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Assessing the risk of recurrence of porcine epidemic diarrhea virus in affected farms on Jeju Island, South Korea

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ABSTRACT

Background: Porcine epidemic diarrhea virus (PEDV) is a swine enteropathogenic coronavirus that has devastated the swine industry in South Korea over the last 30 years. The lack of an effective method to control the endemics has led to a surge in PEDV recurrences in affected farms throughout the country.

Objectives: In the first step toward establishing systematic monitoring of and active control measures over the swine populations, we constructed an assessment model that evaluates the status of (1) biosecurity, (2) herd immunity, and (3) virus circulation in each of the PEDV-infected farms.

Methods: A total of 13 farrow-to-finish pig farms with a history of acute PEDV infection on Jeju Island were chosen for this study. The potential risk of the recurrence in these farms was estimated through on-site data collection and laboratory examination.

Results: Overall, the data indicated that a considerable number of the PEDV-infected farms had lax biosecurity, achieved incomplete protective immunity in the sows despite multi-dose vaccination, and served as incubators of the circulating virus; thus, they face an increased risk of recurrent outbreaks. Intriguingly, our results suggest that after an outbreak, a farm requires proactive tasks, including reinforcing biosecurity, conducting serological and virus monitoring to check the sows' immunity and to identify the animals exposed to PEDV, and improving the vaccination scheme and disinfection practices if needed.

Conclusions: The present study highlights the significance of coordinated PEDV management in infected farms to reduce the risk of recurrence and further contribute towards the national eradication of PEDV.

Keywords: Biosecurity; endemic; PEDV; sow immunity; virus circulation

INTRODUCTION

Porcine epidemic diarrhea virus (PEDV) is a highly communicable coronavirus that almost exclusively affects newborn piglets, causing watery diarrhea, vomiting, and high mortality [1,2]. PEDV is an enveloped, single-stranded, positive-sense RNA virus of the genus *Alphacoronavirus* in the family *Coronaviridae* of the order *Nidovirales* [3,4]. Based on its spike

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Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

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gene sequence, PEDV can be phylogenetically classified into two genotypes with two sub-genotypes: the low pathogenic (LP)-genotype 1 with the classical G1a and recombinant G1b sub-genotypes and the highly pathogenic (HP)-genotype 2 with the local epidemic G2a and global epidemic or pandemic G2b sub-genotypes [1,2]. Since the unprecedented 2013–2014 pandemic that ravaged the American and Asian pig-farming countries, this swine enteric coronavirus has garnered global awareness, posing economic and animal health threats to the pork business worldwide [1].

The first case of PEDV in South Korea was reported in the early 1990s [5], and the virus has been a significant pathogen in the domestic swine industry for the past three decades [1,2]. The most recent nationwide PEDV outbreak occurred in late 2013 due to the invasion of the highly virulent HP-G2b strains nearly identical, at 99.9% sequence homology, to the strains that emerged in the United States [6]. Within five months of the outbreak, the virus had engulfed the entire nation, including the Jeju Province, also known as Jeju Island, causing the losses of approximately one million piglets during 2013–2014 [1,6,7]. Although Jeju Island had maintained its PEDV-free status for a decade and significantly restricted the trade of live pigs from the mainland, the Jeju swine herds were not immune to PEDV. Since the reemergence of PEDV in Jeju Island in early 2014, the virus has been remained endemic in the province, independently undergoing substantial rapid evolution in Jeju pig populations [8-10]. Furthermore, a considerable number of porcine epidemic diarrhea (PED)-affected swine farms have experienced recurrent outbreaks shortly after the initial occurrence, indicating chronic infection of the inhabitant PEDV strain circulating in the herd.

Once PEDV is introduced in swine populations, instead of becoming eliminated quickly, the virus will highly likely persist within the herd. Therefore, it is necessary to monitor PEDV to prevent or control repetitive PEDV infection in the affected herds or both by strengthening the biosecurity protocols and PEDV management, including virus and serological screening. In this study, we aimed to: 1) evaluate the level of biosecurity in pig farms through on-site inspections and questionnaires regarding external and internal biosecurity protocols; 2) quantity herd immunity in each farm through the serological screening of sows; 3) determine the status of endemic infection in the herd through virus and serological monitoring; and 4) estimate the risk of PED recurrence in individual pig farms on Jeju Island.

MATERIALS AND METHODS

Farm information and sample collection

A total of 13 commercial farrow-to-finish swine farms, with 900 to 3,600 heads per farm, in pig-dense areas in Hallim and Daejeong of Jeju Province, were selected in this study. All swine farms have around five buildings at one site for breeding herds, farrowing sows, nursery, growing, and finishing pigs and have adopted a continuous flow system for marketing pigs. These farms all had a history of acute HP-G2b PEDV infection in their animals accompanied by lethal watery diarrhea and mortality in newborn piglets from January to June 2018 [10]. The history of the PED outbreak and vaccination of each farm was obtained and summarized from in-person interviews (**Table 1**). A total of 20 pigs, including five low-parity (parities 1–2), five high-parity (parity ≥ 3) sows, and 10 growing pigs (aged 70–100 days) from different pens in each PED-affected farm, were chosen for sampling. Paired stool and serum samples were obtained from individual pigs in August 2018. The fecal samples (n = 260) from the sows and growing pigs were collected using 16-inch cotton-tipped swabs. The samples were

Table 1. Information on the farms with PEDV outbreak in 2018

Farm	A	B	C	D	E	F	G	H	I	J	K	L	M
Location	Hallim	Hallim	Hallim	Hallim	Hallim	Hallim	Hallim	Hallim	Hallim	Daejeong	Daejeong	Daejeong	Daejeong
No. of heads	1,554	2,469	2,354	1,400	900	1,350	3,500	2,515	1,800	1,946	1,321	2,814	3,647
Neonatal mortality rate*	80%–90%	100%	90%	> 50%	60%–70%	60%	> 50%	> 70%	90%	70%–80%	100%	66.7%	80%
Vaccination†	K/K‡	K/K	K/K	L/K/K§	L/K/K	L/K/K	K/K/K‖	K/K/K	K/K	K/K	Feedback	K/K	F/K¶

*The mortality rate of the suckling piglets (age < 10 days) during the 2018 PED outbreaks. †Vaccinations were done in these farms after initial outbreaks. ‡K/K: Two doses of killed G2b vaccine were parenterally administered. §L/K/K: One dose of live G2a live vaccine and two doses of killed G2b vaccine were parenterally administered. ‖K/K/K: Three doses of killed G2b vaccine were parenterally administered. ¶F/K: Mass vaccination with feedback and parenterally administered killed G2b vaccine.

diluted with phosphate-buffered saline (PBS) to reach 10% (wt/vol) suspensions, vortexed, and centrifuged for 10 min at 4,500 ×g (Hanil Centrifuge Fleta 5; Hanil Scientific Inc., Korea). The supernatants were analyzed with PEDV-specific real-time RT-PCR and further subjected to nucleotide sequence analysis if necessary. The blood specimens (n = 260) were also taken from the same animals and examined by serum neutralization assay and ELISA to describe the PEDV antibody response in each sample.

Biosecurity monitoring

Subjective monitoring encompassed the classic approach based on the data collected from farms and surveys, and verification of the checklists of established tasks. External and internal biosecurity protocols were reviewed with each herd veterinarian through on-site inspection and in-person interviews and evaluated at the yard, staff, and barn levels using purpose-designed checklists with a 1–5 scoring scale, wherein 1 = worst and 5 = best, which is based on the PEDV Biosecurity Quick Fact Sheet (https://www.manitobapork.com/images/producers/pdfs/biosecurity/PEDv-Biosecurity-Quick-Facts.pdf), with some modifications to adjust to the circumstances in the domestic pig farming (**Supplementary Fig. 1**). The biosecurity questionnaire consists of 13, 7, and 6 checklists at the yard, staff, and barn sections, respectively. Each question regarding the biosecurity protocols scored out of 5 points (1 = worst; 5 = best) and the average scores of the yard, staff, and barn biosecurity questions were individually calculated.

Virus neutralization

The presence of PEDV-specific neutralizing antibodies (NAbs) in the serum samples collected from the pigs was determined using a conventional virus neutralization test in 96-well microtiter plates with PEDV isolate KNU-141112 as previously described [11,12] with minor modifications. Vero cells at 2×10^4/well were grown in 96-well tissue culture plates for 24 h. KNU-141112-P5 virus stock was diluted in serum-free α-MEM to achieve 200 $TCID_{50}$ in a 50-μL volume. The diluted virus was then mixed with 50 μL of 2-fold serially diluted (1:2 to 1:512) inactivated serum samples and incubated at 37°C for 1 h. The mixture was added to Vero cells and incubated at 37°C for 1 h. After removing the mixture, the cells were thoroughly rinsed with PBS five times and maintained in a virus growth medium [13,14] at 37°C in a 5% CO_2 incubator for 2 days. The neutralizing endpoint titers were calculated as the reciprocal of the highest serum dilution that inhibited the virus-specific cytopathic effects by ≥ 80% relative to the controls in duplicate wells. The serum samples with neutralizing endpoint titers of ≥ 1:4 were considered positive for the PEDV-neutralizing antibody.

Enzyme-linked immunosorbent assay (ELISA)

Recombinant PEDV S1 protein was purified from PK-rS1-Ig cells as described previously [11]. Anti-PEDV immunoglobulin A (IgA) antibodies in serum were detected using an *in-house*

PEDV G2b S1-based indirect ELISA as described previously [15-17] with minor modifications. Briefly, microtiter plates (Nunc, USA) were coated with 0.5 ng of the S1 antigen diluted in coating buffer (50 mM bicarbonate buffer, pH 9.6) per well and incubated overnight at 4℃. After three washes with PBS containing 0.05% Tween 20 (PBST), the plates were blocked with 5% powdered skim milk (BD Biosciences, USA) in PBST for 2 h at 37℃ and then incubated with each serum sample diluted 1:100 in PBST containing 10% goat serum (Vector Laboratories, USA) for 1 h at 37℃. After washing, a 1:20,000 diluted peroxidase-conjugated goat anti-porcine IgA (Abcam, UK) was added to each well and incubated at 37℃ for 1 h. The peroxidase reaction was visualized using tetramethylbenzidine-hydrogen peroxide as the substrate (R&D Systems, USA) for 20 min at room temperature in the dark and was stopped by adding 2N sulfuric acid (R&D Systems) to each well. The optical density (OD) of each sample was measured at 450 nm using a SPARK 10M multimode microplate reader (TECAN, Switzerland). Positive control, negative control, and blank (sterile water) samples were included in each plate; all the clinical and control samples were tested in duplicates.

Quantitative real-time reverse transcription polymerase chain reaction (rRT-PCR)

Viral RNA was extracted from the fecal samples prepared as described above using an *i*-TGE/PED Detection Kit (iNtRON Biotechnology, Korea) according to the manufacturer's protocol. PEDV S gene-based quantitative rRT-PCR was performed using a One-Step SYBR PrimeScript RT-PCR Kit (TaKaRa, Japan) with the forward primer 5′-ACGTCCCTTTACTTTCAATTCACA-3′, reverse primer 5′-TATACTTGGTACACACATCCAGAGTCA-3′, and a probe 5′-FAM-TGAGTTGATTACTGGCACGCCTAAACCAC-BHQ1-3′ as previously described [13,18,19]. The reaction was performed using a Thermal Cycler Dice Real-Time System (TaKaRa) according to the manufacturer's protocol under the following conditions: 1 cycle of 45℃ for 30 min, 1 cycle of 95℃ for 10 min, and 40 cycles of 95℃ for 15 sec and 60℃ for 1 min. The results were analyzed using an automatic baseline as described previously [13,19]. The samples with the mean cycle threshold [Ct] values of < 35 were considered positive for PEDV.

Risk assessment with the pentagon profile system

A pentagon profile system, used to assess the risk of PED recurrence, was created based on the evaluation scores independently acquired from the biosecurity-related questionnaire (1 = worst; 5 = best), the measurement level of sow immunity (1 = worst; 5 = best), and the endemic status (0 = best; 5 = worst). The subjective monitoring scored how well each swine producer followed all biosecurity protocols at the yard, staff, and barn levels as described above. The level of herd immunity was estimated based on the stability and degree of NAbs and IgA antibodies against PEDV in sows (**Table 2**). The endemic status of the infection was determined according to the circulation of PEDV in the herd, the seroconversion against PEDV in growing pigs, or both (**Table 3**). Individual scores from the five factors were used to construct the five arms of a risk pentagon.

Table 2. Sow immunity categories for the 1–5 scoring scale system

Scores	NAb positive rate*	Protection rate†	IgA antibody kinetics
1	≤ 50% (unstable)	≤ 10% (unstable)	Unstable
2	≥ 90% (stable)	≤ 10% (unstable)	Unstable
3	≥ 90% (stable)	Partial stable	Unstable
4	≥ 90% (stable)	Partial stable	Partial stable
5	≥ 90% (stable)	≥ 90% (stable)	Stable

Nab, neutralizing antibody; IgA, immunoglobulin A.
*Positive rate: an NAb titer higher than or equal to 1:4 was considered positive. †Protection rate: an NAb titer higher than or equal to 1:64 was considered positive for protection.

Table 3. Virus circulation categories for the 1–5 scoring scale system

Scores	PED outbreak	rRT-PCR[*]	Seroprevalence[†]
0	No	Negative	Negative
1	Yes	Negative	Negative
2	Yes	Negative	Low (≤ 10%)
3	Yes	Negative	Medium
4	Yes	Negative	High (≥ 90%)
5	Yes	Positive	High

PED, porcine epidemic diarrhea; rRT-PCR, real-time reverse transcription polymerase chain reaction; PEDV, porcine epidemic diarrhea virus; VNT, virus neutralization test.
[*]PEDV positivity was detected by rRT-PCR using fecal samples from growing pigs. [†]PEDV-seropositive rate was determined by VNT using serum samples from growing pigs.

Ethical statement

No ethical approval was required as sample collection or questionnaires from animals/human has been gathered under the agreement of farm owners.

RESULTS

Evaluation of biosecurity practices

We first inspected the biosecurity measures implemented in 13 pig farms on Jeju Island with a history of PED outbreak and conducted in-person interviews to query and score the extent of adherence to biosecurity manuals in each farm using a designed checklist of "Do's and Do not's," indicating practices that must or not be done to reduce the risk of introduction (external biosecurity) and spread (internal biosecurity) of pathogens such as PEDV. The subjective monitoring scores of each question ranged from 1 to 5, wherein 1 is the worst and 5 is the best, at each yard, staff, and barn level in individual farms. The average scores of the individual questions regarding the biosecurity checklists were calculated at the yard, staff, and barn sections and summarized in **Table 4**. A higher overall score indicated that the corresponding farm was better at implementing biosecurity protocols. The scores of biosecurity performance varied among the farms, with an average of 3.8 (2.2–5), 3.7 (1.3–5), and 3.9 (1.8–5) at the yard, staff, and barn levels, respectively. Fewer than 50% (6 of 13) of the PED-affected farms received scores above the average at external and internal biosecurity levels, whereas three farms (Farms G, K, and L) demonstrated a severe lack of biosecurity practice at all levels.

Evaluation of herd immunity

Assessing the quantity and stability of the specific antibodies against PEDV in sows offers a valuable tool to measure and predict herd immunity among sows that will provide protection for suckling piglets against PEDV through lactation. Individual serum samples were collected from low and high-parity sows and tested for PEDV-specific antibodies using virus neutralization test (VNT) and ELISA. Simultaneously, we also conducted in-person interviews with the swine producers to determine how they vaccinated sows against PED.

Table 4. Biosecurity monitoring scores of the 13 PED-affected pig farms

Farm	Biosecurity score (1–5 scale)[*]													
	A	B	C	D	E	F	G	H	I	J	K	L	M	Average
Yard	3.4	3.9	2.8	4.4	4.4	4.8	2.9	4.8	5	3.8	2.2	3.7	2.9	3.8
Staff	3.3	4	4	5	5	5	2.8	3.8	4	4.3	1.3	2.5	2.5	3.7
Barn	4.5	4.3	3.8	5	4.8	4.8	3.5	5	5	2	1.7	1.8	4.5	3.9

PED, porcine epidemic diarrhea.
[*]The biosecurity management scores ranged from 1 to 5, wherein 1 is the worst and 5 is the best.

Among the 13 farms, 11 farms have implemented various multiple-dose pre-farrow immunization programs using commercial live (L) or/and killed (K) vaccines (**Table 1**). Eight farms parenterally administrated two or three doses of killed G2b vaccines (K/K or K/K/K). Three farms parenterally administrated one dose of live G1a and two doses of killed G2b vaccines (L/K/K). The remaining two farms, Farms K and M, adopted an intentional virus-exposure (feedback) practice or mass vaccination with feedback combined with a G2b killed vaccine, respectively.

Serological assays showed that 90–100% of the farrowing sows developed PEDV-NAbs in their sera (**Fig. 1**). These data indicated that except for one farm (Farm I), almost all the farms (12 of 13) appeared to achieve herd immunity against PEDV. However, all the sow herds showed irregular antibody kinetics, suggesting unstable sow immunity in the PED-affected farms. Furthermore, most seropositive sows maintained lower levels of antibody than the hypothetical protective NAb titer (≥ 64). Based on a cutoff for a protective NAb titer at ≥ 64, none of the farms achieved protective herd immunity (**Table 5**). Six (Farms B–D and I–K), two (Farms A and E), and three (Farms F, G, and L) farms had 0%, 10%, and 20% of their sows, respectively, with a NAb titer of ≥ 64. Meanwhile, two farms had 30% (Farm M) and 50% (Farm H) of their sows, respectively, with NAb titer of ≥ 64. In addition, the kinetics of anti-PEDV IgA antibodies in sow herds were comparable to PEDV-NAbs but much lower and less stable (**Fig. 1**).

Evaluation of virus circulation

The swine populations in the PED-affected farms were further tested for PEDV using molecular and serological assays. The presence of PEDV antigen or antibody or both in the growing herds could be used to determine whether the virus has been circulating or and whether there was a potential risk of recurrence. PEDV-specific rRT-PCR assay was performed on individual fecal specimens (n = 260) obtained from the 13 farms. All of the fecal samples (n = 130) collected from sows were PEDV-negative, whereas five samples (3.8%) from the growing pigs in four (Farms A, B, H, and M) farms (30.8%) were positive for PEDV (**Fig. 2**). The five samples consisted of one growing pig each from Farms A, B, and H and two pigs from Farm M. The rRT-PCR data showed the high Ct values of > 29 in all the positive samples, indicating that the concentration of PEDV was low but still potentially significant in the corresponding farms. Unfortunately, we could not sequence the PEDV in the positive samples, likely due to a low virus concentration. Simultaneously, we carried out serological examinations on the growing pigs using VNT to identify those with past or recent exposure to PEDV. The results revealed that more than half of the farms (7 of 13) had PEDV-seropositive pigs, indicating that their wean-to-finish barns were exposed to PEDV (**Fig. 2**). Moreover, 90%–100% of the growing pigs in these farms were seropositive for PEDV, suggesting the circulation of PEDV within the affected farms (**Table 6**). In summary, our data indicate that a significant number of PED-affected farms are endemic for PEDV infection.

Table 5. Sow immunity scores of the 13 PED-affected pig farms

Farm	A	B	C	D	E	F	G	H	I	J	K	L	M
Positive rate*	90	90	100	90	100	100	100	100	50	100	100	90	100
Protection rate†	10	0	0	0	10	20	20	50	0	0	0	20	30
Score (1–5 scale)‡	2	2	2	2	2	4	3	4	1	2	2	2	3

PED, porcine epidemic diarrhea; NAb, neutralizing antibody.
*Positive rate: an NAb titer higher than or equal to 1:4 was considered positive. †Protection rate: an NAb titer higher than or equal to 1:64 was considered positive for protection. ‡The sow immunity scores ranged from 1 to 5, wherein 1 is the worst and 5 is the best.
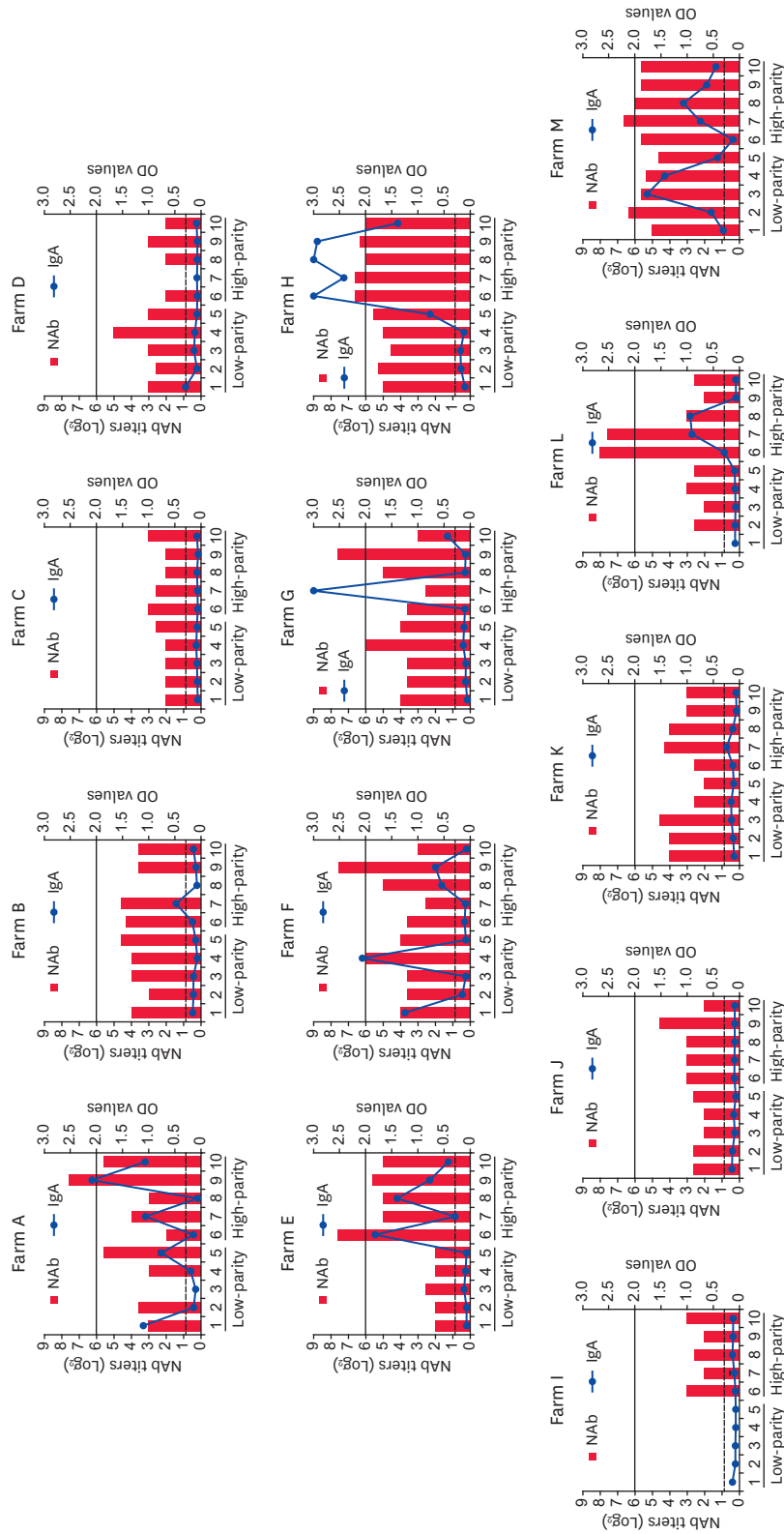
Fig. 1. PEDV-specific antibody kinetics in the serum samples of the sows from 13 farms (Farms A to M). The samples were collected from five low-parity (1–5) sows and five high-parity (6–10) sows and tested with a virus neutralization test (column chart; left y-axis) and the PEDV-S1 IgA ELISA (line chart; right y-axis). NAb titers for individual samples were presented as a $\log_2$ scale. The hypothetical protective NAb titer (1:64) against PEDV was indicated by a bold line. The samples above the OD cutoff value of 0.3 from the IgA ELISA (dashed line) were considered positive. PEDV, porcine epidemic diarrhea virus; IgA, immunoglobulin A; ELISA, enzyme-linked immunosorbent assay; NAb, neutralizing antibody; OD, optical density.
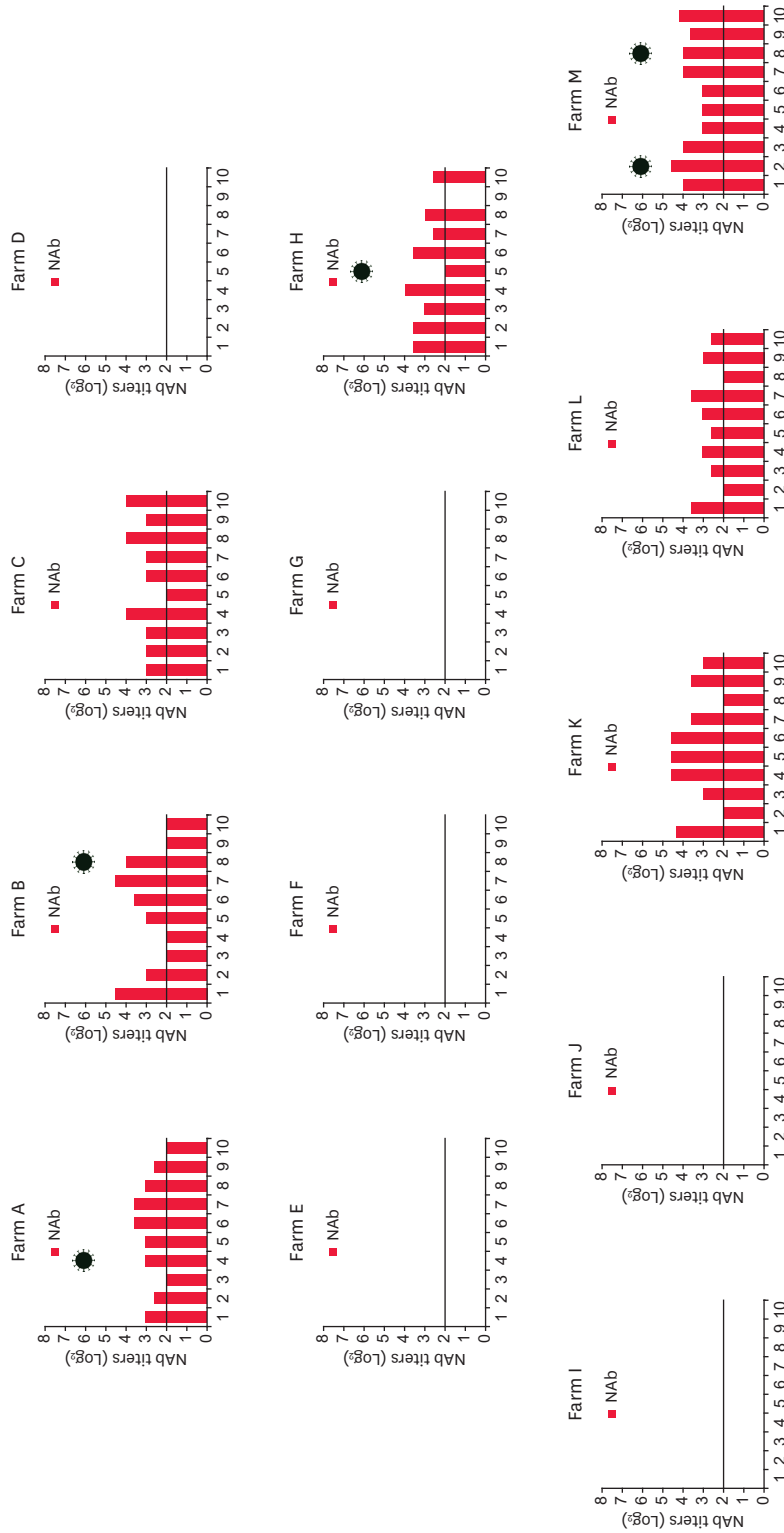
Fig. 2. PEDV-specific antibody responses in serum samples of the growing pigs from 13 farms (Farms A to M). The samples were collected from 10 growing pigs (1–10) and tested with a virus neutralization test. NAb titers for individual samples were presented as a $\log_2$ scale. The samples above the neutralizing endpoint titer 1:4 (dashed line) were considered positive. The diagram illustrating the virus (⬤) represents the case of an animal testing positive for PEDV via the rRT-PCR analysis in rectal swabs from corresponding pigs. PEDV, porcine epidemic diarrhea virus; NAb, neutralizing antibody; rRT-PCR, real-time reverse transcription polymerase chain reaction.

Table 6. Virus circulation scores of the 13 PED-affected pig farms

Farm	A	B	C	D	E	F	G	H	I	J	K	L	M
Seroprevalence*	100	100	100	0	0	0	0	90	0	0	100	100	100
PEDV positivity†	10	10	0	0	0	0	0	10	0	0	0	0	20
Score (0–5 scale)‡	5	5	4	1	1	1	1	5	1	1	4	4	5

PED, porcine epidemic diarrhea; PEDV, porcine epidemic diarrhea virus; NAb, neutralizing antibody.
*Seroprevalence: an NAb titer higher than or equal to 1:4 was considered positive. †PEDV positivity: mean Ct values of < 35 were considered positive for PEDV.
‡The virus circulation scores ranged from 5 to 0, wherein 5 is the worst and 0 is the best.

Assessment of the potential risk of PED recurrence

Based on the evaluation scores of biosecurity performance, sow immunity, and virus circulation (**Tables 4**–**6**), we constructed a risk pentagon profile model with the five components concerning PEDV re-infections in the affected farms. When a farm had higher biosecurity practice and sow immunity scores and a lower endemic infection status score, it was considered to have a low risk of PED recurrence. Conversely, when farm's biosecurity practice and sow immunity scores were lower and its endemic infection status was higher, it was considered to have a high risk of PED recurrence (**Fig. 3**). According to the risk pentagon profile system, the individual farms were classified into three different types of the potential risk of the recurrence: one (Farm F; 7.7%), four (Farms D, E, H, and I; 30.8%), and eight (Farms A–C, G, J, and K–M; 61.5%) farms were classified as having a low, medium, and high risk, respectively, for PED recurrence (**Fig. 4**). Farm A was considered to be high-risk owing to low sow immunity (score 2) and PEDV circulation evident by the detection of fecal PEDV shedding and high seroprevalence in growing pigs (score 5). Despite strict biosecurity performance (scores 4.4–5) and no evidence for virus circulation (score 1), Farm D was considered to be at a medium risk because of low herd immunity (score 2). Although Farm H had relatively high levels of sow immunity (score 4) and biosecurity performance (scores 3.8–5), it was also considered to be a medium risk due to the presence of PEDV and high seroprevalence in growing pigs (score 5). In contrast, Farm F was classified into having a low risk of PED recurrence since this farm fully implemented biosecurity management (scores 4.8–5), maintained high sow immunity (score 4), and had no trace of endemic infection (score 1). The overall risk assessments indicated that numerous PED-affected farms were high-risk for a recurrent outbreak due to their poor biosecurity performance, low herd immunity, and PEDV circulation.

DISCUSSION

PEDV infection causes significant economic losses in affected herds by directly affecting the neonatal mortality and growth and indirectly affecting the sow's reproduction [1]. Following acute PEDV outbreaks, the virus can vanish or remain in farrowing or wean-to-finish barns, and the latter case leads to the endemic infection that results in PED recurrence under suitable conditions [2]. In the absence of active management programs, PEDV has become endemic in South Korea, hindering its control and worsening PED. Notably, seasonal (winter) PED is gradually becoming a year-round occurrence; thus, the number of recurrent outbreaks in PED-affected herds has increased annually across the country. Therefore, it is necessary to implement fundamental control measures that actively monitor biosecurity practice, herd immunity, and virus circulation in swine populations to reduce recurrent PEDV infections in infected farms. The present study analyzes the current state of execution of those measures and assesses the degree of risk of recurrence in PEDV-infected farms based on the evaluation scores.
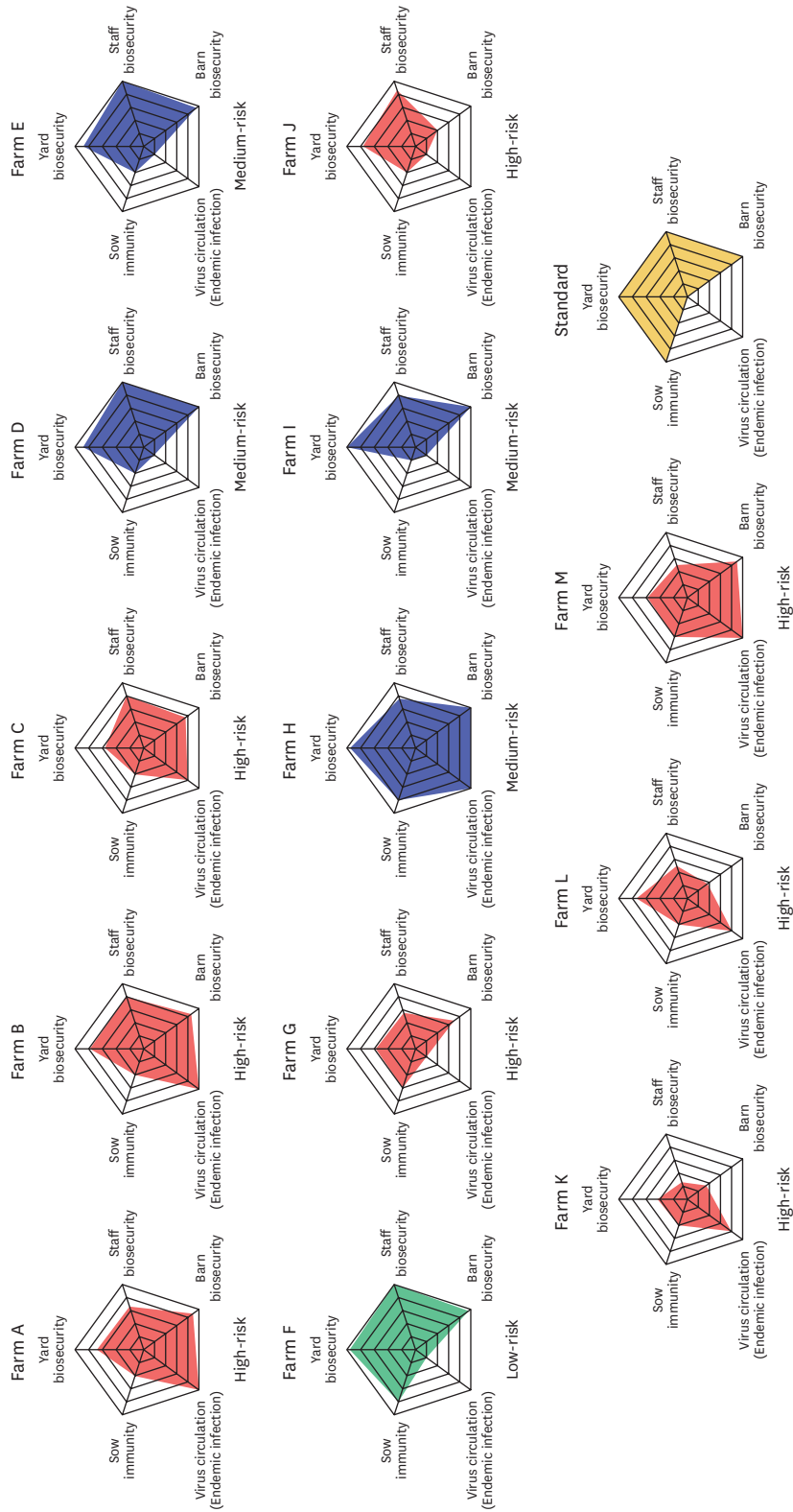
Fig. 3. Illustration of the potential risk of PED recurrence in 13 farms (Farms A to M) with the risk pentagon diagram. The five parameters that were assessed in the risk pentagon system included the levels of yard biosecurity, staff biosecurity, barn biosecurity, sow immunity, and virus circulation (endemic infection). Each factor, indicated at a score of 1–5, was represented by the shaded area relative to the pentagon's total area. The individual farms were indicated as low (green), medium (blue-purple), or high-risk (red) underneath each pentagon. The last pentagon diagram (standard; yellow) represented a normal model with no risk of the occurrence or recurrence of PED.
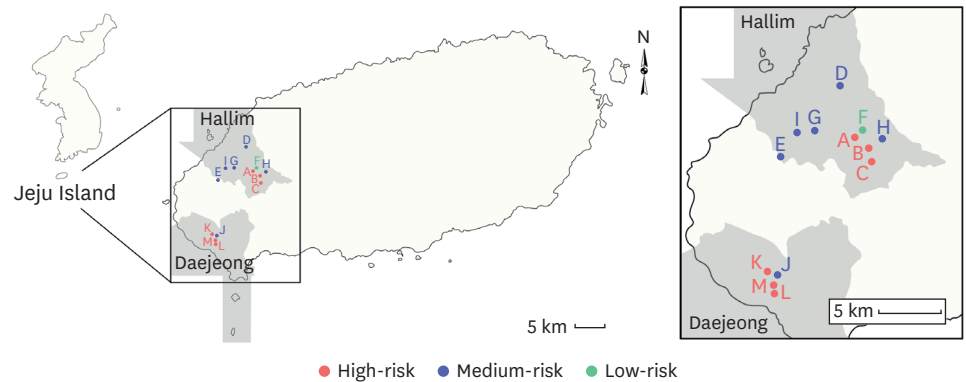PED, porcine epidemic diarrhea.

Fig. 4. The spatial distribution of 13 PED-affected farms on Jeju Island. Jeju Province is the largest island in South Korea off the coast of the Korean Peninsula and had been PEDV-free for a decade, from 2004 to 2013. Different types of risk were marked with dots of different colors. The right image was a magnified version of a boxed area of the picture left.
PED, porcine epidemic diarrhea; PEDV, porcine epidemic diarrhea virus.

The first assessment was to estimate how well swine farmers performed biosecurity procedures using on-site inspection and questionnaire methods. According to their self-evaluations, the farmers tried to follow the biosecurity manuals in their own ways. Among the 13 farms, the farmers in six farms almost completely followed the biosecurity protocols inside and outside the farm, likely due to their recognition of the calamity caused by the previous PED outbreak. However, despite the aftermath of PEDV occurrence, several farms still did not properly execute the protocols, as reflected by their below the average scores at the yard, staff, and barn levels. Collectively, these results indicate the provincial pig herds to be vulnerable to the virus circulating in the farms or introduced outside the farms; thus, the herds face an increased risk of reoccurrence.

Since the passive transfer of maternal antibodies via colostrum and milk is the most effective tool to protect newborn piglets from PEDV, herd immunity in sows is considered a critical indicator of the control and cessation of PED during epidemic or endemic outbreaks [2]. Our study aimed to characterize PEDV serum antibody kinetics in sows and measure the level of sow immunity against PEDV. In South Korea, multiple-dose prime-boost pre-farrow vaccination programs have been a standard recommendation for pregnant sows for decades [1]. In this study, the survey results showed that most farms (11 of 13) conducted different types of prime-boost immunization regimens at 2 or 3-week intervals pre-farrowing. However, our data revealed that despite the multi-dose maternal vaccination schemes, the sow herds of all 13 farms exhibited lower amounts and less stable kinetics of PEDV-specific NAbs and IgA antibodies than expected, probably resulting in an insufficient supply of protective antibody to the neonates. Several factors, including vaccine strain selection, administration doses, adjuvants, vaccination intervals, and the age or parity of the sows, influence the outcome of prime-boost immunization approaches [2,20]. Mainly, several studies on enteric virus vaccines emphasized the oral route of prime immunization in terms of the effectual priming of the gut using a live vaccine or through feedback to augment and sustain lactogenic immunity [17,21-24]. Therefore, the K/K and K/K/K vaccination scheme employed by several farms (6 of 13) may be limited in their effectiveness in fruitfully achieving herd protection against PED. On the other hand, some farms (Farms D, E, and F) implemented the L/K/K vaccination scheme to compensate for the drawback of the K/K scheme; however, they did not attain a satisfactory level of immunity in the sow populations. This result might be due to using a traditional G1a live vaccine that was neither orally

administered nor specific against the HP-G2b strains. Considering these circumstances, it is recommended to adopt the oral priming and parenteral boosting L/K/K regimen to combine the new live and killed commercial G2b vaccines that are commercially available now in South Korea [17]. Altogether, PED-affected farms need to scrutinize and improve their vaccination programs that must be continued before each farrowing to promote protective immunity in vaccinated sows.

Monitoring PEDV will be one of the most important approaches to control its impact on PED-affected farms. PEDV could be detected in infected pigs via rectal swab analysis by rRT-PCR for up to two months since the infection [25,26]; thus, PEDV might remain untraceable in most farms because more than two months had passed since PED ceased in each herd. Consistent with this finding, we could not detect PEDV in many pigs (255 of 260) from the infected farms using rRT-PCR. Even at low concentration, however, PEDV was detected in the fecal of some growing pigs from four different farms, suggesting its presence and circulation within the herds. Since the amount of maternal passive antibodies that piglets receive wane gradually over the post-weaning period, PEDV-specific antibodies found in the pigs during the growing and finishing periods are considered to be produced by the animals in response to the natural infections by the virus circulating in the field. Thus, the serological screening of adult pig herds, which determines the infection status in grower-finisher pigs, is also essential for monitoring the endemic status of PEDV in affected farms [2]. We found PEDV antibody seroconversion in nearly all the growing pigs from more than 50% of the farms tested, including four PEDV-positive farms; these data suggest that the animals have been exposed to the virus. Taken together, our viral and serological tests uncovered that many farms are vulnerable to PEDV even after the termination of PED.

The circulation of PEDV in the herd may be troublesome soon or later and eventually be detonator to trigger re-infections within the same farm or cause new infections in nearby farms. Although PEDV infection is asymptomatic or self-limiting in adult animals, including weaner to finisher pigs, the virus can be shed into the stool and circulate in a subclinical manner in those populations [2]. Since asymptomatic animals can transmit PEDV within a farm, the replacement gilts from external conventional pig breeding herds (purchase) or internal on-farm breeding (own replacement) may provoke PED recurrence in affected farms. Suppose the growing-finishing barn is contaminated with PEDV. In that case, the gilts may risk exposure to PEDV during acclimation after purchase or throughout the entire raising period for own replacement. The PEDV-infected asymptomatic gilts can act as "Trojan Pigs" when they are moved to the farrowing house by continuously shedding viruses in their feces. In this way, the viruses excreted from the "Trojan Pigs" even at low concentrations, can infect and amplify in a clinical manner in susceptible piglets, serving as the source of PED recurrence and ultimately leading to the second wave to increase the death of nursing piglets when they lack protective immunity. Lastly, since clinical manifestations alone are virtually impossible to distinguish the PEDV-infected pigs in wean-to-finish barns, it is necessary to surveil swine populations in affected farms for recent or past virus exposure to the virus with the regular laboratory testing of the individual- or pen-based samples.

Out study is the first to purpose a risk pentagon model that provides a clear visual of the potential risk of recurrence in PED-affected commercial farms based on monitoring biosecurity, herd immunity, and virus circulation. Our evaluations demonstrated that most of the PED-affected farms on Jeju Island were negligent in their practice of biosecurity protocols, inadequate in building sow immunity, and tolerant of virus circulation. As a result,

these farms acted as a virus reservoir that boosted the possibility of PED recurrence as well as the spread to and infection of adjacent farms and even further afield. Thus, pig farmers can wane the risk of recurrence and new infection by coordinating the active and routine monitoring of the swine populations to obtain timely information on pathogen exposure and immune responses. These surveillance measures include: 1) enforcing the compliance of all biosecurity protocols and improving the biosecurity measures; 2) measuring sow immunity using serological assays, such as VNT and ELISA; 3) refining vaccination programs, such as the prime-boost pre-farrow L/K/K scheme using new live oral and killed G2b vaccines; 4) conducting nucleic acid and antibody-based surveillance of the PEDV circulating within the affected farm to identify the animals, particularly in growing and finishing barns, that have been exposed to the viruses or are shedding viruses or both; and 5) taking countermeasures, such as disinfection practices, against the circulation of the virus. Since the farms across South Korea are likely under similar circumstances to those on Jeju Island, the application of risk assessment and management strategies presented in this study is relevant to other regions and should be systematically expanded nationwide. In conclusion, the present study underscores the importance of the regular monitoring and surveillance of PEDV in affected farms; its proposed risk assessment models makes an essential contribution towards launching a government-led regional or national PED eradication policy.

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SUPPLEMENTARY MATERIAL

Supplementary Fig. 1
PEDV biosecurity checklist.

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