introduction

Porcine Reproductive and Respiratory Syndrome (PRRS), also known as "Blue Ear Disease," exhibits a high degree of genetic diversity with multiple lineages. Within Lineage 1, NADC30-like and NADC34-like viruses are currently the predominant strains in China. Their high recombination and mutation characteristics pose significant challenges for the prevention and control in large-scale pig farms, greatly affecting their production performance and economic benefits. The objective of this case study is to examine and implement the "Coscian 4 Measures and 8 Steps PRRSV Eradication Plan" in order to achieve rapid control of infection and the eradication of PRRSV in a PRRSV-negative gestating pig (GP) farm that was exposed to a PRRSV NADC30-like strain and subsequently experienced an outbreak.

The implementation process of the "Coscian 4 Measures and 8 Steps PRRSV Eradication Plan"

The 2400-head GP farm is situated in Changsha City, Hunan Province (hereafter referred to as the "project farm"). The farm is equipped with a boar station, farrowing houses, gestation houses, replacement houses, and isolation houses. The farm employs a continuous production model, with piglets sold after weaning. The project farm was previously a PRRSV dual-negative farm, and infected by PRRSV in November 2023, which was identified as a NADC30-like strain through genetic sequencing. After implementing comprehensive healthcare programs such as Tylvalosin + Chlortetracycline, the anticipated effect has not been achieved. In January 2024, the "Coscian 4 Measures and 8 Steps PRRSV Eradication Plan" was implemented. The key measures are as follows:

Measure 1: Monitoring and Assessment. The evaluation of the infection status of the herd is conducted through PRRSV nucleic acid testing and antibody testing.

Measure 2: Control of Infection. The addition of anti-PRRSV drug *coscian Ⅰ* to the breeding herd for 15 days each month is employed to rapidly clear the PRRSV viremia and virus in the issues. Concurrently, the usage of antibiotics such as Tylvalosin, Tilmicosin, and Chlortetracycline is discontinued.

Measure 3: Herd Close Management. The management of the pig, personnel, and tools in zones and buildings is implemented to reduce movement and cross-contamination.

Measure 4: Biosecurity and Disinfection. Improving the biosecurity management system to prevent the introduction of pathogens. Organizing and disinfecting the internal and external environment of the buildings with a "disinfect-wash-disinfect" process to reduce the spread of PRRSV due to dust and aerosols, and dynamically clearing PRRSV from the environment.

Results and Discussion

The project farm successfully eradicated PRRSV by implementing the "Coscian 4 Measures and 8 Steps PRRSV Eradication Plan" for a period of two months. From 1 April 2024 to 14 June 2024, samples including testicular

fluid, aborted materials, stillbirths and lungs of weak piglets were collected for PRRSV nucleic acid testing, all of which were negative. The level of PRRSV antibody against N protein in the breeding herd decreased rapidly over the course of the implement, with the PRRSV positive rate dropping from 90% in February to 52% in May, and the average S/P value decreased from 1.22 to 0.59.

Following the implementation of the plan, various production indicators exhibited a notable improvement:

1. After 1 month of implementation compared to before, the proportion of dead and poor sperm in boars quickly decreased from 50% before the treatment to current 10%.
2. After 2 months of implementation compared to before, the abortion sows in each month decreased from 11 before the treatment to current 3, and the mortality rate of nursing piglets decreased from 16.8% to 7.1%.
3. After 3 months of implementation compared to before, the average number of healthy piglets per litter increased from 10.3 before the treatment to current 11.6, and the mortality rate of finisher pigs decreased from 24% to 8%.

In summary, after the outbreak caused by the NADC30-like virus, the project farm achieved remarkable results in the rapid stabilization and eradication of PRRSV by utilising the “Coscian 4 Measures and 8 Steps PRRSV Eradication Plan", which provided invaluable experience and a reference for PRRSV-negative large-scale pig farms that have been infected with the NADC30-like strain to quickly stabilize and eradicate PRRSV.

GP5 and M proteins play a major role in the immune protection function of PRRSV structural proteins.

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is an important pathogen that poses a threat to the pig industry in China and globally. In recent years, the NADC30-like strains have frequently undergone recombination with HP-PRRSV and vaccine strains, resulting in an increase in viral diversity and the coexistence of multiple lineages. However, the current vaccines have poor protective effects against heterologous strains, posing a significant challenge to the sustainable development of the pig industry. This study focused on the structural proteins of PRRSV, screening for key antigens that regulate the immune protective effect of the virus, laying a foundation for the development of novel PRRSV vaccines.

Materials and Methods

Using reverse genetics technology, chimeric viruses were successfully constructed by replacing the GP2, GP3, and GP4 proteins (CHsx1401-GP234JX) or the GP5 and M proteins (CHsx1401-GP5MJX) of lineage 8 JXwn06 strain with those of the lineage 1 CHsx1401 virus backbone, and the chimeric viruses were successfully rescued. The chimeric virus group (CHsx1401-SPJX) that was previously rescued in the laboratory by replacing the full-length structural proteins of JXwn06 and a parental virus group CHsx1401 (n=5) were also included for comparison. The four chimeric viruses were immunized at a dose of 2×105 TCID50/mL, and after the viremia was cleared, a challenge test was performed using JXwn06 at a dose of 2×106 TCID50/mL. The DMEM group was immunized with 2mL of DMEM (n=5), and the MOCK group received no treatment (n=3).

Results

After the virus challenge, the body temperature of pigs in the CHsx1401-SPJX group did not increase, while the body temperatures of pigs in the CHsx1401-GP5MJX and GP234JX groups increased to about 40.5°C at the highest. The body temperatures of pigs in the DMEM group and CHsx1401 group rose to 41.7°C. The necropsy results showed that the lung damage in the CHsx1401-SP group was the least. The CHsx1401- GP5MJX group showed milder macroscopic and microscopic lesions in the lungs compared to the CHsx1401-GP234JX group. The CHsx1401-GP5MJX group, like the CHsx1401-SPJX group, did not detect viremia on the third day after the challenge, viremia was detected in 2 pigs of the CHsx1401-GP234JX group. In testing the organ tissue loads of the lungs and inguinal lymph nodes, CHsx1401-GP5MJX showed a titer approximately one lower than that of CHsx1401-GP234JX. In addition, the GP5 and M
proteins have a higher ability in inducing cellular immunity compared to GP2, GP3, and GP4.

Conclusions and Discussion

The conclusion indicates that the structural proteins of PRRSV play a significant role in homologous immune protection, with GP2, GP3, GP4, GP5, and M proteins exerting synergistic protective functions. Among these structural proteins, GP5 and M proteins play the primary role in promoting viral clearance and reducing pathological damage caused by virus infection.

Keywords: porcine reproductive and respiratory syndrome virus; structural protein; immune protection; virus challenge

Proceedings

The effects of wet feed and liquid fermented feed on the blood immunity and mortality rate of weaned piglets infected with reproductive and respiratory syndrome in pigs

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Abstract：Purpose: Porcine Reproductive and Respiratory Syndrome (PRRS), commonly known as "pig blue ear disease". This is a highly contagious disease characterized by reproductive disorders, high fever, and respiratory symptoms in pigs. The disease is prevalent worldwide, causing huge economic losses to the pig farming industry and posing a serious threat to its healthy development. However, there is currently no significant plan for the prevention and treatment of "pig blue ear disease". Therefore, for this disease, improving the immune level of piglets, reducing the mortality rate of diseased piglets, improving piglet production performance, and ensuring the economic benefits of the pig farming industry are particularly important.Currently, an increasing number of studies have shown that wet feed and liquid fermented feed can improve the immune capacity and production performance of weaned piglets, but the impact of these two types of feed on weaned piglets infected with reproductive and respiratory syndrome is not yet clear. Therefore, this study aims to evaluate the effects of wet feed and liquid fermented feed on the blood immunity, mortality rate, and production performance of weaned piglets infected with reproductive and respiratory syndrome in pigs.**Materials and Methods:** In this study, 744 21 day old weaned piglets (5.43 ± 0.14kg) were randomly assigned to a control group (dry feed (CON)), a wet feed group (basic feed mixed with water (LF)), and a liquid fermentation feed group (basic feed fermented (FLF)) for 35 days of production and feeding. Each treatment was repeated 4 times, with 62 weaned piglets repeated each time. **Result:**The results showed that at 35 days, the IFN-r levels in the serum of piglets in the fermentation group were significantly higher than those in the wet and dry feed groups (P>0.05), and the IL-8 levels in the fermentation group were significantly higher than those in the dry feed group. The IFN levels in the wet and fermentation groups were also significantly higher than those in the dry feed group- α The level was significantly higher than that of the dry material group (P>0.05), and the fermentation group had TNF- α The level was significantly higher than that of the wet material group (P>0.05), and the IgA level in the wet material group was significantly higher than that in the dry material group (P>0.05). The levels of IL-6 and IL-1 in the dry material group were also significantly higher β The levels of IL-10 and IgM were significantly higher than those in the wet and fermentation groups (P>0.05), while the IgG levels in the dry group were significantly higher than those in the wet group (P>0.05). At 0-7 days, the mortality rate of piglets in the dry feed group was significantly higher than that in the wet feed group (P>0.05); At 7-14 days, the mortality rate of piglets in the dry feed group was significantly higher than that in the wet feed group and fermentation group (P>0.05); At 14-21 days, the mortality rate of piglets in the wet feed group was significantly higher than that in the fermentation group (P>0.05); At 21-28 days, there was no significant difference in piglet mortality rate among the wet feed group, fermentation group, and dry feed group (P<0.05); At 28 to 35 days, the mortality rate of piglets in the dry feed group was significantly higher than that in the wet feed group (P>0.05).**Conclusion:**Wet feed and liquid fermented feed can enhance the immune capacity of weaned piglets, reduce mortality rates, and thus improve piglet production performance.

Keywords: Porcine reproductive and respiratory syndrome; Weaned piglets; Wet feed; Liquid fermented feed; Immune ability; mortality

Study on the detection methods of swine reproductive and respiratory syndrome virus

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an economically important swine disease caused by the PRRS virus (PRRSV). It can cause severe reproductive disorders in sows and respiratory problems in pigs of different ages, which brings huge economic losses to the global pig industry. PRRSV was first reported in China in 1996, since which the virus has been widely spread in China, and recent studies showed that PRRSV positive rates exceeded 80%. PRRSV infection causes immunosuppression, increasing susceptibility to secondary infections, often leading to elevated mortality in infected animals. Therefore, the rapid, sensitive and reliable detection of diseases in the early stages of infection status always plays an important role in disease prevention and control. This paper summarizes the traditional detection methods such as enzyme-linked immunosorbent method (ELISA), immunoperoxidase monolayer method (IPMA) and immunofluorescence, and some detection methods with many advantages, such as double-stranded real-time quantitative PCR method, quadruple real-time quantitative PCR method and new amplicon deep sequencing method based on ORF 7 gene, in order to provide reference for better detection of pig breeding and respiratory syndrome virus.

conclusion

Although various detection methods such as polymerase chain reaction (PCR) and real-time PCR have been widely used for molecular diagnosis, they are mainly for a single pathogen. Quantitative real-time RT-PCR is a powerful method that combines rapidity, specificity and efficiency and can be used for large-scale screening and strain identification of PRRSV. Thus, this multiplex real-time PCR provides an accurate and sensitive method for the identification of four potential DNA viruses in potential pathogens, enabling their application to diagnosis, surveillance and epidemiology. Furthermore, deep sequencing of the amplicon in highly conserved regions of the PRRSV genome can support laboratory diagnosis and epidemiological surveillance of the disease.

Keywords: PRRS, Real-time RT-PCR, swine industry

Assessment of main productive parameters changes of a breeding herd following a NADC30-like PRRSV infection

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major threat to the global swine industry and has caused enormous losses every year. Since its first emergence in China in 1996, PRRS has been widely spreading and continuing to evolve rapidly in China. NADC30-like PRRSV strains were first detected in Chinese swine herds in 2013, and became the predominant PRRSVs since 2016. In this study, we evaluated the productive impact of a NADC30-like PRRSV infection on a Chinese breeding farm.

Materials and Methods

Information about the PRRS outbreak

The study was performed on a commercial 6000-sow breeding farm with a 21-day batch farrowing interval. After a suckling period of 28 days, all piglets were weaned and sold to other wean-to-finish farms. PRRS outbreak was confirmed in this farm in July 2020 with clinical symptoms including decreased feed-intake, fever, and increased late-term abortions.

Detection and sequencing of PRRSV

Samples including lung tissue, lymph nodes, stillbirths and sera were collected. All samples were detected PRRSV positive by RT-PCR with negative results for classic swine fever virus, pseudorabies virus, and type 2 porcine circovirus. A causative PRRSV strain was isolated using porcine alveolar macrophages (PAMs). ORF5 gene and whole genome were sequenced.

Assessment of production impact

Fourteen main production parameters related to PRRS 12 months before and after the outbreak were collected, including sow mortality (SM), return to heat within 7 days after weaning (%) (RTH), conception rate (CR), farrowing rate (FR), empty sows rate (ES), total born per litter (TB), born alive per litter (BA), average birth weight (ABW), stillbirth per litter (SB), mummies per litter (MM), low birth weight piglets per litter (LBW), pre-weaning mortality (PWM), weaned pigs per litter (WP), and 28-day piglets weight (WW).

Baseline production parameters were conducted based on 12-months prior to the initial of PRRS detection and was used to compare changes afterwards. Changes in a parameter was defined as outside the baseline parameter for at least two months, and returning to baseline parameter was defined as keeping inside for at least two months.

Results

Genetic characteristics of PRRSV

The PRRSV isolate was named GX505 (OM202894). The GX505 isolate was found to be clustered in lineage 1 which was represented by NADC30 and NADC34 according to phylogenetic analysis based on ORF5 and whole genome sequence (Figure 1). Furthermore, recombination events were detected in the genome of GX505, which revealed that GX505 was a recombinant between NADC30-like strain and HP-PRRSV strain (Figure 2).

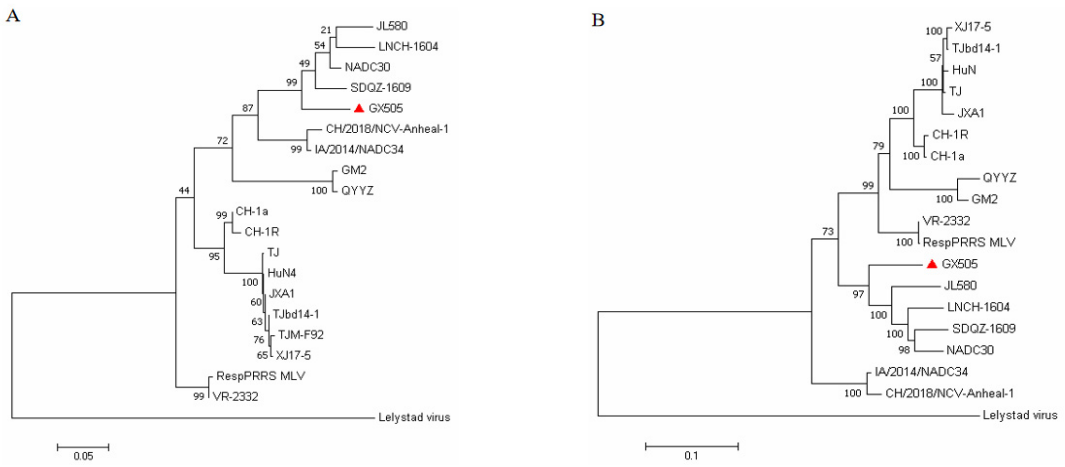

Figure 1 Phylogenetic analysis based on ORF5 (A) and whole gnome (B) sequence of PRRSV. The isolate GX505 recovered in this study is indicated by red triangle.

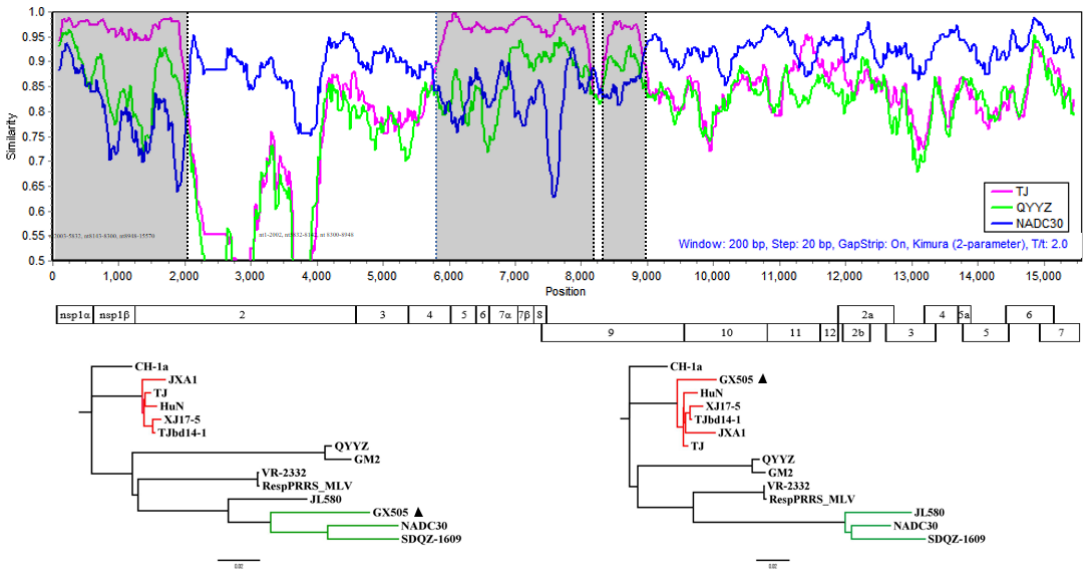

Figure 2 Recombination analysis of GX505. Recombination breakpoints are shown as black dashed lines. The background color of the major parental regions is white, whereas that of the minor parental regions is gray. Below the similarity plot is a full genome structure of PRRSV, with reference to CH-1a,shows the position of the ten ORFS and the fourteen Non-structural proteins. Phylogenetic trees based on different regions of the isolate are sown below the similarity plots. The major parental group is shown in green, the minor parental group is shown in red. The isolate GX505 is indicated by black triangle.

Production impact due to PRRS outbreak

All the fourteen productive parameters were negatively affected. Decreased parameters were identified for RTH, CR, FR, TB, BA, ABW, WP, and WW, while SM, ES, SB, MM, LBW, and PWM were increased (Table 1). Most of the changes were concentrated during months 1-5, and 13 parameters were most affected within 5 months after PRRS infection (Table 2).

Table 1 Main productive parameters changes following PRRS outbreak

Main productive parameters	Before	After	Difference
Sow mortality	0.59%	0.77%	+0.18%
Return to heat within 7 days after weaning (%)	88.40%	86.54%	-1.86%
Conception rate	91.94%	89.15%	-2.78%
Farrowing rate	87.74%	82.51%	-5.23%
Empty sows rate	4.50%	6.25%	+1.75%
Total born per litter (pig)	13.54	13.26	-0.28
Born alive per litter (pig)	12.43	11.60	-0.83
Average birth wight (kg)	1.35	1.29	-0.06
Stillbirth per litter (pig)	0.86	1.12	+0.26
Mummies per litter (pig)	0.29	0.41	+0.12
Low birth weight piglets per litter (pig)	0.36	0.64	+0.27
Pre-weaning mortality	5.47%	8.00%	+2.53%
Weaned pigs per litter (pig)	10.70	9.44	-1.26
28-days piglets weight (kg)	6.80	5.92	-0.87

Table 2 Duration of parameters changes following PRRS outbreak.

Main productive parameters	Months post-outbreak											
	1	2	3	4	5	6	7	8	9	10	11	12
Sow mortality												
Return to heat within 7 days after weaning												
Conception rate												
Farrowing rate												
Empty sows rate												
Total born per litter												
Born alive per litter												
Average birth wight												
Stillbirth per litter												
Mummies per litter												
Low birth weight												
Pre-weaning mortality												
Weaned pigs per litter												
28-days piglets weight												

Conclusions

Our study revealed that NADC30-like PRRSV infection imposed significant productive damage by reducing production performance of both breeding sows and sulking piglets. All the main production parameters analyzed were negatively affected. Time to baseline-production (TTBP), defined as time to recover to the number of pigs weaned per litter that herds had prior to PRRSV-detection, was used to assess time needed to return to PRRS stability. In this study, TTBP was 5 months (21 weeks) (Table 2).

Keywords: PRRS, NADC30, Productive parameters

Diagnostics-Epidemiology

Oral

Emerging of highly lethal PRRSV RFLP 1-4-4 L1C recombinant variant in China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is one of the most significant diseases affecting the pig industry worldwide and is caused by PRRS virus (PRRSV), which has complex genetic variation due to frequent mutations, indels, and recombination. The emergence of the PRRSV RFLP 1-4-4 L1C variant in 2020 has raised worldwide concern.

Materials and Methods

In this study, a PRRSV RFLP 1-4-4 L1C strain, HuN2021, was isolated from a pig farm that was experiencing abortion of pregnant sows and death of piglets; with a mortality rate of 30%, despite these sows having been vaccinated with PRRS MLV vaccine. The genomic features and pathogenicity to piglets of HuN2021 were evaluated.

Results

The HuN2021 was a recombinant strain between NADC30-like PRRSV (lineage 1C) and HP-PRRSV (lineage 8.7). The RFLP 1-4-4 L1C variant from the US was found to be an intra-lineage recombinant strain between NADC30-like PRRSV (lineage 1C) and NADC34-like PRRSV (lineage 1A). Therefore, despite HuN2021 and RFLP 1-4-4 L1C variants belonging to the same RFLP 1-4-4 pattern and L1C phylogenetic tree cluster, their viral origins are different. The SPF piglets challenged with HuN2021 showed severe clinical signs, including loss of appetite, ear cyanosis, dystaxia, and exhibited persistent fever throughout the entire experiment. All piglets (5/5) challenged with HuN2021 were died. Microscopic lung lesions showed marked thickening of the alveolar septa and lymphocyte infiltration in HuN2021-challenged piglets. Considering that HuN2021 is a recombinant variant of HP-PRRSV and NADC30-like PRRSV, we assessed the vaccine protective efficacy of HP-PRRS MLV and NADC30-like candidate MLV. The NADC30-like candidate MLV effectively alleviated the clinical signs, reduced the viral load, and demonstrated better protective efficacy.

Conclusions and Discussion

Currently, there are no commercial NADC30-like MLV in China. Therefore, it is crucial to monitor the epidemic of RFLP 1-4-4 L1C PRRSV and update control measures to prevent the further spread of the RFLP 1-4-4 L1C PRRSV.

Keywords: PRRSV; RFLP 1-4-4 L1C; recombination; pathogenicity; cross-protection

PRRSV genetic variability in the U.S.: how can lineages, sub-lineages and variants help us understand disease epidemiology

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The genetic diversity and frequent emergence of novel genetic variants of PRRSV-2 continue to challenge the industry. Geographical diversity further complicates control and current genetic classification methods have been insufficient for the purposes of epidemiological and on-farm monitoring, assigning the same name to unrelated viruses or creating groups of viruses that are too large and carry little fine-scale epidemiological meaning.

We gathered 21211 PRRSV sequences from the US and organized them based on their phylogenetic structure into lineages and sub-lineages (L/SL). Using Bayesian coalescent SkyGrid models, we analyzed the emergence, peak, and replacement frequencies of different L/SL. We also examined genetic variations in key GP5 sites among L/SL. To enhance resolution, we further divided L/SL into "variants" based on branch support and average patristic distance within clades.

Lineage 1 contains more than 60% of US sequences up to 2018 and more than 88% of sequences from 2019-2023. Due to its prevalence in the US, it is divided into eight sub-lineages (1A to 1J). New sub-lineages within lineage 1 emerged every 1-4 years, reaching peak population sizes after ~4.5 years. Sequential sub-lineage dominance was identified, replacements occurring every ~3 years. Consensus amino acid sequences varied in GP5 sites related to host immunity, suggesting that SL turnover may be due to immune-mediated competition. A variant classification scheme that divides PRRSV-2 in smaller phylogenetically related groups is proposed. A model that classifies sequences into variants was trained and is available but is intended for use in regions where substantial sequence data is available for model training.

Phylogenetic-based PRRSV classification allows for the identification of key epidemiological patterns. Sub-lineages identified within Lineage 1 differed in amino acid sites of the GP5 that are thought to be involved in the immune response to the virus. This may be key for developing better immunization strategies and vaccines. A classification scheme that groups sequences based on their genetic makeup may potentially allow for more cohesive research and communication with stakeholders, while providing opportunities to address questions related to the transmission of PRRSV-2 at a farm level.

Keywords: PRRSV, classification, epidemiology

PRRSV RNA detection patterns in individual sows over time based on tonsil scraping and TOSc results

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Introduction

Common antemortem sample types to detect PRRSV in sows include serum and tonsil scraping. Tonsil scraping was documented as capable of detecting long-term PRRSV carriers due to extended and localized virus RNA presence in lymphoid tissues. However, sampling using serum and tonsil scraping is time-consuming and labor-intensive. We developed a novel sow sampling tool, tonsil oral scrubbing (TOSc), adapted from a sow collector reported to be used for the test-and-removal of ASFV-infected sows in China. TOSc takes biological samples from the oral and tonsillar area within seconds without snaring the sows and shows comparable PRRSV RNA detection rates to tonsil scraping. While most studies on PRRSV dynamics were conducted on growing piglets, little information was known about how PRRSV RNA detection changes over time in commercial sow herds. The objective of this study was to characterize the pattern of PRRSV RNA detection in individual sows over time using tonsil scraping and TOSc samples.

Materials and Methods

The study was conducted in a breeding herd seeking PRRSV elimination. Herd adopted live virus inoculation (LVI) and herd closure after the outbreak. TOSc and tonsil scraping were collected at 30, 60, and 90 days post-LVI (dpi) from each of the 61 conveniently selected sows and tested for PRRSV RNA by RT-rtPCR.

Results

Based on tonsil scraping test results, from 30 dpi to 60 dpi, 21.3% (13/61) positive sows turned negative, while 7.9% (5/61) negative sows turned positive. From 60 dpi to 90 dpi, 52.5% (32/61) positive sows turned negative, while 8.2% (5/61) negative sows turned positive. On the other side, based on TOSc test results, from 30 dpi to 60 dpi, 39.3% (24/61) positive sows turned negative while 21.3% (13/61) negative sows turned positive. From 60 dpi to 90 dpi, 36.1% (22/61) positive sows turned negative while no negative sows turned positive.

Conclusions and Discussion

This study reports an intermittent pattern of PRRSV RNA detection in sows several months post LVI by both tonsil scraping and TOSc, similar to what had been described from suckling pig-based and from growing pigs-based testing. This study also highlights a need for continuous surveillance program on sows to better understand the PRRSV dynamics in sows. Given its ease of use and comparable performance to tonsil scraping in detecting PRRSV RNA, TOSc could be further explored for PRRSV detection and surveillance in sows in the field.

Keywords: PRRSV, tonsil scraping, TOSc, detection

Dissecting genetic diversity, evolutionary trends and pathogenicity of Chinese PRRSV-1

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Introduction

Porcine reproductive and respiratory syndrome has been highly detrimental to the swine industry. Previous studies classified PRRSV-1 into four subtypes: Western European Subtype I, Russian Subtype I, Subtype II, and Subtype III. All Chinese PRRSV-1 belong to Western European Subtype I. The objective of this abstract is to share the epidemiology and pathogenicity of Chinese PRRSV-1.

Materials and Methods

More than 3200 clinical samples were collected in China. PRRSV-1 positive samples was sequenced. Sequences were subjected to analysis by performing phylogenetic analysis, recombination analysis, positive selection analysis and estimation of the evolutionary rate. The PRRSV-1 virus (ZD-1) was isolated using PAM. And Cultures of the third passage in PAMs were used for animal experiments.

Results

Chinese PRRSV-1 subgroup has expanded and there are significant differences in the complete genome among Chinese PRRSV-1 from different pig farms. Chinese PRRSV-1 can be divided into seven subgroups and BJEU06-1-Like PRRSV has been the predominant strains. BJEU06-1-Like and NMEU09-1-Like strains have extensively participated in recent recombination events. The mean rate of Chinese PRRSV-1 were 4.11×10-3 substitutions/site/year. The analysis of positive selection indicated that BJEU06-1-Like PRRSV possessed more combinations of amino acid physicochemical properties in the positively selected sites of the GP5 ectodomain than other PRRSV-1 strains from China. Animal experiments suggested that the BJEU06-1-like PRRSV-1 strain ZD-1 is moderately pathogenic to piglets.

Conclusions and Discussion

The genetic diversity of PRRSV-1 strains from China has increased. BJEU06-1-Like PRRSV has been the predominant strains. The recombination patterns of PRRSV-1 strains from China are quite complex. PRRSV-1 strains from China possesses a high mutation rate. The pathogenicity of PRRSV-1 strains from China is generally low, but some strains exhibit moderate pathogenicity.

Keywords:PRRSV-1, epidemiology, pathogenicity

Posters

Development of a quadruplex TaqMan real-time PCR system for simultaneous differentiation of HP-PRRSV-like, PRRSV NADC30-like, NADC34-like and PRRSV-1

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Introduction

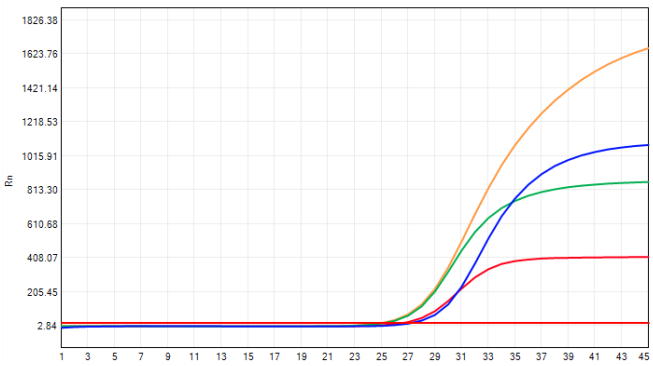
Porcine reproductive and respiratory syndrome (PRRS), commonly known as "Blue ear disease", is an acute infectious disease caused by PRRS virus (PRRSV), with reproductive disorders in pregnant sows and respiratory symptoms in growing pigs. PRRSV belongs to the genus *Arterivirus* of the family *Arteriviridae*, which is a single-stranded positive-sense RNA virus. According to the latest classification of ICTV, PRRSV is divided into two species: *Betaarterivirussuid* 1 (PRRSV-1) and *Betaarterivirussuid* 2 (PRRSV-2). PRRS is one of the most economically devastating viral diseases in the global pig industry. In 1987, the first case of PRRS was reported in North America, and PRRSV was first isolated from abortion samples in China in 2006. Nowadays, PRRSV-2 is the mainly prevalent virus in China, among which highly pathogenic PRRSV-like (HP-PRRSV-like), NADC30-like and NADC34-like are the dominant viruses in the field. Meanwhile, PRRSV-1 and co-infection of the two species exist on pig farms, leading to great obstacles for the prevention and control of PRRS. Therefore, differential diagnosis between different lineages of PRRSV-2 or PRRSV-1 and PRRSV-2 is of great significance for the prevention and control of PRRS.

Materials and Methods

The genome sequences of four strains of PRRSV were downloaded from GenBank database, and multiple sequence alignment analysis was performed to screen conserved gene sequences. According to these sequences, specific primers and TaqMan probes for four strains of PRRSV were designed and synthesized. To prepare the standard positive controls, four PCR products of the quadruplex assay were cloned into the pUC57 vector, respectively and transformed into DH5α chemically competent cells according to the standard procedures. The recombinant plasmids were purified and quantified. Then, the plasmids were diluted with ddH2O to obtain the stock solutions containing 10^9 copies of each standard plasmid DNA per microliter. The standard curves for quadruplex PRRSV real-time PCR assays were generated using 10-fold dilutions (10^3–10^7 copies/μL) of each standard plasmid. The sensitivity of the quadruplex RT-qPCR assay was determined by detecting serially diluted 10-fold standard plasmids. By optimizing the detection conditions (concentrations of primers and probes, concentrations of Mg^{2+} and dNTP, concentrations of Taq DNA polymerase and M-MLV reverse transcriptase, PCR reaction procedure and volume), finally, a quadruplex RT-qPCR system for simultaneous differentiation of four PRRSV strains was established and was preliminarily verified in the laboratory.

Results

A quadruplex RT-qPCR system has been established for simultaneous detection and differentiation of HP-PRRSV-like, NADC30-like, NADC34-like, and PRRSV-1 strains. In this system, the final concentration of primers was 0.3 μM, the final concentration of probes was 0.15 μM, the total reaction volume was 30 μL, the final concentrations of Mg^{2+} and dNTP were 2.5 mM and 2 mM, respectively. The final concentrations of Taq DNA polymerase and M-MLV reverse transcriptase were 0.15 U/μL and 0.2 U/μL, respectively. The optimal annealing temperature was 56 °C, and the number of cycles was set to 45. Recombinant plasmids containing partial genes of different viruses were used, and the standard curves of the four strains were constructed. The qPCR amplification efficiency of the four strains was greater than 95%, and the R^2 was greater than 0.99. The limit of detection of HP-PRRSV-like, NADC30-like, NADC34-like and PRRSV-1 were 500 copies/mL, 1000 copies/mL, 1000 copies/mL and 500 copies/mL, respectively.



Amplification curves of four recombinant plasmids.

FAM (blue): HP-PRRSV-like, HEX (green): PRRSV NADC30-like, ROX (orange): PRRSV NADC34-like, CY5 (red): PRRSV-1

Conclusion

This study has preliminarily established a quadruplex RT-qPCR system for simultaneous differentiation of three major strains of PRRSV-2 (HP-PRRSV-like, NADC30-like, NADC34-like) and PRRSV-1 strains with an ideal sensitivity and reliability. The clinical performance of the newly developed system will be further verified by clinical samples in our next work. The development of differential diagnosis system for the main epidemic strains of PRRSV can provide an effective tool for pig farms to monitor PRRSV infection and implement prevention and control strategy.

Keywords

PRRS, PRRSV-1, PRRSV-2, HP-PRRSV-like, NADC30-like, NADC34-like, RT-qPCR

Differential Diagnosis and Control of PRRS Complicated by Haemophilus parasuis and Streptococcus suis Infection

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Introduction

Since Porcine reproductive and respiratory syndrome virus (PRRSV) was first isolated in China in 1996, various strains have been reported to be circulating, including classic and highly pathogenic (HP-PRRSV) and NADC30-like and NADC34-like[1]. At present, mixed or concurrent infection of PRRSV with *Haemophilus parasuis* (HPS), *Streptococcus suis* (SS) and other bacteria has become a common phenomenon in pig farms[2-3]. In November 2022, a large-scale pig farm in Anhui Province with 2000 sows was immunized with PRRSV live vaccine (CH-1R strain), and sows and piglets aged 40 to 50 days suffered from an outbreak of disease. The symptoms were mainly manifested as high fever, depression, dyspnea, loss of appetite or cessation of food, with an incidence of about 15%. The case fatality rate is about 8%.

Materials and Methods

In order to identify the pathogens causing respiratory symptoms in a large-scale swine farm，and to develop a scientific prevention and control plan，serum and lung samples were collected to detect antibodies and antigens of common respiratory pathogens，meanwhile，bacteria isolation，identification and typing were carried out，then drug susceptibility test was conducted for the isolates.

Results and discussion

Antibody detection results showed that the positive rates of antibodies against porcine reproductive and respiratory syndrome virus(PRRSV)，porcine circovirus type 2(PCV2)and classical swine fever virus(CSFV)were 60.0%(45/75)，98.67%(74/75)and 97.33%(73/75)，respectively. The results of virus nucleic acid test showed that the positive rate of PRRSV was 36.0%(9/25)，both PCV2 and CSFV were detected negative. The results of bacterial isolation and identification showed that the morphological characteristics of the cultured bacteria conformed to those of *Haemophilus parasuis*(HPS)and *Streptococcus suis*(SS). The results of bacteria typing showed that highly pathogenic PRRSV(HP-PRRSSV)and NADC30-like PRRSV were contributive to the infection；HPS and SS were determined as serotypes 12 and 9，respectively. The drug sensitivity test results showed that HPS was more sensitive to doxycycline，ceftriaxone and cefotaxime，while SS was more sensitive to penicillin，ceftriaxone，cefotaxime and cephalexin. In conclusion，the outbreak in the farm was caused by coinfection of HP-PRRSV，NADC30-like PRRSV，HPS-12 and SS-9. As the classical vaccine strain of PRRSV CH-1R immunized in the farm failed to match with the isolate，and the dispersion of antibodies after immunization was large，resulting in low protection effect of pigs，then immune suppression complicated by HPS and SS infection，followed by a large number of infected or dead pigs. The outbreak was effectively controlled through strengthening biosecurit，implementing comprehensive emergency vaccination with PRRSV live and inactivated vaccines，and administration of sensitive drug，ceftiofur. A successful experience was provided for

future differential diagnosis and precise prevention and control of co-infection with PRRSV and other pathogens in large-scale swine farms.

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Repeat Offenders: understanding PRRSV clinical re-breaks

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Introduction

Immunization-based control efforts on sow farms exist to ensure that herds have been exposed to and developed immunity against the "resident" PRRSV variants. However, repeated clinical outbreaks of single PRRSV variants do occur, which suggests that the virus has changed, allowing it to reinfect a herd with preexisting immunity. The objective of this study is to identify clinical re-breaks of near identical PRRSV variants on single sow farms and to compare the variants' whole genomes for identification of patterns of change that could be related to viral immune escape.

Methods and Materials

A US system provided weekly abortion counts and diagnostic data (surveillance CT values and ORF5 sequences) for sow farms from 2014 to 2023. A "clinical break" is defined as weekly abortion counts above the weighted average baseline from EWMA analysis with concurrent dropping processing fluid PCR CT values. A clinical "re-break" is defined as above with additional criteria: 1) the second clinical break must occur within 3-12 months of a previous break, and 2) the ORF5 gene from the second clinical break must be ≥97% similar, at the nucleotide level, to the first clinical break. Upon fulfilling these criteria, viral isolates from these breaks were utilized for whole genome sequencing and comparison.

Results

Viral isolates were from 13 clinical re-breaks on 12 farms between 2017 and 2023. The median genetic distance between rebreak pairs was 1.82% (IQR: 0.67-3.62%) across the whole genome. The median days elapsed between each sample was 213.5 days (IQR: 139.5-230.8). Amino acid sequence comparisons for GP5 revealed positions 32, 33, 58, 94, 104, and 121 substituted more frequently in re-break pairs than expected amongst closely related viruses. Substitutions in other structural proteins were also present in a high number of re-break pairs, though no expected values could be calculated.

Conclusions

In this analysis, we characterize genomic differences that frequently accrue *between* re-break events on single farms. These include numerous sites that have been identified experimentally to underly immune escape *in vitro* and *in vivo*. To our knowledge, this is amongst the first evidence that sites associated with immune escape experimentally also occur in field clinical scenarios where immune escape is suspected.

Molecular epidemiological characteristics of porcine reproductive and respiratory syndrome virus circulated in Gansu, China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is caused by porcine reproductive and respiratory syndrome virus (PRRSV), an endemic virus that

significantly impacts the swine industry, remaining one of the most devastating diseases of swine globally. Recombination and mutation, the two common phenomena in RNA viruses, are undesirable events that constantly alter the genome sequence and may enhance PRRSV virulence. The prevention and control of PRRS is becoming increasingly difficult, as novel PRRSV variants keep emerging. The new strains, especially NADC30-like strains, may cause severe symptoms and continuously challenge the pig industry. Given the global concerns surrounding the continuous emergence of PRRSVs, comprehensive monitoring of the whole genome, is imperative. Here, we report the information of ORF5 gene and whole-genome of field PRRSV strains detected in 2020~2023 in Gansu, which will enrich our knowledge on PRRSV epidemiology and contribute to the prevention and control of PRRSVs.

Materials and Methods

Fifty-two clinically suspected PRRSV samples were collected from different farms in 2020~2023 in Gansu, China. Sanger sequencing approach was used for the amplification of ORF5 gene and the full-length genome. Phylogenetic trees were constructed with the neighbor-joining method by MEGA software (MEGA11, USA). Recombination events were initially detected by Recombination Detection Program version 4.67 (RDP4). Potential recombination was further verified by SIMPLOT (version 3.5.1, USA). ClustalW was conducted with Lasergene software (DNASTAR Inc., Madison, WI, United States) to achieve amino acid alignment.

Results

Phylogenetic analysis

In this study, 30 clinical samples were confirmed as PRRSV positive by ORF5- targeted RT-PCR. Phylogenetic analysis of ORF5 gene showed that all 30 strains from Gansu were belonged to PRRSV-2 genotype, among which including sublineage 1.5 (4 strains), sublineage 1.8 (22 strains), sublineage 5.1 (2 strains), and sublineage 8.7 (2strains) (Fig.1). The sequence identity between strains in sublineage 1.5, sublineage 1.8, sublineage 5.1 and sublineage 8.7 were 97.5-98%, 84.9-96.4%, 99.2% and 98%, respectively (results not showed).

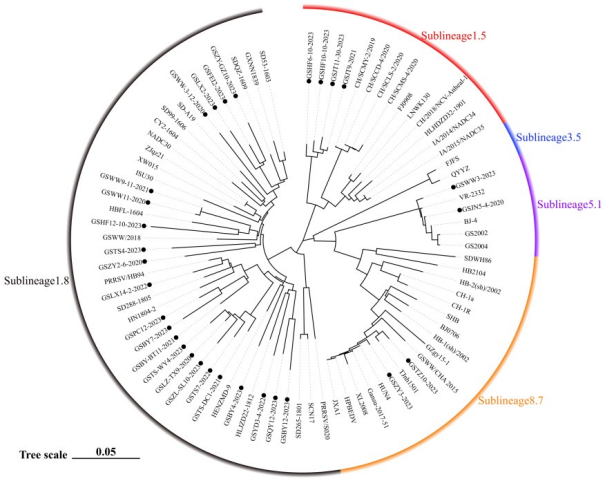

Fig.1. Phylogenetic analysis of ORF5 fragments obtained in Gansu with 52 reference strains. Sublineage 1.5, sublineage 1.8, sublineage 3.5, sublineage 5.1 and sublineage 8.7 were marked with red, black, blue, purple and orange respectively. Strains obtained in Gansu were annotated with “ ”.

Amino acid alignment of NSP2 protein

For NSP2 protein, most strains exhibited similar characteristics, with a total length of 3195 bases and encoded 1064 amino acids (results not showed). Notably, a unique amino acid deletion pattern (1+1+101+1+19) formed with 133 amino acids was characterized and scattered into five different positions in NSP2 protein of GSQY12- 2023, among which the 15th deletion of GSQY12-2023 was consistent with those in strains from the sublineage 8.7, and the 308th amino acid deletion was novelly detected in NSP2 protein in PRRSV (Fig. 2).

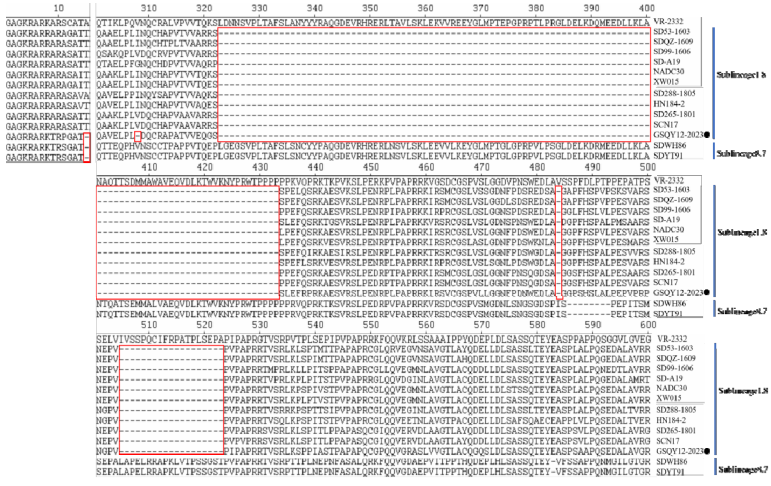

Fig.2. Alignment of the translated amino acid sequence of the NSP2 protein among PRRSVs within sublineage 1.8 and sublineage 8.7 (not fully displayed). GSQY12-2023 was annotated with “ ”.

Whole-genome sequencing and recombination analysis

Considering the high possibility of recombination in NADC30-like PRRSVs, the whole-genome of five NADC30-like strains (including GSTS4-2023, GSLX2-2023, GSFEI2-2023, GSBY4-2023 and GSQY12-2023) were amplified and sequenced for recombination analysis. The complete sequences of GSTS4-2023, GSLX2-2023, GSFEI2-2023, GSBY4-2023 and GSQY12-2023 were 15061 nt, 15025 nt, 15014 nt, 14981 nt and 15032 nt in length respectively, including 5′ untranslated region and 3′ untranslated region.

Recombination signal was detected for all five strains, and at least
6 detection methods in RDP software showed a high degree of reliability in recombination, with p-values lower than 4.554×10-6, exhibiting a high extent of intra- lineage recombination (results not showed). As expected, NADC30 was the major parent of the five strains, and the minor parents were JXA1-like strains, VR-2332-like strains and QYYZ-like strains. Simplot analysis was performed to confirm the results from RDP4. Analyses results showed that four recombination breakpoints were detected in GSTS4-2023, which divided its whole genome into four segments related to NADC30-like strains and JXA1-like strains (Figure 3A). In addition, six breakpoints were revealed in GSLX2-2023, which is a recombinant product of JXA1-like strains and VR-2332-like strains (Figure 3B). In addition, two breakpoints were identified within GSFEI2-2023, which separated its whole genome into three parts related to NADC30-like strains and VR-2332-like strains (Figure 3C). Interestingly, six breakpoints were identified in GSBY4-2023, and the recombination events were between NADC30-like strains, JXA1-like strains and QYYZ-like strains (Figure 3D). Moreover, eight recombination breakpoints were detected in GSQY12-2023 and divided the whole genome into nine regions related to JXAI and NADC30 respectively (Fig. 2a~c). Collectively, all of the five strains are recombinant strains with different recombination patterns, which were displayed in Fig.3F.

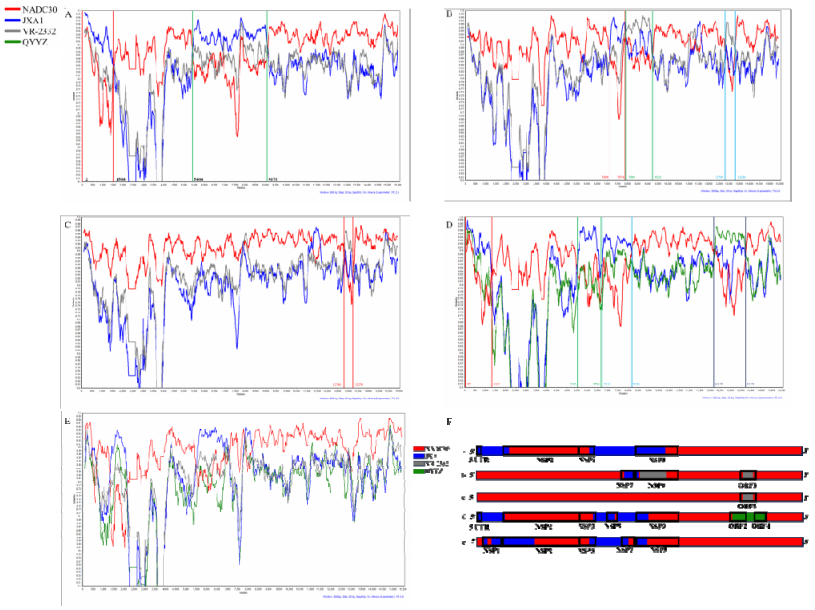

Fig.3. Recombination analysis of GSTS4-2023 (A), GSLX2-2023 (B), GSFEI2-2023 (C), GSBY4-2023 (D) and GSQY12-2023 (E). The parent strains were NADC30 (red), JXA1 (blue), VR-2332 (grey) and QYYZ (green). Recombination events are marked within lines with identical color, and the recombination breakpoints are annotated with the same colors at the bottom with nucleotide positions consistent with VR-2332. (F) Simplified illustrations showing the location of the recombination breakpoints. (a) GSTS4-2023. (b) GSLX2-2023. (c) GSFEI2-2023. (d) GSBY4-2023. (e) GSQY12-2023.

Conclusions

Simultaneous circulation of PRRSV-2 within multiple lineages was identified in Gansu, among which strains of sublineage 1.8 (NADC30-like strains) may be the main epidemic strains.A unique deletion pattern of NSP2 protein was characterized in detail, which may contribute to the evolution of PRRSVs.Most NADC30-like strains were recombinant products, which exhibited different genomic characteristics and recombinant patterns in Gansu.

References (Omitted**)**

A preliminary study on the the relationships between the severity of pulmonary gross lesions, histological changes and and viral quantities in PRRS

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Introduction：

PRRS is the most serious infectious disease in the global pig industry.
The diagnosis of PRRS can be established by the clinical examination and PCR and histopathology. In this study, several clinical cases were used to study the correlation between the severity of pulmonary gross lesions, histological changes and viral quantities.

Materials and methods：

In the four field cases, the diagnosis of PRRS was made by the clinical examination, PCR and histopathology. In case 1, the lung was grossly unremarkable；In case 2, the lung was affected by mild lesions；In case 3, the lung was grossly affected by moderate interstitial pneumonia；In case 4, the lung was affected by severe interstitial pneumonia.Histopathology and quantitative real time PCR for PRRSV was conducted on all 4 cases.

Results:

In case 1, the quantity of PRRSV was 1.0E+05 copies/uL (Ct=25.20); However, no obvious microscopic lesions were observed in the lungs. In case 2, the quantity of PRRSV was 4.7E+06 copies/uL (Ct=17.86); In histopathology, there were some eosinophilic necrotic materials in the alveoli, and the degree of histological lesions was assessed to be mild to moderate. In case 3, the PCR was negative. In histopathology, there were prominent necrotic material the alveoli, and the degree of microscopic lesions were moderate. In case 4, the quantity of PRRSV was 4.7E+06 copies/uL (Ct=17.10); In histopathology, there was severe necrotic interstitial pneumonia.

Conclusion:

The viral quantities do not necessarily correlate to gross or histological lesions, but the agreement between gross and histological changes are good. When the PCR was positive but there were no obvious histological changes, the pigs were probably still protected by maternal antibodies, or the PCR might have detected vaccine virus. When necrotic interstitial pneumonia was present but PCR was negative, the viremia might have been gone, or there could be other etiologies causing similar lesions. These observations emphasize the importance of using different methods to achieve accurate diagnoses.

Keywords：

PRRS,pulmonary gross lesions,histological changes,viral quantities

Metatranscriptomic sequencing analysis and identification of an example of co-infection with European-type and North American-type porcine reproductive and respiratory syndrome

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Introduction

A pig farm in Guangxi conducted sampling inspection on piglets in the farrowing room, which were then sent to the High-Throughput Disease Testing Center at South China Agricultural University for high-throughput Metatranscriptomic sequencing. Targeted analysis of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) was performed to reveal the infection status of PRRSV, providing a basis for the prevention and control of PRRSV.

Materials and Methods

The collected samples in this study included umbilical cord blood, castration fluid, and serum samples from a total of 14 piglets. These samples were combined into four high-throughput sequencing samples based on the mixing in the pigpen.The sequencing platform used was MGI.

Results

The results showed the simultaneous detection of multiple lineages of North American-type PRRSV and BJEU06-1-like European-type PRRSV within this pig farm.Phylogenetic analysis of whole genomes and ORF5 genotyping showed consistent lineage results. Interestingly, different pigpens exhibited variations in the detected North American-type lineages, with instances of different lineages even within the same pen. The detected North American-type lineages comprised lineage 1 (representing strains such as NADC30) and lineage 5 vaccine strains (VR2332). Recombination analysis revealed complex recombination events in the detected viral sequences. Apart from recombination events between the two prevalent lineages, additional recombinations involving lineage 3 (GM2) and highly pathogenic blue ear disease lineage 8.7 (JXA1 and CH-1a) were identified within the farm.

Conclusions and Discussion

The prevalent strains in this pig farm were determined to be recombinants of vaccine and endemic strains. The farm conducts routine monitoring for PRRS to ensure timely detection and control of potential epidemic spread. Additionally, the pig farm needs to strengthen the management and isolation of the pig population to reduce the risk of viral transmission and spread. Furthermore, strengthening collaboration with disease detection centers, enhancing monitoring, and sharing information are crucial for timely responses to potential outbreak scenarios, thereby minimizing the impact of outbreaks on the pig farm.

Keywords: Piglets, Metatranscriptomic sequencing, PRRSV, Lineage, Recombination

Evaluation of commercial qPCR kits for detection of PRRSV-1 and PRRSV-2 strains in China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economically significant diseases affecting swine production, with PRRSV-1 and PRRSV-2 strains circulating in China. This study investigates the performance of a newly designed multiplex qPCR assay for detection and differentiation of PRRSV-1 and PRRSV-2 using characterized PRRSV strains from China.

Materials and Methods

A total of eight well-characterized PRRSV samples were collected. Samples included PRRSV-2 NADC30-like, PRRSV-2 NADC34-like, PRRSV-2 QYYZ-like, PRRSV-2 CH1a, PRRSV-2 HP, PRRSV-2 MLV and two different PRRSV-1 isolates.

Samples were tested using RealPCR PRRSV Type 1 and Type 2 Multiplex RNA Test (IDEXX qPCR), and results compared with ten commercial qPCR kits available in China for detection of PRRSV-1 and PRRSV-2. Samples were also tested using five in-house qPCR methods used in the big pig farm for diagnosis of PRRSV in China.

Results

IDEXX qPCR correctly identified the six PRRSV-2 and two PRRSV-1 Chinese strains (Ct values 22-33). Other commercial qPCR kits failed to correctly detect all the samples tested: five kits missed one sample, two kits missed two samples, one kit missed three samples and two kits missed four samples. Compared with IDEXX qPCR, other commercial kits showed later Ct values when testing this set of samples. Similarly, none of the in-house qPCR methods correctly identified all the samples: one in-house qPCR missed one sample, another method missed two samples, two in-house methods missed 3 samples and another method missed five samples.

Conclusions and Discussion

The comparison of commercial qPCR assays for detection of PRRSV-1 and PRRSV-2 showed that IDEXX qPCR reliably detected all of the Chinese PRRSV strains included in this study. Detection of circulating PRRSV strains should be prioritized when selecting qPCR assays to support effective management and control strategies against PRRSV.

Keywords: PRRS, PRRSV, PCR, qPCR, RT-qPCR

Prevalence, Time of Infection, and Diversity of Porcine Reproductive and Respiratory Syndrome Virus in China

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine viral pathogen that affects the pig industry worldwide. Control of early PRRSV infection is essential, and different types of PRRSV-positive samples can reflect the time point of PRRSV infection. This study aims to investigate the epidemiological characteristics of PRRSV in China from Q4 2021 to Q4 2022, which will be beneficial for PRRSV control in the swine production industry in the future.

Materials and Methods

An active cross-sectional study was performed in 100 intensive pig farms (ranging from 500 to 6000 sows) from 21 provinces in China. The investigated farms covered all five pig production regions classified by the Ministry of Agriculture and Rural Affairs in China. A total of 7518 samples, which included three types of samples (i.e., processing fluid, weaning serum, and oral fluid from 437 batches/farms. RNA was extracted from all samples by using extraction kits (TianLong Science and Technology Co., Ltd., Xi'An, China). Real-time PCR was performed to detect the presence of PRRSV nucleic acids by using a commercial PRRSV RT-PCR detection kit (VetMAX™ PRRSV EU & NA 2.0 Kit; Thermo Fisher Scientific, Waltham, MA, USA). The sequences with mixed or overlapped signals were implemented into multiplex RT-PCR for the differential detection within lineages. All PCR-positive samples with Ct values less than 30 were collected and sent to Sangon Biotechnology Co., Ltd., Shanghai, China for ORF5 sequencing aiming to differentiate of modified live vaccine with wild PRRSV.

Results

Independent of sample type, 24% (1780/7518) of the total samples were PCR-positive for wild PRRSV. The rate of wild PRRSV-positive batches was 58% (254/437). On the basis of the time of infection, 49% batches of 3-5-days-old and 25% 3-4-weeks-old piglets showed PRRSV infection before weaning age. The sequencing analysis results indicate a wide range of diverse PRRSV wild strains in China, with lineage 1 (60%) as the dominant strain.

Conclusions and Discussion

The study demonstrates a high prevalence of wild PRRS in batches and samples in China. It also confirms a high early PRRSV infection rate and offer comprehensive information on PRRSV diversity.

Keywords: PRRSV; prevalence; time of infection; wild strain diversity

Reference: Li C, Fan A, Liu Z, Wang G, Zhou L, Zhang H, Huang L, Zhang J, Zhang Z, Zhang Y. Prevalence, Time of Infection, and Diversity of Porcine Reproductive and Respiratory Syndrome Virus in China. Viruses. 2024 May 13;16(5):774. doi: 10.3390/v16050774. PMID: 38793655; PMCID: PMC11125865.

Tail blood swabs can be utilized for precise localization of PRRSV-positive litters during the perinatal period in order to enhance the effectiveness of PRRS elimination programs

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major swine viral pathogen that affects the pig industry worldwide. Implementing test and remove intervention strategies when approaching positive stable (2) status, the time required to achieve stability may be reduced. Currently, newborn piglet blood, and umbilical cord tissue/blood are commonly used samples for identifying positive PRRSV during the perinatal period. However, collecting blood from newborn piglets may cause invasive damage and compromise internal biosecurity management. Additionally, obtaining umbilical cord blood is labor-intensive and prone to cross-contamination. Therefore, it is crucial to identify a sample type that is both easy to use and capable of precisely localizing PRRSV-positive litter. In this study, tail blood swabs were collected alongside umbilical cord blood samples from sows during the perinatal period in order to compare their respective correlation with PRRSV detection rates.

Materials and Methods

Tail blood swabs and umbilical cord blood samples (5-6 piglets per litter) were collected from 23 sows housed in the same farrowing room in a weekly batch production farm. The umbilical cord blood samples, pooled based on litter, were promptly sent to the laboratory within 24 hours for PRRSV RT-PCR detection. A self-developed PRRSV RT-PCR method was employed with a cut-off Ct-value of below 37 considered as positive.

Results

The detection rates for tail blood swabs and umbilical cord blood samples were found to be 34.7% and 30.4%, respectively. All sows with negative tail blood swabs also tested negative for umbilical cord blood samples, while all sows with positive umbilical cord blood samples had corresponding positive results in tail blood swabs analysis. The coincidence rate between both sample types was determined to be 95.2%.

Conclusions and Discussion

In PRRS elimination process, timely identification and precise elimination of PRRS-positive litters before implementing cross-fostering practices can reduce the risk and frequency of horizontal transmission in farrowing rooms, thereby shortening the time required to achieve stability. Collection of umbilical cord blood samples entails higher labor costs and potential operational errors; alternatively, sow tail blood swabs may be selected to guide and locate positive sows and their litters. However, it should be noted that the limited sample size in this study may impose certain limitations on drawing definitive conclusions. Further studies with larger sample sizes are warranted to statistically analyze the correlation between these two types of samples.

Keywords: PRRSV, Tail blood swabs, precise elimination, test remove

Genomic Characterization of HLJDZD55: The First L1B PRRSV in China

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) critically threatens the pig industry in China. Lineage 1 PRRSV, which is divided into L1A-L1F and L1H-L1J, is widely recognized as the most extensively genetically diverse lineage globally. L1A (NADC34-like) and L1C (NADC30-like) PRRSVs have become the current major circulating strains in China. Notably, strains from other branches of L1 have not been reported in China. During our epidemiological investigation of PRRSV, we identified a new strain named HLJDZD55. The objective of this abstract is to share the genomic characteristics of newly discovered PRRSV.

Materials and Methods

In 2023, blood and lung samples were collected from approximately 20% of piglets with respiratory symptoms at a pig farm in Heilongjiang Province. Five samples (three serum samples and two lung samples) were collected from the piglets. The samples were processed for RNA extraction, cDNA preparation, RT-PCR and genome sequencing. DNAStar, MEGA7.0, Simplot(v3.5) and other biological software were used for sequence splicing, alignment, recombination analysis and phylogenetic analysis.

Results

We selected one of the strongly positive samples (from the lungs), HLJDZD55, for further study. Phylogenetic analysis of the ORF5 gene revealed that HLJDZD55 belongs to the L1B branch. Alignment of deduced amino acid sequences based on the NSP2 gene indicated that HLJDZD55 has a discontinuous deletion of 131 amino acids (111+1+19). Phylogenetic analysis based on the whole-genome sequence revealed that HLJDZD55 belongs to the L1C branch. Recombination analysis of the whole genome demonstrated that HLJDZD55 is a recombinant strain of TJZH-1607 (L1C, identified in China) and Minnesota 14 (L1B, identified in the USA).

Conclusions and Discussion

HLJDZD55 is a newly emerged lineage 1 PRRSV in China, which may have been introduced from the U.S. strain and subsequently recombined with the local Chinese strain and underwent evolution. Taken together, these results demonstrated the emergence of L1B PRRSV in China for the first time.

Keywords: L1B PRRSV, recombination, novel strain, genomic characteristics

Pathogenicity studies of Two Recombinant Viruses of the Porcine Reproductive and Respiratory Syndrome Virus in China

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Introduction

At present, a variety of recombinant variants of porcine reproductive and respiratory syndrome virus (PRRSV) have emerged in China, and their epidemic rules and pathogenicity are different, which has increased the difficulty of prevention and control of PRRS as a whole. In this study, we analyzed the whole gene sequence and recombination pattern of two PRRSV strains (JS-2020-1 and ZJ-2021-1) isolated from different pig farms and found that JS-2020-1 is a recombinant between the JXA1-like and NADC30-like strains and ZJ-2021-1 is the result of recombination between JXA1-like, QYYZ-like, VR2332-like and NADC30-like strains circulating in China. Postmortem examinations underscored the typical lung pathology associated with PRRSV, indicating that the lungs were the primary affected organs. Animal experiments revealed discrepancies in virulence between JS-2020-1 and ZJ-2021-1. The recombinant strain with JXA1 as the parent is more pathogenic than the recombinant strain with NADC30 as the parent. In summary, this study could provide critical insights into preventing and controlling PRRSV in this region.

Materials and Methods

Our laboratory had collected pig tissue homogenate suspensions from several large-scale pig farms in the Yangtze River Delta region and obtained the PRRSV using traditional virus isolation methods.

Homology analysis, phylogenetic analysis, and recombination analysis were performed on the strain using software such as MEGA, RDP4, and SIMPLOT.

To evaluate the pathogenicity of the recombinant strains, nine 30-day-old healthy piglets with negative PRRS antigen and antibody were divided into three groups with three piglets in each group and raised separately in a biosafety room to avoid mutual interference. Two groups were inoculated with two different identification strains (JS-2020-1 and ZJ-2021-1), the dose was intramuscular injection of 2 mL/pig and nasal drop of 2 mL/pig ($10^{2.5}$ copies/mL) and the negative control group was inoculated with the same volume of DMEM. Based on the clinical symptoms and viremia of piglets, we comprehensively assessed the pathogenicity of the recombinant virus strain.

Results

The comparative analysis of the whole genome sequence showed that JS-2020-1 shared the highest homology with NADC30 (92.6%) and ZJ-2021 shared the highest homology with JXA1 (86.7%).

Regarding the restructuring situation，JS-2020-1 is a recombinant between the JXA1-like and NADC30-like strains and ZJ-2021-1 is the result of recombination between JXA1-like, QYYZ-like, VR2332-like and NADC30-like strains circulating in China.

Regarding pathogenicity experiments，the ZJ-2021-1 strain included a persistent fever (>40°C) from 2 to 5 days post-inoculation (dpi), with a peak of 40.7°C at 5□dpi. The JS-2020-1 group began to drop in body temperature after 5 dpi and continued until the end of the experiment. All ZJ-2021-1-infected pigs died at 14 dpi, 15 dpi and 20 dpi, whereas no mortality was observed in JS-2020-1-infected group.

Regarding the virus content in the nasal secretions of the infected group, the virus content in the ZJ-2021-1 group

continued to increase and reached the peak at 14 dpi (1.34□×□10^3 copies/mL). The results revealed that the lungs exhibited the highest viral load (1.9□×□10^6 copies/mL), whereas the lowest viral load was detected in the kidney (1×□10^4 copies/mL) of the ZJ-2021-1 group. In contrast, only lower levels of viral load were detected in the JS-2020-1 group.

Conclusions and Discussion

By analyzing the recombination patterns and pathogenicity of two separate PRRSV strains, it can be concluded that the recombinant framework of ZJ-2021-1 is JXA1 with a high mortality rate; the recombinant framework of JS-2020-1 is NADC30 with low pathogenicity. The pathogenicity of the recombinant strain has similar virulence characteristics to its parental skeleton. Conventional ORF5 assays may be inaccurate for the errors in the analysis of recombinant strains. Therefore, in the face of the current trend of PRRSV strain recombination, it is recommended that the whole genome sequencing analysis of the detected strains or the comprehensive sequencing analysis of multiple PRRSV genes, such as ORF1a, ORF5 and ORF6, should be performed to provide a strong reference for more accurate identification of PRRSV strains.

Keywords: PRRS、genetic recombination、phylogenetic analysis、pathogenicity

Genomic characteristics of a novel emerging PRRSV branch in sublineage 8.7 in China

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Introduction

During the continuous monitoring of PRRSV, a new PRRSV strain type with novel characteristics was first identified in three different regions of Shandong Province. These strains presented a novel deletion pattern (1+8+1) in the NSP2 region and belonged to a new branch in sublineage 8.7 based on the ORF5 gene phylogenetic tree.

Materials and Methods

In 2020–2022, 31 clinical samples of pigs suspected of PRRSV infection, including lung, lymph node and serum samples, were collected from farms in three different regions of Shandong Province in northern China. Seven pairs of overlapping primers were designed based on sublineage 8.7 and used to amplify the complete genome. Ultimately, the entire genome sequence was meticulously analyzed through sequence alignment, phylogenetic analysis, and recombination analysis.

Results

Based on the phylogenetic analysis of the whole genome, these strains formed a new independent branch in sublineage 8.7, which showed a close relationship with HP-PRRSV and intermediate PRRSV according to nucleotide and amino acid homology but displayed a completely different deletion pattern in NSP2. Recombinant analysis showed that these strains presented similar recombination patterns, all of which involved recombination with QYYZ in the ORF3 region. Furthermore, we found that the new-branch PRRSV retained highly consistent nucleotides at positions 117–120 (AGTA) of a quite conserved motif in the 3’-UTR; showed similar deletion patterns in the 5’-UTR, 3’-UTR and NSP2; retained characteristics consistent with intermediate PRRSV and exhibited a gradual evolution trend.

Conclusions and Discussion

In summary, we identified a new type of PRRSV strain with novel deletion patterns in the 5′-UTR, 3′-UTR and NSP2 regions, which showed the same origin and formed a new independent branch in sublineage 8.7. They may be similar to HP-PRRSV also evolved from intermediate PRRSV, but represent separate strains that evolved simultaneously with HP-PRRSV. In addition, they have undergone rapid evolution and recombined with other strains and have the potential to become epidemic strains, which should receive more attention.

Keywords: PRRSV, new branch, sublineage 8.7, 1+8+1, genomic characteristics

Prevalence and genetic evolution of porcine reproductive and respiratory syndrome virus in commercial fattening pig farms in China

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Introduction

To investigate the prevalence and evolution of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) at commercial fattening pig farms, PRRSV monitoring was carried out on seven commercial fattening pig farms in five provinces of China for a single fattening cycle (approximately 150 days), and a total of 1397 clinical samples were collected from 2020 to 2021.

Materials and Methods

Seven commercial fending pig farms adopted the closed management mode of all in and all out, and PRRSV vaccine CH-1R strain, HuN4-F112 strain and TJM strain were used in Heilongjiang B Farm, Heilongjiang C Farm and Hubei C Farm , respectively. Sequence analysis mainly included multiple sequence alignment using MAFFT, phylogenetic tree construction using MEGA, and selection pressure analysis using Datamonkey.

Results

The results revealed that PRRSV was present on all seven farms, and the percentage of PRRSV-positive individuals was 17.54–53.33%. A total of 344 NSP2 gene sequences and 334 complete ORF5 gene sequences were obtained from the positive samples. The statistical results showed that PRRSV-2 was present on all seven commercial fattening farms, and PRRSV-1 was present on only one commercial fattening farm. A total of six PRRSV-2 subtypes were detected, and five of the seven farms had two or more PRRSV-2 subtypes. L1.8 PRRSV was the dominant epidemic strain on five of the seven pig farms. Sequence analysis of L1.8 PRRSV from different commercial fattening pig farms revealed that its consistency across farms varied substantially. The amino acid alignment results demonstrated that there were 131 aa discontinuous deletions in NSP2 between different L1.8 PRRSV strains and that the GP5 mutation in L1.8PRRSV was mainly concentrated in the peptide signal region and T-cell epitopes. Selection pressure analysis of GP5 revealed that the use of the PRRSV MLV vaccine had no significant episodic diversifying effect on L1.8 PRRSV.

Conclusions and Discussion

PRRSV infection is common at commercial fattening pig farms in China, and the percentage of positive individuals is high. There are multiple PRRSV subtypes of infection at commercial fattening pig farms in China. L1.8 is the main circulating PRRSV strain on commercial fattening pig farms. The pressure on the GP5 of L1.8 PRRSV may not be directly related to the use of the vaccines.

Keywords: PRRS, Control, Commercial fattening pig farms, L1.8(NADC30-like PRRSV)

Proceedings

Genetic variation and recombination analysis of the *NSP11* gene of PRRSV-2 Strains in China from 1996 to 2022

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Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), a highly contagious pathogen affecting swine populations globally, has significant economic implications. Particularly in China, the emergence of highly pathogenic PRRSV strains has had a profound impact on the swine industry. Porcine reproductive and respiratory syndrome (PRRS) is caused by the PRRS virus (PRRSV). It leads to reproductive disorders and respiratory failure in sows and piglets. As an endoribonuclease, the PRRSV non-structural protein 11 (NSP11) is crucial in replication and assists immune system evasion. NSP11, which is relatively more conserved than NSP2, could potentially cause a new round of PRRSV epidemics, given a major mutation occurs. This study analyzes 489 PRRSV-2 NSP11 sequences collected from 1996 to 2022. The objective of this abstract is to elucidate the genetic diversity, phylogeny, and recombination events of PRRSV-2 NSP11, providing critical insights for epidemiological surveillance and control measures in China.

Materials and Methods

From the nucleotide database, 489 strains of PRRSV-2 (n=489) were selected. These included 476 Chinese and 16 American PRRSV-2 strains. Utilizing these strains, a phylogenetic analysis of NSP11 was conducted using the neighbor-joining (NJ) method, and trees were constructed using DNAStar and MEGA software. Potential recombination events were analyzed and detected using the RDP software (version 4.0, USA), employing various methods including RDP, GeneConv, SiScan, MaxChi, BootScan, Chimaera, and 3Seq. Detected recombination events were further confirmed using SimPlot (version 3.5.1). Sixty PRRSV-2 strains were then selected from the 489 strains based on the following criteria to ensure representation of each lineage: commonly cited representative strains and equal representation. The ClustalW technique within the MegAlign function of DNAStar software (version 7.0, Madison, WI) was utilized to conduct nucleotide and amino acid homology analyses on the selected 60 strains.

Results

We analyzed the PRRSV-2 NSP11 genetic diversity in China between 1996–2022. The NSP11 nucleotide sequence was analyzed in 60 PRRSV-2 strains, revealing a similarity of 83.6%–100%. Similarly, amino acid sequences exhibited homology ranges of 91.0%–100.0%. Amino acid sequence alignment analysis revealed multiple substitutions in NSP11. NSP11 phylogenetic analysis of 489 PRRSV-2 strains revealed that Lineages 8 and 1 were the predominant strains of PRRSV circulating in China. These two lineages exhibit relatively close genetic relationships. Although unsupported by SimPlot analysis, recombination analysis suggested a potential

recombination event in the 489 PRRSV-2 NSP11 sequences. Recombination analysis and amino acid sequence alignment confirmed the PRRSV NSP11 conservation. Our findings provide genetic diversity of PRRSV-2 NSP11 in China and contribute to effective strategy development to prevent and control PRRSV.

Conclusions and Discussion

Based on NSP11 variations, phylogenetic tree analysis of PRRSV-2 isolates in China between 1996–2022 revealed four distinct lineages: 1, 3, 5, and 8. Lineages 1 and 8 were the most common, with relatively small genetic distances between them. PRRSV NSP11 sequence conservation was confirmed using nucleotide and amino acid homology analyses along with amino acid sequence alignment. Although a potential recombination event was identified in PRRSV-2 NSP11 using recombination analysis, SimPlot revealed that no such events occurred. This study utilized NSP11 to classify PRRSV strains in China into lineages and investigated the prevalence of different lineages. Furthermore, through amino acid and nucleotide homology analyses, amino acid mutation analysis, and recombination analysis, the conservation of NSP11 was confirmed. NSP11 conservation is a factor that ensures the fundamental PRRSV life characteristics and functions. Given the complex genetic diversity of PRRSV, NSP11 is a key target for drug and diagnostic reagent development owing to its minimal changes and significant biological functions. Continuous monitoring of NSP11 genetic variations is crucial for understanding the prevalence dynamics of PRRSV-2 in China, enabling timely prevention and control of future PRRS outbreaks.

Keywords: PRRSV; NSP11; Genetic variation; Recombination

Variations in the NSP1 Gene of PRRSV-2 Strains Isolated in China from 1996 to 2022

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Introduction

Nonstructural protein 1 (NSP1) is an important protein in porcine reproductive and respiratory syndrome virus (PRRSV) that inhibits interferon synthesis and signaling, promotes PRRSV replication. This abstract examines the genetic evolution of Chinese strains of NSP1 in a comprehensive manner by comparing the nucleotide and amino acid sequence homology of domestic strains and studying the genetic variation of the *NSP1* gene and protein in the last 20 years, which will provide the basis for an in-depth understanding of the genetic variation of *NSP1* gene as well as the development of safe drugs.

Materials and Methods

NSP1 sequences of 193 PRRSV-2 strains were selected from the GenBank database on the NCBI website, including strains of lineages 1, 3, 5 and 8. For CH-1R strain, the main physicochemical properties of NSP1 were analysed using the ProtParam tool from the ExPASy Resource Portal and signal peptides were predicted using SignalP-6.0. Fifteen representative PRRSV strains were selected from the 193 PRRSV-2 strains and subjected to nucleotide and amino acid homology analysis and amino acid sequence comparison using DNAStar software; phylogenetic trees were constructed using the Neighbour-Joining (NJ) and Maximum Likelihood (ML) methods in MEGA software, and the phylogenetic trees were annotated using iTOL.

Results

NSP1 is a stable hydrophobic protein with a molecular weight of 43,060.76 Da, and lacks a signalling peptide. NSP1 from different strains showed high nucleotide similarity (79.6%-100%), and amino acid similarity (78.6%-100%), with 94.2% nucleotide similarity between the lineage 3 strain FJFS and the lineage 8 strain TJ. In an amino acid sequence comparison of 15 representative PRRSV-2 strains, multiple amino acid substitution sites were identified in NSP1. In an amino acid sequence comparison of 15 representative PRRSV-2 strains, multiple amino acid substitution sites were found in NSP1, and no insertions or deletions were detected. The strains could be classified into lineages 1, 3, 5, and 8 in phylogenetic tree and analysis showed that lineages 1 and 8 have different evolutionary branches and are genetically distant, and long evolutionary branch lengths of the virulent strains of lineage 1.

Conclusions and Discussion

During the evolution of PRRSV, NSP1 has a relatively high degree of conservatism, but the phenomenon of genetic variation still occurs. Nucleotide homology analysis showed that the representative strain TJnh1501 of lineage 1 had high homology with the representative strain TJ of lineage 8, of which was as high as 99.2%, indicating that the homology of the strains between different lineages was high and that the NSP1 sequences might be involved in genetic recombination. Lineage 3 is the strain that appeared late in China, and lineage 8 is the main epidemic lineage in China, but the representative strains of lineage 3 and lineage 8 also showed high homology, and it is assumed that NSP1 is not the main factor leading to the different prevalence of strains from

different lineages, which needs to be further investigated. The amino acid sequence of NSP1 has a high degree of homology too. Of the differences within the amino acid sequence lineages of NSP1, the largest is found in lineage 3 and the smallest in lineage 8. All homologies within lineages are high compared to those between lineages. Comparative analysis of the amino acid sequences shows that NSP1 has amino acid substitutions at individual sites and no regions of insertion or deletion of amino acids. The variation in NSP1 is more conserved than that in the amino acid sequneces of NSP2 and ORF5. Phylogenetic tree analysis showed relatively large genetic distances between lineage 1 and lineage 8 and long evolutionary branch lengths of the virulent strains of lineage1, evidencing changes in the *NSP1* genes of lineage 1. In-depth study of the NSP1 variant region is expected to identify sites that inhibit PRRSV replication and signalling, which contributes to the development of safe drugs.

Keywords: PRRSV, PRRS; NSP1; Variations

Genetic variation and recombination analysis of the *GP5(GP5a)* gene of PRRSV-2 strains in China from 1996 to 2022

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease caused by the porcine reproductive and respiratory syndrome virus (PRRSV). PRRSV
mainly causes spontaneous abortion, stillbirth, and fetal mummification in pregnant sows. Additionally, respiratory insufficiency, interstitial pneumonia, and immunosuppression are also common symptoms in piglets. PRRS was first discovered in the United States in 1987 and rapidly spread worldwide after its detection in Europe in 1990, causing significant economic losses to the global pig industry. PRRSV was first identified in China in 1996, and since then, it has been reported throughout the country. In 2006,a highly pathogenic PRRSV (HP-PRRSV) outbreak occurred in China, causing a significant increase in the severity of lesions and mortality rates among infected pigs. GP5 (GP5a) is highly variable and thus is commonly used as a target gene for analyzing genetic variation in PRRSV.

Materials and Methods

In this study, GP5 sequences of 517 PRRSV-2 strains (501 Chinese PRRSV-2 strains and 16 US. PRRSV-2 strains) were selected from the GenBank database on the NCBI website, which included strains from lineages 1, 3, 5 and 8. From the 517 PRRSV-2 strains, 60 representative strains were selected for nucleotide and amino acid homology analysis and amino acid sequence comparison of the *GP5 (GP5a)* gene using DNAStar software; the phylogenetic tree was constructed using the neighbor-joining (NJ) and maximum-likelihood (ML) methods of MEGA software, and the generated phylogenetic tree was annotated using iTOL. Finally, the recombination analysis of 517 PRRSV-2 GP5 sequences was performed using RDP software to detect potential recombination events, and the detected recombination events were further verified using SimPlot software.

Results

To monitor the genetic variation of PRRSV-2 in China, the GP5 sequences of 517 PRRSV-2 strains from 1996 to 2022 were analyzed and phylogenetic trees were constructed. Furthermore, a total of 60 PRRSV strains, originating from various lineages, were carefully chosen for nucleotide and amino acid homologies analysis. The results showed that the nucleotide homologies of the PRRSV *GP5* gene ranged from 81.4 to 100.0%, and the amino acid homologies ranged from 78.1 to 100.0%. Similarly, the PRRSV *GP5a* gene showed 78.0~100.0% nucleotide homologies and70.2~100.0% amino acid homologies. Amino acid sequence comparisons of GP5 and GP5a showed that some mutations, such as substitutions, deletions, and insertions, were found in several amino acid sites in GP5, these mutations were primarily found in the signal peptide region, two highly variable regions (HVRs), and near two T-cell antigenic sites, while the mutation sites of GP5a were mainly concentrated in the transmembrane and intramembrane regions. Phylogenetic analysis showed that the prevalent PRRSV-2 strains in China were divided into lineages 1, 3, 5, and 8. Among these, strains from lineage 8 and lineage 1 are currently the main prevalent strains, lineage 5 and lineage 8 have a closer genetic distance. Recombination analysis revealed

that one recombination event occurred in 517 PRRSV-2 strains, this event involved recombination between lineage 8 and lineage 1. In conclusion, this analysis enhances our understanding of the prevalence and genetic variation of PRRSV-2 in China.

Conclusions and Discussion

The results showed that the nucleotide homology of the PRRSV *GP5 (GP5a)* gene ranged from 81.4% to 100.0% (78.0% to 100.0%), and the amino acid homology ranged from 78.1% to 100.0% (70.2% to 100.0%). When the amino acid sequences of the PRRSV *GP5 (GP5a)* gene were compared, it was found that several amino acid sites in GP5 (GP5a) were mutated, including amino acid substitutions, deletions and insertions. Mutation is the main driver of PRRSV evolution, and mutation leads to immune escape from the virus, which results in vaccine immunization failure. Phylogenetic analysis showed that the PRRSV-2 epidemic strains in China belong to four lineages, 1, 3, 5 and 8, respectively, of which the strains of lineage 8 and lineage 1 dominate the PRRS epidemic. Recombination analysis showed that 517 PRRSV-2 strains underwent one recombination event, which manifested itself as recombination between lineage 8 and lineage 1. Recombination can lead to a significant reduction in the effective protection rate of vaccines in pig farms, thus making PRRS prevention and control more difficult. From 1996 to 2022, PRRSV-2 strains circulating in China have

been classified into lineages 1, 3, 5, and 8. Among these, lineage 8 and lineage 1 are the most widespread and susceptible to recombination. The GP5 (GP5a) amino acid mutation is a major driver of PRRSV evolution and contributes to viral evolution. Therefore, in order to enhance disease prevention and control measures in the future, it is imperative to intensify monitoring of PRRSV genetic variations.

Keywords: PRRSV, *GP5* gene, *GP5a* gene, genetic variation, recombination, phylogeny

Analysis of Genetic Variations in NSP4 of Type 1 Porcine Reproductive and Respiratory Syndrome Virus in China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease caused by PRRS virus (PRRSV), resulting in abortions, stillbirths, mummified fetuses, and respiratory illnesses in pigs, particularly in piglets. PRRSV encodes the non-structural protein 4 (NSP4), which exhibits 3C-like serine proteinase (3CLSP) activity, featuring a conserved catalytic triad of His39-Asp64-Ser118. As a crucial protease encoded by PRRSV, NSP4 plays a vital role in suppressing the host's innate immune response. Apart from its ability to inhibit IFN-I signaling pathways, NSP4 also possesses the capability to activate the Notch signaling pathway, regulate antigen presentation pathways, and induce cell apoptosis. The objective of this abstract is to analyze 24 domestic strains of PRRSV-1 that were uploaded to GenBank. The NSP4 sequences of these strains were compared with typical strains from China and other countries, and their genetic variations were analyzed. This provides valuable data to support the study of PRRSV-1 NSP4 and valuable reference information for molecular epidemiological research on domestic PRRSV-1, providing a theoretical basis for effective prevention and control of PRRS.

Materials and Methods

This study selected 40 complete genome sequences of PRRSV from the GenBank database, including 32 PRRSV-1 and 8 representative PRRSV-2 sequences, with a specific focus on the NSP4 gene derived from 24 PRRSV-1 sequences originating from China. Homology analysis of NSP4 nucleotide sequences was conducted using the MegAlign function of the DNASTAR software package and the Clustal W method. Simultaneously, the translated amino acid sequences of these 40 PRRSV-1 NSP4 nucleotide sequences were subjected to amino acid homology analysis. Sequence alignment using BioEdit software identified amino acid differences at specific sites among the 40 PRRSV-1 NSP4 sequences, providing a comprehensive assessment of variation in the PRRSV-1 NSP4 amino acid sequences. To gain a more comprehensive understanding of the evolutionary relationship between the complete genome of PRRSV-1 and its NSP4 sequences, this study obtained 86 PRRSV genome sequences from GenBank, including 66 PRRSV-1 and 20 representative PRRSV-2 genome sequences, and constructed a phylogenetic tree using the maximum likelihood method in MEGA software. Finally, the Tree Of Life website was utilized to display, manipulate, and annotate the results of the phylogenetic tree analysis.

Results

The nucleotide homology of NSP4 between 32 PRRSV-1 and 8 PRRSV-2 strains ranged from 57.3% to 62.4%, and the amino acid homology ranged from 58.6% to 64.5%. The nucleotide homology of NSP4 among the 32 PRRSV-1 strains ranged from 75.4% to 100.0%, and the amino acid homology ranged from 81.3% to 100.0%. Comparison of the NSP4 amino acid sequences of 32 PRRSV-1 and 8 PRRSV-2 strains revealed that the biggest difference was a deletion at the 83rd amino acid residue in PRRSV-1, and other differences manifested as varying degrees of mutations at multiple amino acid sites. Phylogenetic analysis showed that the PRRSV-1 strains isolated

in China clustered into four subgroups of Subtype 1 Global: Amervac-like, BJEU06-1-like, HKEU16-like, and NMEU09-1-like strains.

Conclusions and Discussion

This study delves into the prevalence and genetic evolution of PRRSV-1 in China, specifically focusing on the NSP4 protein. Despite PRRSV-2 being the primary strain, the detection rate of PRRSV-1 has been steadily rising each year, with both types co-circulating within the same regions. The findings reveal that the nucleotide and amino acid homology of NSP4 sequences of Chinese PRRSV-1 strains with Subtype 1 Global strains are relatively high, although lower with other subtype representative strains. Notably, a deletion was observed at the 83rd amino acid residue in the amino acid sequence alignment of PRRSV-1, along with variations at multiple amino acid sites. Additionally, vaccination emerges as a critical measure for PRRS control, though the efficacy of PRRSV-1 vaccines warrants further investigation. With the expansion of international trade, heightened surveillance is imperative to curtail the spread and prevalence of PRRSV-1 at its source. This study provides a thorough examination of PRRSV-1 prevalence and the genetic evolution of its NSP4 protein, shedding light on transmission mechanisms, vaccine development, and control strategies for PRRS. It also lays a theoretical foundation for future research in this area.

Keywords: PRRSV-1, PRRSV-2, NSP4, genetic variation, phylogenetic analysis

Variations in the NSP4 gene of the Type 2 Porcine Reproductive and Respiratory Syndrome Virus Isolated in China from 1996 to 2021

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) has been continuously mutating since its first isolation in China in 1996, posing challenges for infection prevention and control. Infections caused by PRRSV-2 strains are the main epidemic strains in China. The non-structural protein 4 (NSP4) of PRRSV is highly conserved, and its antibody can be detected early in infection. As an important protease encoded by PRRSV, NSP4 also participates in various immunosuppressive reactions and plays a significant role in antagonizing the natural immune response of the host through mechanisms other than antagonizing the type I interferon (IFN-I) signaling pathway. NSP4 can activate the Notch signaling pathway, regulate the antigen presentation pathway, and induce apoptosis and other cellular responses to help the virus escape host immunity. Additionally, 3CLSP plays an essential role in regulating PRRSV replication, inhibiting the production of host IFN, and inducing host cell apoptosis. Therefore, it has emerged as a crucial protein warranting further investigation into the pathogenesis of PRRSV. The objective of this abstract is to explore the genetic variation of PRRSV-2 NSP4, providing insights for understanding viral immune evasion and informing strategies for PRRS prevention and control.

Materials and Methods

This study focused on the prevalence and genetic variations of PRRSV-2 NSP4 in China over the past two decades. It predicted the biological properties of NSP4, conducted comprehensive analyses of its sequence and structure using bioinformatics tools, including physical and chemical properties, signal peptide prediction, secondary and tertiary structure prediction, as well as nucleotide and amino acid sequence alignments. Phylogenetic tree analysis assessed genetic variations among 123 strains, providing comprehensive insights into NSP4 diversity and aiding in understanding PRRSV evolution for informed prevention and control strategies.

Results

This study investigated the genetic variations of PRRSV-2 NSP4 from the main prevalent strains in China over the past 20 years. The predicted molecular weight of NSP4 protein was determined to be 21.1 kDa, and it was predicted to be a stable hydrophobic protein lacking a signal peptide. Nucleotide homology analysis of 123 PRRSV-2 NSP4 sequences revealed a homology range of 81.0% to 100.0%, while amino acid homology analysis showed a range of 85.8% to 100.0%. PRRSV-2 NSP4 consists of 204 amino acids, exhibiting relative conservation with only occasional amino acid substitutions at specific sites and no insertions or deletions. The NSP4 sequences from lineage 1 and lineage 8 strains showed a distant genetic distance, while those from lineage 1 and lineage 3 strains showed a closer genetic distance.

Conclusions and Discussion

Sequence analysis of the NSP4 protein reveals its strong antigenicity and flexibility, indicating the presence of multiple B-cell epitopes, rendering it suitable for generating high-quality antibodies. Homology analysis of

nucleotide and amino acid sequences among 123 PRRSV-2 NSP4 strains demonstrates high similarity, with nucleotide homology ranging from 81.0% to 100% and amino acid homology from 85.8% to 100%. NSP4 lacks insertion or deletion sites, with only occasional amino acid substitutions, indicating its relative conservation during evolution within Chinese PRRSV strains. Phylogenetic tree analysis suggests distant genetic distances between lineages 1 and 8, prevalent strains prone to genetic recombination, facilitating their survival during long-term evolution and mutation processes, though further research is needed to elucidate specific mechanisms. Analyzing the spatial structure and genetic evolution of NSP4 can aid in identifying critical amino acid sites, serving as important antiviral targets for drug design, and providing theoretical foundations for future vaccine development or NSP4 gene detection methods. NSP4 plays a crucial role in combating host innate immune responses, and analyzing its genetic variations may provide theoretical insights into its involvement in regulating host innate immune responses, elucidating its role in pathogenesis, which warrants further investigation.

Keywords: porcine reproductive and respiratory syndrome virus, NSP4 gene, genetic variation, amino acids sequence, homology, phylogeny

Establishment an Indirect ELISA Detection Method for Porcine Reproductive and Respiratory Syndrome Virus NSP4

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) induces fever and anorexia in infected pigs. Late-stage pregnant sows may experience miscarriages, premature births, and stillbirths, while neonates are particularly vulnerable to respiratory complications. Highly pathogenic strains cause symptoms like severe fever, cyanosis, and sudden death in adult pigs. The non-structural protein 4 (NSP4) of PRRSV possesses 3C-like serine protease (3CLSP) activity, which can influence PRRSV replication, suppress host IFN-β production, induce host cell apoptosis, and play a crucial role in PRRSV detection. Antibodies induced by NSP4 can effectively serve as indicators of infections caused by the wild-type virus. The objective of this abstract is to establish an indirect enzyme-linked immunosorbent assay (ELISA) detection method for PRRSV NSP4. Utilizing the PRRSV NSP4 to establish an ELISA antibody detection method was deemed more conducive for sustained antibody monitoring in pig farms over time. Therefore, the establishment of an NSP4 indirect ELISA detection method provides technical support for the detection of PRRSV antibodies.

Materials and Methods

Utilizing the NSP4 gene sequence from the PRRSV XH-GD strain, specific primers were designed for amplification, resulting in the construction of a recombinant plasmid, pET-28a-NSP4. Following induction of NSP4 expression in E. coli BL21, the protein underwent purification and validation steps. Optimization procedures were conducted to establish the ideal antigen coating concentration and serum dilution, alongside the assessment of various ELISA experimental conditions. The critical value of the ELISA method was determined, with thorough evaluations of its specificity, sensitivity, and reproducibility. Ultimately, the efficacy of the established indirect ELISA was validated through comparison with a commercial PRRSV IDEXX ELISA kit using 230 clinical porcine serum samples.

Results

The recombinant plasmid pET-28a-NSP4 was successfully constructed, and the target recombinant protein was expressed in E. coli BL21 (DE3) following induction with 0.8 mmol/L IPTG at 37°C for 7 hours, predominantly in the form of inclusion bodies. SDS-PAGE analysis confirmed a band at 27.1 kDa, consistent with the expected size. After purification and ultrafiltration concentration, Western blotting revealed specific binding of the recombinant protein to both His-tag antibody and PRRSV-positive serum, confirming its immunogenicity. An indirect ELISA method for PRRSV NSP4 was established using the purified recombinant NSP4 protein as the coating antigen. The ELISA method underwent optimization, with the optimal coating concentration of NSP4 recombinant protein determined as 1.0 mg/L. Additionally, optimal parameters included a serum dilution of 1:400, a blocking buffer of 3.0% BSA with a 3-hour incubation, a serum reaction time of 75 minutes, an enzyme-labeled secondary antibody dilution of 1:10000, a secondary antibody incubation time of 60 minutes, and a color

development time of 8 minutes. The method exhibited excellent specificity, sensitivity, and repeatability. Testing with 230 porcine serum samples resulted in a total concordance rate of 91.8% compared to the IDEXX ELISA kit.

Conclusions and Discussion

Currently, the predominant market offerings include imported PRRSV antibody-testing kits that serve their purpose but are expensive and sometimes lack precision in detecting neutralizing antibody levels. In this study, an advanced indirect ELISA targeting the PRRSV NSP4 was established to address the challenges associated with the high cost of commercially available PRRSV antibody diagnostic kits and their analytical limitations. By refining the detection process, our approach guarantees accurate and dependable PRRSV antibody results, thereby improving foundational PRRSV antibody testing and monitoring. Our method also offers increased testing efficiency, which is suitable for comprehensive clinical sample analyses. When compared with conventional test kits, this method is a cost-effective diagnostic alternative for swine production, enabling the development of more precise preventive strategies against PRRSV outbreaks. In summary, the NSP4 indirect ELISA detection method developed in this study has significant application value both to researchers and pig farming industries.

Keywords: porcine reproductive and respiratory syndrome virus, NSP4, prokaryotic expression, ELISA

Genetic Variability and Recombination of the *NSP2* Gene of PRRSV-2 Strains in China from 1996 to 2021

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Introduction

Genetic variability and recombination of the *NSP2* gene are of great significance in gaining an in-depth understanding of the prevalence of PRRSV in China over the past 25 years. We compared the nucleotide and amino acid homologies of the NSP2 sequences of different PRRSV-2 lineages, and examined phylogenetic relationships based on an analysis of the NSP2 sequences of 122 strains. What is more, recombination analysis revealed the occurrence of five recombinant events among the 135 selected PRRSV-2 strains. These results provide a theoretical foundation for evolution and epidemiology of the spread of PRRSV.

Materials and Methods

One hundred and twenty-two Chinese PRRSV-2 strains and 13 US PRRSV-2 strains in the NCBI website GenBank database were chosen, including those of lineage 1, 3, 5, and 8. Utilizing these strains, a phylogenetic analysis of NSP2 was conducted using the neighbor-joining (NJ) and Maximum Likelihood (ML) methods, and trees were constructed using MEGA and PhyloSuite software. We compared the nucleotide and amino acid homologies of the NSP2 sequences of different PRRSV-2 lineages. When potential recombinant events were detected based on RDP software (version 4.0), GENECONV, BootScan, MaxChi, Chimera, SiScan, and 3eq analyses, five or more methods were identified as genetic recombination and $P < 0.05$ in RDP software. The strains thus identified were considered recombinant strains. In addition, we used SimPlot (version 3.5.1) to confirm the detected recombination events.

Results

The phylogenetic tree revealed that the PRRSV-2 strains prevalent in China could be classified into four lineages, namely, lineages 1, 3, 5, and 8. Among these, lineage 3 is closely related with lineage 8, whereas lineage 1 and lineage 8 strains appear to be separated by comparatively large genetic distances. We selected different representative strains for comparisons of nucleotide and amino acid sequences, which revealed nucleotide and amino acid homologies of 72.5% to 99.8% and 63.9% to 99.4%, respectively, among the NSP2 proteins of different PRRSV-2 strains. Recombination analysis revealed the occurrence of five recombinant events among the 135 selected PRRSV-2 strains.

Conclusions and Discussion

From 1996 to 2021, PRRSV-2 isolates in China have been categorized into lineages 1, 3, 5, and 8, based on variations in their NSP2 genes. Lineages 1 and 8 have been found to be predominant in the prevalence of PRRS in China. Among these, lineage 3 is represented by GM2-2011, QYYZ-2011, and FJFS-2012, which appear to be closely related, whereas lineage 1 and lineage 8 strains appear to be separated by comparatively large genetic distances. For each of the four assessed lineages, we selected different representative strains for comparisons of nucleotide and amino acid sequences, which revealed nucleotide and amino acid homologies of 72.5% to 99.8% and 63.9% to 99.4%, respectively, among the NSP2 proteins of different PRRSV-2 strains. We speculate that these

strains have undergone relatively limited mutation during the course of genetic evolution, and accordingly have been unable to effectively evade vaccine-mediated immunity and host immune surveillance, although further studies are necessary to ascertain specific details in this regard. In recent years, recombination has played a significant role in the evolution of PRRSV-2. Recombinant analysis performed for PRRSV-2 NSP2 revealed a total of five recombinant events among the 135 selected strains, a majority of which appear to have occurred between lineage 1 and 8 strains, possibly because there are many types of PRRSV vaccines in the Chinese market, and the abuse of vaccines is also the main reason why PRRSV is prone to recombination. The findings of this study enabled us to gain an in-depth understanding of the prevalence of PRRSV in China over the past 25 years and will contribute to providing a theoretical basis for evolution and epidemiology of the spread of PRRSV.

Keywords: PRRS, *NSP2* gene, genetic variability, recombination, phylogeny

Recombination and Genetic Diversity Analysis of PRRSV-1 NSP2 in China

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) has existed in China for about 30 years, resulting in substantial annual financial losses to the swine enterprise due to continuous mutation of the PRRS virus (PRRSV). The objective of this study was to analyze the recombination patterns and genetic variation of the PRRSV-1 non-structural protein 2 (*NSP2*) gene in China. In total, sixty-nine PRRSV-1 strains were collected, including twenty-five from China and forty-four from other countries. This study evaluated similarities in amino acid and nucleotide sequences among these strains. Multiple sequence alignment was performed on the NSP2 sequence to determine whether PRRSV-1 and PRRSV-2 differ in their amino acid sites. Furthermore, phylogenetic analysis of ninety PRRSV NSP2 sequences revealed that genetic relationships between NMEU09-1-like and BJEU06-1-like strains have been progressively approaching but distantly related to HKEU-16-like and Amervac-like strains. In the ninety selected PRRSVs, four recombination events were detected using recombination analysis, two of which occurred in Chinese PRRSV-1 strains.

Materials and Methods

Sixty-nine PRRSV-1 NSP2 strains (twenty-five from China and forty-four from overseas) and twenty-one PRRSV-2 strains were carefully selected from the NCBI website. We examined sixty-nine PRRSV-1 NSP2 sequences for nucleotide and amino acid similarities. This study was conducted using the Cluster W method within the DNAStar software (version 7.0, Madison, WI). Thirty-four PRRSV-1 NSP2 sequences and six PRRSV-2 NSP2 sequences were compared using the GENEDOC sequence comparison software (version 2.7). Phylogenetic evaluation of ninety PRRSV NSP2 sequences (Table 1) was performed using the neighbor-joining (NJ) method of MEGA software (version 7.0.26, Mega Limited, Auckland, New Zealand) and the maximum likelihood (ML) approach of PhyloSuite software (version 1.2.2) with 1000 bootstrap replicates. Seven methods of RDP software (version 4.101) were used to identify potential recombination events. Recombination events that were detected by at least five methods with a p-value of less than 0.05 were significant. In addition, recombination events identified through SimPlot (version 3.5.1) were subjected to validation.

Results

The findings revealed that the nucleotide homology of the PRRSV-1 NSP2 was 42.1%-97.0%. The amino acid similarity of PRRSV-1 NSP2 was between 59.4% and 100.0%. The variation in NSP2 among PRRSV-1 did not display a specific pattern, and deletion of NSP2 was the highest at the 288-463 site. PRRSV-2 and PRRSV-1 were in two distinct evolutionary branches. Additionally, genetic relationships between NMEU09-1-like and BJEU06-1-like strains were found to be closely related to PRRSV-1, whereas the relationship between HKEU-16-like and Amervac-like strains was more distantly related to PRRSV-1 than that between the BJEU06-1-like and HKEU-09-1-like strains. We revealed four possible occurrences of recombination.

Conclusions and Discussion

From 1997 to 2022, researchers in China have categorized PRRSV-1 isolates into four distinct gene subgroups: NMEU09-1-like, HKEU-16-like, BJEU06-1-like, and Amervac-like. The PRRSV-1 NSP2 sequence exhibits a higher degree of replacement, deletion, and variation regions compared to PRRSV-2 NSP2 sequences. In our investigation, we conducted a comparative analysis of amino acid sites between PRRSV-1 and PRRSV-2 NSP2 by aligning thirty-four PRRSV-1 and six PRRSV-2 NSP2 sequences. The findings indicated that PRRSV-1 NSP2 showed a higher number of deletion and substitution sites than PRRSV-2. Moreover, the deletion patterns in PRRSV-1 NSP2 exhibited additional irregularities, potentially presenting a hurdle for research on the PRRSV-1 vaccine. For PRRSV-1, there are many international vaccines such as Suvaxyn PRRS MLV, ReproCyc PRRS EU, Ingelvac PRRSFLEX EU, UNISTRAIN PRRS, and Porcilis PRRS. However, the use of live attenuated vaccines carries the risk of reversion to a virulent state and can lead to its recombination with wild strains, which complicates control and prevention efforts. The PRRSV NSP2 region is of particular importance in vaccine research because it can tolerate missing fragments and maintain stable expression of inserted genes. In addition, PRRSV-1 exhibits a lower recombination rate in the NSP2 region than PRRSV-2. Although there is extensive knowledge on the mechanisms of immune escape of PRRSV, pathogenesis, viral proliferation, molecular epidemiology, immune response, and clinical pathogenesis, timely monitoring of genetic changes in PRRSV can serve as a valuable reference for the effective management of PRRS in China in the forthcoming years.

Keywords: Genetic analysis, Recombination, *NSP2* gene, China, PRRSV

Diagnosis and prevention of porcine reproductive and respiratory syndrome

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Abstract: Porcine reproductive and respiratory syndrome (PRRS) is an acute viral disease. The disease is caused by porcine reproductive and respiratory syndrome virus (PRRSV), which mainly affects the reproductive system and respiratory system of pigs, leading to abortion, premature delivery, elevated body temperature, anorexia and other symptoms.Since its first outbreak in North Carolina in 1987, PRRS has been widely spread around the world, and has shown a high incidence trend in China in recent years, becoming an important threat to the development of pig industry.PRRS mainly depends on clinical symptom observation, laboratory testing and histopathological examination. Clinical symptoms include reproductive disorders in sows and respiratory abnormalities in pigs. Laboratory tests accurately determine whether pigs are infected with PRRSV by means of serological identification, virus isolation and PCR technology. Histopathological examination can further observe the lesions and provide a basis for diagnosis. Vaccination is the main means to prevent PRRS, which can effectively improve the immunity of pigs and reduce the risk of viral infection.In addition, provenance control is also an important part, strict introduction detection and isolation observation to reduce the possibility of virus introduction into pig farms. At the same time, we will strengthen the sanitary disinfection of the pig farm environment and reduce the risk of virus transmission and infection.Although porcine reproductive and respiratory syndrome has brought great challenges to the pig industry, through the comprehensive application of diagnostic techniques and immune prevention and control measures, we can effectively reduce the occurrence and spread of the disease.This article first describes the epidemiological characteristics of PRRS ; then, the structural characteristics, genetic evolution, interaction between virus and host protein, and regulation of host immunity of PRRSV were introduced. Finally, the diagnosis of PRRS was introduced, including clinical symptom observation, laboratory examination and molecular biology techniques, and the prevention and control suggestions of PRRS were put forward, in order to provide reference for the sustainable development of pig industry.

Keywords: PRRS; diagnosis; prevention; control; detection

Epidemiological and genetic characteristics of swine reproductive and respiratory syndrome viruses in different regions

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of porcine reproductive and respiratory syndrome (PRRS). Porcine reproductive and respiratory syndrome virus type 2 (PRRSV2) is a major threat to the global pig industry. The genome of PRRSV consists of single-stranded positive-strand RNA of approximately 15 kb in length, containing at least 10 open reading frames (open reading frame, ORF), including ORF 1 a, ORF 1 b, ORF 2 a, ORF 2 b, 3,4,5,5a, 6 and 7 Genotype 2 PRRSV is very prevalent in China. Continuous surveillance of the epidemiological and genetic characteristics of PRRSV epidemic strains facilitates the prevention and control of infection.

Methods

Pig samples of different types were collected from 2428 pig farms in 27 provinces of China. Among the 7980 samples collected, 2080 (26.07%) were tested for PRRSV2 ORF5 positive by RT-PCR. The positive rate of PRRSV in different regions of China was 8.12% -29.33%, and 7.96% -55.50% in different months. The phylogenetic analysis based on ORF 5 gene shows that the current circulating PRRSV2 strains in China belong to 5 branches, and most of the PRRSVs detected were the highly pathogenic PRRSVs (HP-PRRSVs, clade IV) and PRRSV NADC30-like strains (clade I). Furthermore, all 479 ORF 5 sequences detected in South China from 2017 to 2021 were PRRSV2. This shows that the highly pathogenic strains are still the main PRRSV in South China, and the overall detection rate is increasing year by year. Similarly, 231 samples were collected from different scale pig farms in East China from 2017 and 2022 and showed that the PRRSV positive rate was 24% (54 / 231). Phylogenetic analysis (13 positive samples) showed that all the isolates belonged to genotype 2, and that the circulating PRRSV strains in East China were still HP-PRRSV, while the proportion of NADC30-like and NADC34-like strains increased. When investigating the epidemiology and genetic characteristics of PRRSV strain PRRSV, we found that the prevalence of PRRSV in healthy pigs was relatively low but higher in diseased pigs. Several lineages of PRRSV were prevalent in Hunan and Hebei Province, among which NADC30-like PRRSV strains (sublineage 1.8) and HP-PRRSV strains (sublineage 8.7) were the main lineages. In addition, we isolated and reported four PRRSV strains in this study, of which a novel NADC30-like strain was reconstituted from both NADC30-like and HP-PRRSV-like strains. This was also reported in foreign studies, with a total of 5062 serum and tissue samples collected from different regional provinces of the Republic of Korea between 2018 and 2022. , A total of 5062 serum and tissue samples were collected from different regions and provinces between 2018 and 2022. Open reading frame 5 (ORF 5), the sequence indicates subgroup A (42%), followed by lineage 1 (21%), lineage 5 (14%), lineage C (LKC) (9%), lineage B (LKB) (6%) and subtype 1C (5%). High virulence lineage 1 (NADC30 / 34 / MN 184) and 8 were also detected. These viruses often mutate or recombine with other viruses. Other studies indicated that serum samples were collected from epidemic areas in different regions of Nepal, and phylogenetic analysis showed that PRRSV strains belong to PRRSV-2 and lineage 8 strains. The sequences of Nepalese PRRSV strains showed high similarity to strains isolated from India, China and Vietnam. The PRRSV strain from Nepal belongs to the PRRSV-2 species (previously a North American genotype).

Results

In conclusion, this review summarizes the epidemiological and genetic characteristics of swine reproductive and respiratory syndrome viruses in recent years, aiming to understand the prevalence of PRRSV in different regions and to provide reference for further prevention and control, epidemiological research and vaccine development of PRRS.

Keywords: PRRS, Epidemiology ,PRRSV2,Prevention and control

Methods for the treatment and diagnosis of porcine reproductive and respiratory syndrome virus

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a severe global pig farming disease. Clinically, this virus can cause reproductive disorders in sows and severe respiratory diseases in piglets. PRRSV can be divided into two species: Betaarterivirus suid 1 (PRRSV-1) and Betaarterivirus suid 2 (PRRSV-2), which share 60% nucleotide (nt) identity at the whole-genome level. Over the past two decades, PRRSV has persisted in Chinese pig farms through mutation or recombination, causing tremendous economic losses to the Chinese pig industry. Currently, PRRSV-2 strains are the dominant circulating strains, including NADC30-like strains and Chinese HP-PRRSV strains. The infection rate of PRRSV in commercial fattening farms was very high from 2020 to 2021, and there were multiple types of PRRSV coexisting in Chinese farms. Currently, there is no fully effective vaccine or antiviral drug against this disease. To mitigate the burden of PRRS, there is an urgent need for more research on disease prevention and treatment, focusing on the interaction between PRRSV and its host. This article mainly summarizes the research on the treatment and diagnostic methods of porcine reproductive and respiratory syndrome virus in recent years, aiming to provide new ideas for the prevention and control of porcine reproductive and respiratory syndrome.

Methods

Nanobodies are considered a promising new method for treating diseases due to their ease of production and low cost. Zinc oxide-selenium nanoparticles (ZnO-Se NPs) have inhibitory effects on PRRSV proliferation. Mechanism studies have shown that ZnO-Se NPs mainly target the replication process by upregulating the expression of NLRX1 (a protein that interacts with PRRSV Nsp9) and targeting the replication process by inhibiting PRRSV-induced ROS upregulation, rather than stimulating innate immunity. Additionally, tea polyphenols (TPP) are effective drugs against PRRSV infection and have broad application value in the pig industry. TPP exhibits effective anti-PRRSV activity both before and after treatment. TPP was also shown to inhibit the entry of PRRSV-induced p65 into the nucleus, suppressing the activation of the NF-κB signaling pathway, ultimately leading to suppressed expression of inflammatory cytokines. Furthermore, TPP restricts the synthesis of the core component of the viral replication transcription complex, non-structural protein 2 (nsp2), which may contribute to inhibiting viral RNA replication.

Conclusion

In summary, nanobodies, zinc oxide-selenium nanoparticles, and tea polyphenols can inhibit the proliferation of PRRSV and have the potential to develop into novel drugs for preventing and controlling PRRSV infection in the future.

Keywords: PRRS; ; pig; PRRSV

Differential diagnosis of interstitial pneumonia

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Introduction：

Two cases of interstitial pneumonia were used to distinguish PRRS from porcine circovirus disease.
Materials and methods：

Materials and Methods

Through two on-site cases, pig farm patrols, pig dissection, fluorescence quantitative PCR detection, and histopathology, a comprehensive diagnosis is conducted. Case One: A pig farm in Zhejiang Province, where 70-day-old piglets had coarse and messy fur during the conservation stage. Clinical symptoms: difficulty breathing and slow growth. Case 2: In a pig farm in Zhejiang province, the 60-day-old piglets in the conservation stage had coarse and messy fur. Clinical symptoms: Difficulty breathing, slow growth, emaciation, and exposed bones. Both cases were sampled for fluorescence quantitative PCR detection, and pathological tissue sections were also sent for examination.

Results:

Case 1:
Symptoms of autopsy: Interstitial pneumonia of the lungs.
PCR: Severe interstitial pneumonia in the lungs. Fluorescence quantitative PCR detected a PRRSV content of 1.4E+06 copy/uL, with a Ct value of 18.9198.
Pathological section: Significant reduction in alveolar space; The alveolar interstitium is significantly thickened, and there are necrotic cell masses in the alveoli.
Case 2:
Symptoms of autopsy: Interstitial pneumonia of the lungs, spotted kidney.
PCR: PRRSV was negative, and PCV-2 was positive.
Pathological section: Extreme lymphocyte failure in the tonsils with extranuclear inclusion bodies; Degenerated renal tubules. Epithelial cells have cytoplasmic inclusion bodies and granulomatous lesions of the lungs.

Conclusion:

In case one, through comprehensive diagnosis, a clear PRRS typical lesion (interstitial widening of the lungs and eosinophilic necrotic masses in the alveoli) was observed in pathology, which confirmed the positive PRRSV result of PCR and confirmed that interstitial pneumonia in the lungs was caused by the PRRS. In Case 2: Through comprehensive diagnosis, obvious granulomatous lesions, lymphocyte failure, and granulomatous lesions were observed in pathology, confirming the positive result of the PCR ring and confirming that interstitial pneumonia in the lungs was caused by Porcine circovirus disease.
The difference between interstitial pneumonia caused by blue ear and circular ring disease: (1) In clinical practice, porcine circular ring disease is mainly characterized by emaciation and exposed bone; (2) In terms of anatomical symptoms, porcine circovirus disease can cause enlargement of the spotted kidney and lymphatic organs in the kidneys; (3) PCR can distinguish; (4) Pathologically, PRRS is mainly characterized by interstitial widening of the lungs and eosinophilic necrotic masses in the alveoli, while Porcine circovirus disease is mainly characterized by granulomatous strain and lymphocyte failure in the lungs and lymphoid organs.

Keywords：

PRRS；Porcine circovirus disease；differential diagnosis；interstitial pneumonia；

Epidemiological investigation and genetic evolutionary analysis of PRRSV-1 on a pig farm in China

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Introduction

The prevalence and features of PRRSV-1 on Chinese farms are unclear. We continuously monitored PRRSV in a pig farm with strict biosafety measures in Henan Province, China, in 2020.

Materials and Methods

A pig-finishing farm (500 head) that has been monitored for PRRSV was investigated in the study. The farm is located in Henan Province, China, and there are no neighboring pig farms within 3 km. The 500 piglets (42–49 days old) entered the farm at the same time. Clinical samples, including serum, lung and submaxillary lymph nodes, are collected by a professional veterinarian at each farm and laboratory tests are performed every 15 days.

Results

The results showed that multiple types of PRRSV coexisted on this single pig farm. PRRSV-1 was one of the main circulating strains on the farm and was responsible for infections throughout nearly the entire epidemic cycle. Phylogenetic analysis showed that PRRSV-1 isolates from this pig farm formed an independent branch, with all isolates belonging to BJEU06-1-like PRRSV. The analysis of selection pressure on ORF5 on this branch identified 5 amino acids as positive selection sites, indicating that PRRSV-1 had undergone adaptive evolution on this farm. According to the analysis of ORF5 of PRRSV-1 on this farm, the evolutionary rate of the BJEU06-1-like branch was estimated to be 1.01×10^{-2} substitutions/site/year. To further understand the genome-wide characteristics of PRRSV-1 on this pig farm, two full-length PRRSV-1 genomes representative of pig farms were obtained. The results of amino acid alignment revealed that although one NSP2 deletion was consistent with BJEU06-1, different new features were found in ORF3 and ORF4. According to the above results, PRRSV-1 has undergone considerable evolution in China.

Conclusions and Discussion

PRRSV-1 was first reported as one of the main endemic strains on a pig farm where multiple PRRSV subtypes coexist. The PRRSV-1 strains of this farm belong to the BJEU06-1-like branch of subtype 1, and this clade presents high strain diversity. This study is the first to report the prevalence and characteristics of PRRSV-1 on a large farm in mainland China, which will provide a reference for the identification and further prevention and control of PRRSV-1.

Keywords: PRRSV-1, first detection, main epidemic strain, Chinese pig farm, evolution and genetic diversity

Host Genetics

Oral

Isolation, identification and pathogenicity analysed of a NADC34-like porcine reproductive and respiratory syndrome virus

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Introduction

Currently, NADC34-like strains is prevalent in many provinces of China, which has a significant impact on the swine industry of China. Recent studies have shown that NADC34-like PRRSV strain have recombined with domestic strains and spread rapidly, which increase the difficulty of PRRSV prevention and control. In this study, we isolated and identified a PRRSV NADC34-like strain, and conducted an investigation of the pathogenicity, to provide reference for the prevention and control of PRRSV.

Materials and methods

The lungs of infected pigs were collected from one large-scale pig farm in Jilin Province, amplified and identified by qPCR. We filtered the positive samples, and used to inoculate and culture into Marc-145 cell line. After 3 blind passages, the Marc-145 samples with cytopathic effect (CPE) were subjected to examination by indirect immunofluorescence assay (IFA). PRRSV ORF3, ORF5 and Nsp2 (hypervariable region) genes were amplified by RT-PCR and sequenced, and phylogenetic evolution and homology analysed with the nucleotide sequences published on the NCBI.

Results and cinclusion

The cycle threshold values (Ct) of PRRSV antigen was 28.21, which showed that PRRSV was positive. The CPE of Marc-145 cells were appeared after 72h of post-infection. Antigenicity positive were proved by IFA test. PRRSV ORF3, ORF5 and Nsp2 (hypervariable region) genes were sequenced, ultimately, obtained one ORF3 gene, one ORF5 gene, none Nsp2(hypervariable region) gene. The comparative analysis of the resulting sequences and the reference strains sequences found out both ORF3 and ORF5 sequences had the highest amino acid sequence homology with NADC34 strain, which was 92.5% and 96.1%, respectively. The evolutionary tree demonstrated all the samples were in the same evolutionary branch with the NADC34 strain. We finally proved that the isolated strain was PRRSV NADC34-like strain.

The results of pathogenic investigation showed that the symptoms of abortion were well controlled after the injection of inactivated PRRSV vaccine to the sows. The main harm was to the nursery pigs, such as emaciation, hair growth, eyelid edema, secretion increased, limping and hind legs paralysis increased, overt thoracic and abdominal fibrinous exudate, hydropericardium with fibrinous exudate.

Switching immune target: applying MJPRRS classifications to characterize how PRRSV GP5-epitope C changes over time

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Introduction

PRRSV is the most economically significant disease of swine in the US and evolves rapidly, which poses a major barrier to effective immunization by generating genetic and antigenic diversity leading to poor cross-protection. The surface protein GP5 is of interest for immunity because it contains several epitopes, including epitope C, which is thought to be a target for homologous neutralization and overlaps with a hypervariable region (HVR2) in the ORF5 gene. Our objective was to quantify how epitope C varies across time and assess the extent to which these changes were associated with emergence, which would support the hypothesis that lineages have different immunological phenotypes.

Materials and Methods

A dataset of 14,552 PRRSV-2 ORF5 sequences from the upper Midwest USA (2004-2021) were assigned to epitope C immune groups according to the MJPRRS typing scheme (developed by Phibro Animal Health), which characterize different amino acid patterns present in epitope C. We investigated the rates of such switching events between different MJPRRS groups using Bayesian Evolutionary Analysis Sampling Trees (BEAST) discrete trait analysis.

Results

BEAST analysis showed that pattern D1 was the likely ancestral epitope pattern for non-lineage 1 PRRSV strains, with other epitope patterns emerging within each non-lineage 1 clade. In contrast, pattern D4 was the most likely ancestral epitope for lineage 1, with early epitope pattern switches emerging with sub-lineages, e.g., pattern D7 emerged with sub-lineage 1A and pattern S1 emerged with sub-lineage 1F. Within each sub-lineage, there are additional micro-emergences associated with pattern switching, e.g., separate clades within sub-lineage 1C switching to pattern D5 or to pattern S2 and on to S1.

Conclusions

Characterizing PRRSV evolution through the lens of MJPRRS epitope pattern classifications can provide insight into how the virus has been changing from an antigenic perspective. This may allow for more precise immunization strategies in the field by targeting the circulating epitope pattern and those that it is most likely to transition to. Investigating other regions of the PRRSV genome, particularly those that are likely antigenic, may highlight patterns of emergence of potentially immunologically distinct strains.

Keywords: PRRS, MJPRRS, antigenic evolution

PRRSV modulates autophagy via multiple mechanisms to optimize viral proliferation

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Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is known for its immunosuppressive nature. Autophagy plays a crucial role in viral infections and immunity. However, it remains unclear whether PRRSV infection triggers complete autophagy and the role of autophagy in PRRSV-mediated immune suppression. Understanding the interplay between autophagy and PRRSV can provide valuable insights for combating PRRSV.

Materials and Methods

The effects of PRRSV nonstructural protein (nsp5) and E protein (a viral structural protein) on autophagy were investigated through assessing LC3-II levels via Western blotting and analyzing the colocalization of autophagosomes and lysosomes using IFA. The role of p62 in autophagic degradation of DDX10 was evaluated using p62 knockout cell lines. The impact of DDX10 on PRRSV proliferation was detected through $TCID_{50}$ assays and RT-qPCR.

Results

Nsp5 activated incomplete autophagy by blocking autophagosome-lysosome fusion. Specifically, nsp5 interacted with STX17 to inhibit its interaction with SNAP29, thereby impairing the formation of autolysosomes. In contrast, E protein induced p62-mediated selective autophagy to degrade antiviral proteins such as DDX10. The autophagic degradation block the anti-PRRSV activity of DDX10, ultimately enhancing viral proliferation.

Conclusions and Discussion

Nsp5 induces incomplete autophagy, while E protein promotes complete autophagy. Nsp5 is a nonstructural protein generated in the early stages of PRRSV infection, while E protein is a structural protein productid in the late stages. This suggests that PRRSV may utilize various proteins at different infection stages to modulate autophagy. Notably, DDX10 can increase IFN-β levels, and PRRSV initially induces low levels of IFN-β but decreases them in the later stages. These findings imply that E protein-mediated degradation is an important immune evasion mechanism by PRRSV.

Keywords: PRRSV, Autophagy, Immune evasion, Replication

Performance of a newly designed qPCR for multiplex detection of PRRSV-1 and PRRSV-2: field study evaluation

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) continues to be one of the most economically significant diseases affecting the swine industry worldwide. Effective management strategies for controlling PRRSV rely on the use of diagnostic assays, with ELISA and qPCR being the most common. This study investigates the performance of a newly designed multiplex qPCR assay for detection and differentiation of PRRSV-1 and PRRSV-2 and compares this to a reference qPCR.

Materials and Methods

A total of 370 swine samples, including serum, oral fluids, lung lavage, processing fluids and tongue tip exudates, were obtained from several farms in the US. Samples were extracted and independently tested using two different qPCR diagnostic assays using the same qPCR instrument. qPCR-A refers to the RealPCR PRRSV Type 1 and Type 2 Multiplex RNA Test, IDEXX Laboratories while qPCR-B refers to another commercially available qPCR test for PRRSV-1 and PRRSV-2.

Results

PRRSV-1 results: 101 samples were PRRSV-1 positive by one or both qPCRs. 269 samples were negative by both PCR assays. The percentage of agreement was 95.1%. The average Ct values for positive samples was 30.8 for qPCR-A and 32.1 for qPCR-B. qPCR-A identified six more positive samples (confirmed by an alternate method) than qPCR-B.

PRRSV-2 results: 170 samples were positive by one or both qPCRs. 200 samples were negative by both assays. The percentage of agreement between qPCR-A and qPCR-B was 95.1%. The average Ct values for positive samples was 29.8 for both assays. Discrepant results corresponded to high Ct values (> 36).

Conclusions and Discussion

The comparison of two qPCR assays for detection of circulating strains of PRRSV-1 and PRRSV-2 in North America showed that both tests identified the same number of PRRSV-2 RNA positive samples with the same average Ct value. However, qPCR-A identified more PRRSV-1 positive samples compared to qPCR-B and had a lower average Ct value. According to this evaluation, qPCR-A showed high performance for detection of PRRSV-1 and PRRSV-2 strains circulating in North America.

Keywords: PRRS, PRRSV, PCR, qPCR, RT-qPCR

PRRSVSeq: Multiplex PCR-Based Whole-Genome Sequencing for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Background

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) imposes a substantial economic burden on the global swine industry. PRRSV is primarily categorized into two genotypes: European (PRRSV-1) and North American (PRRSV-2), each containing multiple subtypes. The high genetic variability and rapid spread of this virus pose significant challenges to existing monitoring and control measures. Therefore, enhancing genomic surveillance techniques for PRRSV is crucial for understanding the virus's evolution and dissemination. This study presents PRRSVSeq, a novel amplicon sequencing method that enables efficient and cost-effective whole-genome sequencing of PRRSV and is applicable to various PRRSV strains.

Materials and Methods

Primer schemes for PRRSV-1 and PRRSV-2 were designed for multiplex tiling PCR. Each scheme consists of 102-105 primer pairs with an average amplicon length of 250 bp. The primers were validated using PRRSV virus stocks and clinical samples from multiple institutions, representing diverse genotypes. A Snakemake-based bioinformatics pipeline was developed for Illumina/MGI sequencing data analysis, including read mapping, primer trimming, consensus genome generation, and variant detection.

Results

The developed primer schemes are suitable for PRRSV and can be used for individual strain sequencing or combined for pan-strain sequencing. The method was validated using virus stocks and clinical samples with diverse genetic backgrounds. Except for a few strains, all achieved genome coverage above 95%. The PRRSVSeq method showed comparable sensitivity and coverage to other commonly used primer schemes. PRRSVSeq can be used for sequencing samples of unknown strains, detecting multiple strains within a single sample, and is compatible with various library preparation kits and sequencing instruments.

Conclusions and Discussion

Through comprehensive validation, PRRSVSeq was demonstrated to be an efficient, low-cost, and sensitive whole-genome amplicon sequencing method that can cover the genetic diversity of PRRSV. This method can seamlessly integrate with existing amplicon sequencing workflows and is suitable for global PRRSV genomic surveillance. Future work will focus on further optimizing this method to address newly emerging viral variants and promoting its application in low-resource settings.

Keywords: PRRSV, Whole-genome sequencing, Multiplex PCR

Fidelity Characterization of Highly Pathogenic Porcine Reproductive and Respiratory Syndrome Virus and NADC30-like Strain

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Introduction

Viral recombination accelerates evolutionary processes, enhancing genetic diversity and adaptability. Recombinant viruses derived from highly pathogenic PRRSV (HP-PRRSV) and NADC30-like strains are prevalent in fields with high proportions. The mechanism is unclear, so the replication fidelity of HP-PRRSV and NADC30-like strain were characterized both in vitro and in vivo. Moreover, the fidelity determinant region was also investigated.

Materials and Methods

The viral genome of the HP-PRRSV strain JXwn06 and the NADC30-like strain CHsx1401 was extracted from infected PAMs with MOI of 0.01 at 24 hpi, followed with Next-Generation Sequencing (NGS) and Virus Recombination Mapper (ViReMa) analysis. The titers and genome copies of JXwn06 and CHsx1401 were quantified to compare the amount of defective viral genome (DVG). The sera samples of JXwn06 or CHsx1401 infected pigs were collected at 7 days post-infection, for NGS and ViReMa analysis to investigate the fidelity differences of PRRSV in vivo. In addition, the fidelity of two chimeric viruses with swapped nsp9 and nsp10 between HP-PRRSV and NADC30-like virus was also investigated and compared with the parental strains by testing the nucleotide analog resistance and NGS with ViReMa analysis.

Results

JXwn06 generated more accumulative mutations both in vivo and in vitro, while CHsx1401 generated more recombination junctions and DVG. The chimeric strain JSn9n10 was slightly more sensitive to analogs compared to the parental strain RvJXwn and SJn9n10 was significantly more sensitive to analogs compared to the parental strain RvCHsx1401. Additionally, JSn9n10 had higher junction frequency and junction types and lower accumulative mutation numbers than RvJXwn, whereas SJn9n10 showed the opposite characteristics compared to the RvCHsx1401.

Conclusions and Discussion

The data reveal that NADC30-like strain CHsx1401 generates more recombination while JXwn06 generates more accumulative mutations, suggesting a propensity for different types of genetic variations between these strains. Furthermore, the fidelity evaluation of chimeric viruses with swapped nsp9-10 segments between JXwn06 and CHsx1401 further indicated the nsp9-10 region as a critical determinant of their fidelity.

Keywords: PRRSV, fidelity, recombination, mutation

Evaluation of the resistance of Liang Guang small Spotted pigs with partial deletion of the CD163 SRCR5 Domain to Porcine Reproductive and Respiratory Syndrome Virus 2 Infection

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Porcine reproductive and respiratory syndrome viruses (PRRSVs) has posed a serious threat to the swine industry. CD163 has been identified as essential receptor for PRRSV infection mainly through the interaction ofthe scavenger receptor cysteine-rich domain 5 (SRCR5) region with virus. Therefore, we previously employed CRISPR/Cas9 to deleted a 41-aa fragment containing the ligand-binding pocket (LBP) in the SRCR5 domain of CD163 in Chinese indigenous pig breed Liang Guang small Spotted pig. Here, we describe the evaluation of in vivo and in vitro viral challenge of gene edited pigs.

The porcine alveolar macrophages (PAMs) were isolated for PRRSV JXA1 strain challenge. Through cytopathic effect (CPE) analysis, immunofluorescent staining and western blot analysis of viral protein expressions, and detection of viral nucleic acids, we found the PRRSV was absent in PAMs derived from gene edited homozygotes at any time point, indicating that the homozygous PAMs were fully resistant to PRRSV infection in vitro. In contrast, PAMs derived from both the gene edited heterozygous and wild type pigs did were susceptible to PRRSV infection.

Furthermore, we found the heterozygotes are more susceptible to PRRSV infection, as reflected by pig death occurred first on day 5 after challenge, and all died on day 7, with high viremia and fever throughout the animal viral challenge. While the first death of wild type pig occurred on day 10 post challenge, and the survival rate was 66.7%. In contrast, the gene edited homozygotes pigs did not present fever and viremia, and all survived after viral challenge. Finally, the necropsy showed that severe lesions were found in lungs of gene edited heterozygous and wild-type pigs, while no obvious lesion was found in lungs of gene edited homozygous pigs.

Our results indicate the small deletion in SRCR5 of CD163 can confer fully resistance to PRRSV infection at homozygous state, whereas the gene edited heterozygotes were more susceptible to viral infection. The underlying mechanisms will be further investigated.

KEYWORDS： Liang Guang small Spotted pigs，Anti-PRRSV，CD163 SRCR5

Emerging Viruses

Posters

Novel characteristics of Chinese NADC34-like PRRSV during 2020-2021

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Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a major disease-causing significant economic loss in the global swine industry. Before 2020, an overwhelming majority of PRRSV-2 strains in China could be divided into four lineages: sublineage 8.7 (JXA1-like and CH1a-like), sublineage 5.1 (VR-2332-like), sublineage 3.5 (QYYZ-like), and sublineage 1.8 (NADC30-like). NADC34-like(sublineage 1.5) strains were first detected in China in 2017, was reported to be a potential pandemic strain in China.

Materials and Methods

From 2020 to October 2021, we collected 828 clinical samples of pigs with suspected PRRSV infection from different pig farms in 14 provinces of China. Then RT-PCR was used to identify whether the samples were PRRSV positive, and some NADC34-like PRRSV positive samples were selected to complete the whole genome sequencing. DNAStar, MEGA7.0, Simplot(v3.5) and other biological software were used for sequence splicing, alignment, recombination analysis and phylogenetic analysis.

Results

From 2020 to October 2021, 82 NADC34-like PRRSV isolates were obtained from 433 PRRSV-positive clinical samples. These strains accounted for 11.5% and 28.6% of positives in 2020 and 2021, respectively, and have spread to eight provinces. All Chinese NADC34-like strains cluster with sublineage 1.5 strains. Six of fifteen complete genome sequences were derived from recombination between NADC34-like and NADC30-like or HP-PRRSV.

Conclusions and Discussion

Since 2021, NADC34-like PRRSV has become one of the main epidemic strains in some regions of China. NADC34-like PRRSV has recombined with local strains in China and shows complex recombination. Novel changes in NADC34-like PRRSV pose a great challenge to monitoring as well as preventing and controlling PRRSV. Therefore, we should prioritize the continuous monitoring of NADC34-like PRRSV and the strengthening of PRRSV prevention and control.

Keywords: NADC34-like PRRSV, novel characterization, recombination, endemic strain

Novel characterization of NADC30-like and NADC34-like PRRSV strains in China: epidemiological status and pathogenicity analysis of L1A variants

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Introduction

NADC34-like PRRSV which first appeared in China in 2017, is currently one of the main epidemic strains in China. We found that a new variant of NADC34-like PRRSV evolved, named the L1A variant. The phylogenetics, epidemic status, and pathogenicity of the L1A variants were subsequently comprehensively evaluated.

Materials and Methods

The samples tested positive from different regions from 2021 to October 2023 were amplified and the DNA fragments were connected to PMD-18T vector for sequencing. DNA Star, MEGA7.0, Simplot (v3.5) and other biological software were used for sequence splination, comparison, recombination analysis and phylogenetic analysis.

Results

Based on the results of the ORF5 phylogenetic analysis, the L1A variants were classified as NADC34-like PPRSV. Recombination analysis revealed that the L1A variants were recombinant viruses that contained an NADC30-like PRRSV skeleton, a nonstructural protein-encoding gene region obtained in part from JXA1-like PRRSV and a ORF2-ORF6 gene region partly obtained from NADC34-like PRRSV and that exhibited similar recombination patterns. In animal experiments, L1A variant TZJ2756 exhibited moderate pathogenicity in piglets, causing obvious clinical symptoms, namely, persistent fever, significantly reduced body weight, interstitial edema and severe interstitial pneumonia in the lungs, and prolonged high-load viremia.

Conclusions and Discussion

The L1A variant strains are a class of recombinant viruses that contain a viral skeleton obtained from NADC30-like strains and a partial recombination fragment obtained from the NADC34-like and JXA1-like strains, exhibit similar viral recombination patterns, and have the same characteristic 131-aa deletion in the Nsp2 region. The L1A variant TZJ2756 exhibits moderate pathogenicity. Our findings contribute to the understanding of the evolutionary characteristics of Chinese NADC34-like PRRSV strains.

Keywords: NADC34-like PRRSV, NADC30-like PRRSV, L1A variant, recombination, pathogenicity