

SHORT COMMUNICATION

Assessment of PRRSV and PCV2 seroprevalence and antigen prevalence in minipigs at laboratory-animal production facilities

Yoon Beom Lee¹ , Ji Woon Kim¹ , Woori Jo¹ , Tae-Ku Kang¹ , MinKyoung Sung¹ , KilSoo Kim² ,
Na-Hye Park¹ , Gwang-Hoon Lee¹ 

¹Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Republic of Korea

²Department of Veterinary Toxicology, College of Veterinary Medicine, Jyungpook National University, Daegu, Republic of Korea

ABSTRACT

Objective: Pigs are used in various biomedical research fields because of their anatomical and physiological similarities to humans. While farm pigs are raised outdoors for several months, minipigs are typically raised in indoor barrier facilities for several years. Although numerous studies have investigated the prevalence of major pathogens, including porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2), in farm pigs, similar studies targeting minipigs are lacking.

Materials and Methods: We imported 57 minipigs to our institution from three experimental animal production facilities and immediately assessed the serological and antigenic prevalence of PRRSV and PCV2.

Results: PRRSV and PCV2 had seroprevalences of 80.7% and 94.7%, respectively, with 0% antigen positivity rates for PRRSV types 1 and 2 and high-pathogenic PRRSV and PCV2.

Two factors could account for the high seroprevalence rates: the majority of individuals may have been vaccinated despite official claims from the origin facilities or exposed to PRRSV and PCV2. Preventing microbial infections is crucial for obtaining accurate and reproducible results.

Conclusion: As the first investigation of microbial prevalence in minipigs, our findings indicate that minipigs raised in barrier facilities are not necessarily free from vaccines or infections. These results will significantly enhance the credibility of future biomedical research.

ARTICLE HISTORY

Received May 28, 2024

Revised June 23, 2024

Accepted July 20, 2024

Published December 28, 2024

KEYWORDS

Experimental animal; Production facilities; Minipigs; Porcine circovirus type 2; Porcine reproductive and respiratory syndrome virus.







© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0>)

Introduction

Animal research facilities are dedicated to scientific exploration and experimentation involving animals and serve various disciplines, such as medicine, biology, and life sciences. A wide array of animal species has been employed for research purposes, including mice, rats, rabbits, dogs, pigs, and monkeys [1,2]. Pigs are particularly noteworthy because of their anatomical and physiological resemblance to humans. They are used to assess the efficacy of drugs for cardiovascular diseases, ocular diseases, skin disorders, reproductive diseases, and even organ transplantation studies because their organs are similar in size to human organs. Additionally, because pigs have dimensions similar to those of humans, they have been used in diverse preclinical evaluations of medical devices [3,4]. However,

laboratory pigs are very different from farm pigs in terms of their size and rearing environment.

Farm pigs are raised for consumption, can weigh more than 300 kg, and are typically reared for 6 months before being slaughtered at weights ranging from 100 to 120 kg [5,6]. In contrast, experimental pigs (referred to as minipigs) can be nurtured for prolonged durations and generally achieve weights between 30 and 70 kg by 2 years of age, making them substantially different from farm pigs [6,7]. Furthermore, farm pigs are reared in outdoor farm environments, whereas minipigs are housed indoors in controlled facilities. These facilities employ HEPA filters for air purification, and the environmental conditions are maintained at approximately 23°C ± 3°C and 50 ± 10% humidity. Minipigs are provided with sterilized feed and

Contact Gwang-Hoon Lee  ghl@kmedihub.re.kr  Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Republic of Korea and Na-Hye Park  pnh0211@kmedihub.re.kr  Preclinical Research Center, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Republic of Korea.

How to cite this article: Lee YB, Kim JW, Jo W, Kang TK, Sung M, Kim K, Park NH, Lee GH. Assessment of PRRSV and PCV2 seroprevalence and antigen prevalence in minipigs at laboratory-animal production facilities. *J Adv Vet Anim Res* 2024; 11(4):1017–1022.

filtered water to ensure their health and protect them from microbial contaminants. Such stringent cleanliness measures safeguard the health of minipigs, allowing for controlled research variables and ensuring the accuracy and reliability of the experimental outcomes, crucial for evaluating the efficacy of pharmaceuticals and medical devices [8].

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus (PCV) are significant diseases affecting pigs worldwide, including Korea, where the swine industry is prominent. Because these diseases are particularly important in areas where the pig industry is developed and difficult to control, each authority of the countries is making efforts to manage and prevent them [9,10]. PRRSV was initially identified in North America (type 2) in the late 1980s and Europe (type 1) in the early 1990s, with nucleotide sequence similarities between the two types ranging from 55% to 70%, highlighting their substantial differences. In Korea, PRRSV types 1 and 2 cocirculate [11].

PRRSV is a positive-stranded RNA virus categorized under the family Arteriviridae within the order *Nidovirales*. It is characterized by reproductive disorders and respiratory issues and occurs in two main genotypes: the European prototype (EU-type, type 1), represented by the Lelystad virus, and the North American prototype (NA-type, type 2), identified as VR-2332 [12]. The Circoviridae family comprises small, icosahedral, nonenveloped viruses with single-stranded circular DNA genomes. Within the Circoviridae family, two genera (Gyroviruses and Circoviruses) are distinguished based on differences in genome size and organization. PCV types 1 and 2 (PCV1 and porcine circovirus type 2 (PCV2), respectively) belong to the genus Circovirus. PCV1 is considered nonpathogenic and is not linked to naturally occurring diseases, whereas PCV2 is the primary pathogen responsible for post-weaning multisystemic wasting syndrome [13,14].

According to previous studies, the antigen prevalence of PRRSV was approximately 69.8% in farm pigs in Korea from 2018 to 2022 [15], whereas in 2022, the antigen prevalence of PCV2 was 62.3% in farm pigs [16], indicating that PRRSV and PCV2 are prevalent diseases in farm pigs. Although numerous studies have explored the prevalence rates of PRRSV and PCV2 in farm pigs, no studies have explored microbial infections, such as PRRSV and PCV2, in minipigs maintained in experimental animal production facilities with barrier systems [11,16-20].

The health status of experimental animals significantly affects the reproducibility and accuracy of research outcomes. Microbial infections can alter the physiological state of animals and activate the immune system, potentially biasing experimental results. Moreover, infected animals can transmit infections to others during the research

period, emphasizing the importance of maintaining animal health and minimizing microbial infections to enhance research reliability. Therefore, appropriate surveillance and preventive measures are required.

Our study aimed to evaluate the seroprevalence of PRRSV and PCV2 in pig populations from experimental animal production facilities to evaluate the immune status of these pigs. Additionally, PCR testing was used to investigate the antigen positivity rates in individuals with a positive antibody status and to ascertain whether they had been vaccinated or infected. Specifically, we sought to obtain information on the antibody and antigen positivity rates of pigs in controlled environments in experimental animal production facilities to enhance the accuracy and reliability of the experimental results.

Materials and Methods

Ethical approval

The animal study protocol was approved by the Institutional Review Board of the Daegu Gyeongbuk Medical Innovation Foundation (protocol code: KMEDI-24032701; approval date: 03 March 2024).

Isolation of serum

From 2021 to 2024, pigs ($n = 57$) from three experimental animal production facilities in Korea were imported to the Daegu-Gyeongbuk Medical Innovation Foundation. Immediately upon arrival, whole blood was collected via jugular vein puncture into the serum-separating tube (SST, BD, 367815, Netherlands). The SST tubes were then centrifuged at 3000 rpm for 10 min at 4°C to separate the serum. The separated serum sample was stored at -70°C until further analysis. The samples were categorized according to age (Table 1).

Reagents

All enzyme-linked immunosorbent assay (ELISA) and virus detection kits were procured from South Korea. The PRRSV ELISA kit (Bionote PRRS Ab ELISA 4.0; Cat No. EB4404PO) was purchased from BIONOTE (Hwasung, Korea). The PCV2 ELISA kit (VPro® PCV2 AB ELISA, Cat no. ES-PCV-01) was purchased from MEDIAN, and the PRRSV / PCV2 Detection Kit (Prime-Q, Cat no. ADP-1113Q2) was purchased from GENETBIO (Daejeon, Korea). Reagents from the RNeasy

Table 1. The number of animals according to age.

Facility	<1 years	1–2 years	>2 years	Total
A	4	34	7	45
B	6	2	0	8
C	0	4		4

Mini Kit (Qiagen, Hilden, Germany; 74104) and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Basingstoke, UK) were used to prepare the diagnoses.

Serology

All sera were analyzed using ELISA kits: the Bionote PRRS Ab ELISA 4.0, which can detect antibodies against type 1 PRRSV. Samples with sample-to-positive (S/P) ratios of ≥ 0.4 (cutoff value) were considered positive for the antibody against PRRSV. The VPro® PCV2 AB ELISA kit can only detect antibodies against PCV type 2. The cutoff value for this ELISA was 0.4, as for the PRRSV ELISA kit.

Confirmation of infection

Viral RNA was extracted using an RNeasy Mini Kit (Qiagen, USA). The extracted RNA was diluted 2-fold in diethyl pyrocarbonate-treated water. The RNA concentration (ng/ μ l) and purity were determined using a U-2800 spectrophotometer (Hitachi High Technologies, Japan). Complementary DNA was synthesized from 100 ng of RNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's protocol.

Subsequently, a reaction mixture containing 10 μ l of One-step qRT-PCR Premix (Prime-Q PRRSV/PCV2 Detection Kit, GENETBIO), 5 μ l (10 pmol) of primer/Probe Mixture of the target gene, and 5 μ l of cDNA was subjected to real-time PCR analysis. The primer used to detect the expression of the pathogens and their condition, including annealing temperatures, is listed in Table 2.

Identification of highly pathogenic PRRSV using PCR

Primers were prepared according to Table 3. Primers encoding the following adhesins: a highly pathogenic form of PRRSV isolated from Korea. Commercial PCR master mixes (AccuPower PCR PreMix, Bioneer, Daejeon, South Korea) were used for amplification; a 20- μ l PCR reaction was carried out with the following amplification conditions: 1 cycle at 95°C for 5 min, 35 cycles at 95°C for 20 s, 60°C for 30 s, and 72°C for 30 s (Table 3). The PCR product was visualized on a 1.5% agarose gel at 100 V for 30 min.

Table 2. Conditions of real-time PCR analysis.

Step	Temperature (°C)	Time	Cycle
cDNA synthesis	50	20 min	1
Pre-denaturation	95	10 min	1
Denaturation	95	10 sec	40
Annealing/extension	60	30 sec	

Table 3. The primer sequences for PCR.

Target gene	Sequence (5'-3')	Tm (°C)
JBNU-22-N01	CAGGAACGGTGCTTGTGTTG	60
	TCACCCAGCTAGTCGATCA	
JBNU-22-N02	ACAGCGAAGGATGCAAGTGA	60
	CACTTGTGAGTGCCAAACCG	
JBNU-22-N03	ACAGCGAAGGATGCAAGTGA	60
	CACTTGTGAGTGCCAAACCG	

Results

Serology of PRRSV and PCV antibodies by ELISA

To determine the formation of PRRSV and PCV antibodies in laboratory swine, all serum samples were analyzed using ELISA (Table 4 and Figure 1). PRRSVs were detected in 46 animals, and 54 pigs showed antibodies against PCVs. The S/P values of ELISA for each group are shown in Figure 1 (c) and (d). Each group was separated by regions of the laboratory animal facility. Of the 57 samples from each group, 46 (80%) were PRRSV antibody-positive tested by ELISA, and 54(95%) were positive for PCVs. Results of PCV are shown in Figure 1 (b) and (d) in terms of contingency tables. As a result of dividing each group by age, no statistically significant differences were identified according to specific age (Figure 1 c and d).

Confirmation of PRRSV and PCV infection using real-time PCR

Viral infection was confirmed using real-time PCR. In total, 57 serum samples were isolated from laboratory swine in three facilities. According to this test, PRRSV and PCV type 2 were not detected in any animal (Table 5).

Confirm the infection of NADC34-Like PRRSV by PCR

Outbreaks of NADC34-Like PRRSV have been reported in Korea [21]. Therefore, this study also tried to confirm the NADC34-like PRRSV infection through the PCR, but it was not detected in all of the serums. This ultimately suggests that PRRSV infection was not confirmed in the laboratory animal facility.

Discussion

This study represents the first investigation of the microbial prevalence in minipigs from experimental animal production facilities. The study focuses on PRRSV and PCV, which cause systemic symptoms, including respiratory issues, in pigs. These viruses are the most prevalent infectious agents on pig farms, causing fever, weight loss, and death upon infection. Particularly in minipigs used in experiments, PRRSV and PCV infection cause significant

academic and economic damage [12,13]. Despite the assumption that mini pigs are raised in barrier facilities and are not infected, there have been no studies to prove this in biomedical research.

Official information provided by laboratory animal supply facilities states that minipigs are raised in barrier facilities devoid of vaccinations. According to our findings, the antigen prevalence of PRRSV in minipigs supplied by laboratory animal facilities was 80.7%, whereas that of PCV Type 2 (PCV2) was 94.7% (Table 4). These are higher than the antibody rates (PRRSV: 69.8% and PCV2: 62.3%) of farm pigs in general [15,16]. This discrepancy suggests the possibility of PRRSV or PCV2 infection or vaccination. However, the fact that the antigen positivity rate is 0% may suggest that vaccination is more likely than infection. Additionally, the fact that the subjects were housed in a barrier facility may support this possibility. Nevertheless, the prevalence of antibodies varied among the facilities. For PRRSV, Facility A exhibited a seroprevalence of 84.4%, Facility B exhibited a seroprevalence of 25%, and Facility C exhibited a seroprevalence of 87.5%. Similarly, for PCV2, Facility A had a seroprevalence of 100%, Facility B 25%,

and Facility C 100% (Figure 1). However, only one out of four minipigs from Facility B showed antibodies against both PRRSV and PCV2, suggesting lower antibody formation, possibly due to vaccination.

Pigs have historically served as valuable animal models for various infectious diseases [22,23]. Vaccines can induce cross-immunity, which is altered by the host immune response, with variations based on sex, genetic factors, and age.

Severe microbial infections in experimental animals can affect parameters such as weight gain and anesthetic depth, potentially causing adverse effects in veterinary management. Pigs are crucial animal models for evaluating vaccine efficacy [22,24,25].

PRRSV and PCV2 have high transmissibility rates and can localize upon infection [9,18]. Given the high infectivity of PRRSV and PCV2 and their persistence following infection, vaccination in experimental animal production facilities may be considered more beneficial than risky. Maintaining pig health through vaccination rather than increasing susceptibility to microbial infections can be advantageous for research involving minipigs. However, when conducting research such as vaccine evaluations, considering cross-immunity plays a pivotal role in preserving the precision and consistency of experimental findings [26,27]. This study revealed that the antibody rates are significantly high in experimental minipigs. Therefore, it is advisable to conduct antibody tests to verify vaccination

Table 4. Number of pigs in which PRRSV and PCV antibodies were detected ($n = 57$).

Results	PRRSV	PCV
Positive	46	54
Negative	11	3

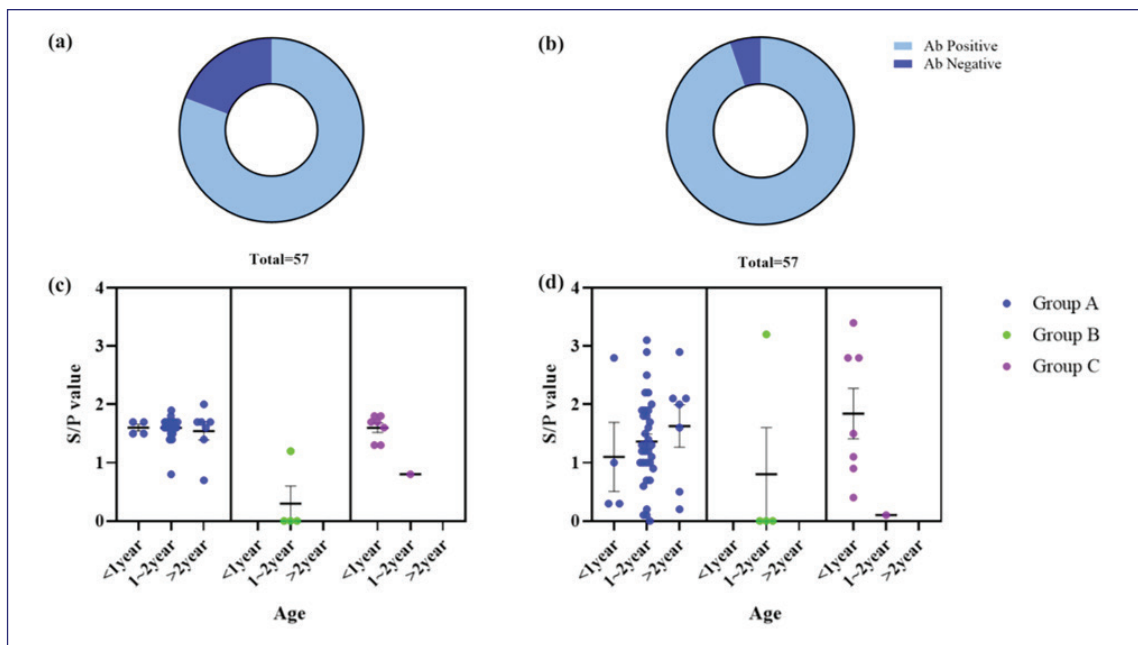


Figure 1. Results of serology. This figure shows PRRSV and PCV antibody positivity rates (a and b) and the distribution of S/P values (c and d).

Table 5. Results of PRRSV and PCV detection.

Results	PRRSV	PCV
Positive	0	0
Negative	57	57

against prevalent microbes such as PRRSV and PCV2 before starting research.

Therefore, before conducting research, it is advisable to perform antibody testing to confirm vaccination against microbes prevalent in the country, such as PRRSV and PCV2. For farm pigs, periodic monitoring is necessary to establish epidemiological measures owing to the presence of PRRSV and PCV2. In Korea, surveillance is conducted based on the genotype of PCV2 (PCV2a, PCV2b, PCV2c, and PCV2d) to devise national-level epidemiological measures [16,28].

However, our study did not detect antigens of PRRSV types 1 and 2 or the more pathogenic NADC-like PRRSV and PCV2 (Figure 1). The NADC-like PRRSV of lineage 1 is distinct from the highly pathogenic PRRSV of lineage 8, which is currently observed in China. Because no reports of highly pathogenic PRRSV have emerged in Korea and clinical symptoms were absent in minipigs, additional testing was not pursued. While clinical symptoms may not be evident in cases of singular PRRSV infection, animals with highly pathogenic PRRSV typically exhibit symptoms [29-31]. Thus, the results of this study indicate that PRRSV and PCV infections were not confirmed in the experimental minipigs. This suggests that the breeding and research facility environments are maintained at a reliable level that prevents exposure to infectious agents.

One limitation of this study lies in the restricted sample size, with a relatively high proportion from Facility A (78.9%), followed by Facility C (14.0%) and Facility B (7.0%). However, our study is significant because it is the first to investigate seroprevalence and antigen prevalence in pigs from laboratory animal supply facilities. Future studies should increase the sample size and test for PRRSV and PCV2 antigens in specific organs, such as lymph nodes, during pig necropsy to enhance the reliability of the experimental results.

Conclusion

This study investigated the seroprevalence and antigen prevalence of PRRSV and PCV in minipigs housed in an experimental animal production facility. Our findings revealed a notable seroprevalence of PRRSV and PCV2 in the minipig population, indicating exposure to these pathogens. Interestingly, the observed antibody prevalence exceeded the levels suggested by the official records of the facility, suggesting potential vaccination practices

contrary to the described barrier conditions. Furthermore, significant variations in seroprevalence were observed among facilities, indicating potential differences in management practices or exposure levels. Although vaccination may offer benefits in preventing infections, its implications for research outcomes, particularly in studies involving cross-immunity, require careful consideration. Therefore, we recommend antibody testing for prevalent microbial infections, such as PRRSV and PCV2, before conducting experiments to ensure accurate and reproducible results. This groundbreaking study provides insights into the prevalence of microbial infections in minipigs within laboratory animal production facilities, emphasizing the importance of proactive health management and surveillance to ensure research integrity and animal welfare.

List of Abbreviations

ELISA, enzyme-linked immunosorbent assay; EU-type, type 1, European prototype; NA-type, type 2, North American prototype; PRRSV, porcine reproductive and respiratory syndrome virus; PCV2, Porcine Circovirus Type 2; PCV1, PCV types 1; PCV2, PCV types 2.

Acknowledgments

This study was supported financially by the Daegu-Gyeongbuk Medical Innovation Foundation.

Conflict of interest

The authors declare no conflicts of interest in data.

Authors' contributions

Y.B.L. and J.W.K. collected data and edited the manuscript. W.J., T.K.K., and M.S. were involved in collecting data. K.S.K. and G.H.L. conceptualized this study. G.H.L. prepared manuscript. N.H.P. and G.H.L. administrated the project and edited the manuscript.

References

- [1] Hasenau JJ. Definitions of research animal, laboratory animal, laboratory animal veterinarian, research veterinarian, and research animal veterinarian. *J Am Vet Med Assoc* 2021; 258(3):254-5; <https://doi.org/10.2460/javma.258.3.254>
- [2] Swaters D, van Veen A, van Meurs W, Turner JE, Ritskes-Hoitinga M. A history of regulatory animal testing: what can we learn? *Alternat Lab Anim* 2022; 50(5):322-9; <https://doi.org/10.1177/02611929221118001>
- [3] Kobayashi E, Hishikawa S, Teratani T, Lefor AT. The pig as a model for translational research: Overview of porcine animal models at Jichi Medical University. *Transplant Res* 2012; 1:1-9; <https://doi.org/10.1186/2047-1440-1-8>
- [4] Mukherjee P, Roy S, Ghosh D, Nandi S. Role of animal models in biomedical research: a review. *Lab Anim Res* 2022; 38(1):18; <https://doi.org/10.1186/s42826-022-00128-1>

- [5] Stygar A, Dolecheck K, Kristensen A. Analyses of body weight patterns in growing pigs: a new view on body weight in pigs for frequent monitoring. *Animal* 2018; 12(2):295–302; <https://doi.org/10.1017/S1751731117001690>
- [6] Singh VK, Thrall KD, Hauer-Jensen M. Minipigs as models in drug discovery. *Expert Opin Drug Disc* 2016; 11(12):1131–4; <https://doi.org/10.1080/17460441.2016.1223039>
- [7] Pawlowsky K, Ernst L, Steitz J, Stopinski T, Kögel B, Henger A, et al. The Aachen minipig: phenotype, genotype, hematological and biochemical characterization, and comparison to the Göttingen minipig. *Eur Surg Res* 2017; 58(5-6):193–203; <https://doi.org/10.1159/000471483>.
- [8] Li R, Wang F, Zhang Y, Li C, Xia C, Chen H, et al. Comparison of hematologic and biochemical reference values in specific-pathogen-free 1-month-old Yorkshire pigs and Yorkshire-Landrace crossbred pigs. *Can J Vet Res* 2019; 83(4):285–290.
- [9] Dietze K, Pinto J, Wainwright S, Hamilton C, Khomenko S. Porcine reproductive and respiratory syndrome (PRRS). Rome: FAO's Emerg Prev Syst. 2011; 1:13–8. <https://doi.org/10.1016/j.theriogenology.2006.04.024>.
- [10] Opriessnig T, Karuppannan AK, Castro AM, Xiao C-T. Porcine circoviruses: recent status, knowledge gaps and challenges. *Virus Res* 2020; 286:198044; <https://doi.org/10.1016/j.virusres.2020.198044>
- [11] Kang H, Yu JE, Shin J-E, Kang A, Kim W-I, Lee C, et al. Geographic distribution and molecular analysis of porcine reproductive and respiratory syndrome viruses circulating in swine farms in the Republic of Korea between 2013 and 2016. *BMC Vet Res* 2018; 14:1–11; <https://doi.org/10.1186/s12917-018-1480-6>
- [12] Liu Z, Shan B, Ni C, Feng S, Liu W, Wang X, et al. Optimized protocol for double vaccine immunization against classical swine fever and porcine reproductive and respiratory syndrome. *BMC Vet Res* 2023; 19(1):14. <https://doi.org/10.1186/s12917-022-03559-z>
- [13] Ren W, Li Y, Yu X, Luo W, Liu G, Shao H, et al. Glutamine modifies immune responses of mice infected with porcine circovirus type 2. *Br J Nutr* 2013; 110(6):1053–60; <https://doi.org/10.1017/S0007114512006101>
- [14] Martínez-Lobo FJ, Díez-Fuertes F, Simarro I, Castro JM, Prieto C. The ability of porcine reproductive and respiratory syndrome virus isolates to induce broadly reactive neutralizing antibodies correlates with in vivo protection. *Front Immunol* 2021; 12:691145; <https://doi.org/10.3389/fimmu.2021.691145>
- [15] Lee M-A, Jayaramaiah U, You S-H, Shin E-G, Song S-M, Ju L, et al. Molecular characterization of porcine reproductive and respiratory syndrome virus in Korea from 2018 to 2022. *Pathogens* 2023; 12(6):757; <https://doi.org/10.3390/pathogens12060757>
- [16] Moon B-M, Chu K-S, Kim S-C, Kim H-J, Kim D-J, Kim W-I. Genotypic diversity and prevalence of Porcine circoviruses for slaughter pigs in Korea. *Korean J Vet Ser* 2023; 46(4):315–24; <https://doi.org/10.7853/kjvs.2023.46.4.315>
- [17] Kim H-R, Park J, Kim W-I, Lyoo YS, Park C-K. Prevalence and co-infection status of three pathogenic porcine circoviruses (PCV2, PCV3, and PCV4) by a newly established triplex real-time polymerase chain reaction assay. *Korean J Vet Ser* 2022; 45(2):87–99; <https://doi.org/10.7853/kjvs.2022.45.2.877>
- [18] Park SC, Kim S, Jeong TW, Oh B, Lim CW, Kim B. Prevalence of porcine circovirus type 2 and type 3 in slaughtered pigs and wild boars in Korea. *Vet Med Sci* 2024; 10(1):e1329; <https://doi.org/10.7853/kjvs.2022.45.2.87>
- [19] Kang I, Ham H. Age-specific prevalence of porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and *Mycoplasma hyopneumoniae* in Korea Pig Farms. *J Bacteriol Virol* 2020; 50(1):9–16; <https://doi.org/10.4167/jbv.2020.50.1.009>.
- [20] Cho H, Kang I, Oh T, Yang S, Park KH, Min K-D, et al. Comparative study of the virulence of 3 major Korean porcine circovirus type 2 genotypes (a, b, and d). *Can J Vet Res* 2020; 84(3):235–40.
- [21] Kim S-C, Kim H-J, Moon S-H, Cho H-S, Kim W-I. First identification and genomic characterization of NADC34-like PRRSV strains isolated from MLV-vaccinated pigs in Korea. *Transb Emerg Dis* 2023:9995433; <https://doi.org/10.1155/2023/9995433>
- [22] Gerdts V, Wilson HL, Meurens F, van Drunen Littel-van den Hurk S, Wilson D, Walker S, et al. Large animal models for vaccine development and testing. *ILAR J* 2015; 56(1): 53–62; <https://doi.org/10.1093/ilar/ilv009>
- [23] Conti F, Abnave P, Ghigo E. Unconventional animal models: a booster for new advances in host-pathogen interactions. *Front Cell Infect Microbiol* 2014; 4:142; <https://doi.org/10.3389/fcimb.2014.00142>
- [24] Habiela M, Seago J, Perez-Martin E, Waters R, Windsor M, Salguero FJ, et al. Laboratory animal models to study foot-and-mouth disease: a review with emphasis on natural and vaccine-induced immunity. *J Gen Virol* 2014; 95(11):2329–45; <https://doi.org/10.1099/vir.0.068270-0>
- [25] Mengeling WL, Lager KM, Vorwald AC, Koehler KJ. Strain specificity of the immune response of pigs following vaccination with various strains of porcine reproductive and respiratory syndrome virus. *Vet Microbiol* 2003; 93(1):13–24; [https://doi.org/10.1016/s0378-1135\(02\)00427-3](https://doi.org/10.1016/s0378-1135(02)00427-3)
- [26] Franzo G, Segalés J. Porcine circovirus 2 genotypes, immunity and vaccines: multiple genotypes but one single serotype. *Pathogens* 2020; 9(12):1049; <https://doi.org/10.3390/pathogens9121049>
- [27] Yu C, Cao M, Wei Y, Liu J, Zhang H, Liu C, et al. Evaluation of cross-immunity among major porcine circovirus type 2 genotypes by infection with PCV2b and PCV2d circulating strains. *Vet Microbiol* 2023; 283:109796; <https://doi.org/10.1016/j.vetmic.2023.109796>
- [28] Shin G-E, Park J-Y, Lee K-K, Ko M-K, Ku B-K, Park C-K, et al. Genetic diversity of porcine reproductive and respiratory syndrome virus and evaluation of three one-step real-time RT-PCR assays in Korea. *BMC Vet Res* 2022; 18(1):327; <https://doi.org/10.1186/s12917-022-03407-0>
- [29] Li Y, Wang X, Bo K, Wang X, Tang B, Yang B, et al. Emergence of a highly pathogenic porcine reproductive and respiratory syndrome virus in the mid-eastern region of China. *Vet J* 2007; 174(3):577–84; <https://doi.org/10.1016/j.tvjl.2007.07.032>
- [30] Han J, Zhou L, Ge X, Guo X, Yang H. Pathogenesis and control of the Chinese highly pathogenic porcine reproductive and respiratory syndrome virus. *Vet Microbiol* 2017; 209:30–47; <https://doi.org/10.1016/j.vetmic.2017.02.020>
- [31] Duinhof T, Van Schaik G, Van Esch E, Wellenberg G. Detection of PRRSV circulation in herds without clinical signs of PRRS: comparison of five age groups to assess the preferred age group and sample size. *Vet Microbiol* 2011; 150(1-2):180–4; <https://doi.org/10.1016/j.vetmic.2011.01.001>