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Monitoring and evaluation of provincial classical swine fever immunization implementation with an E2 subunit vaccine in Jeju Island, South Korea

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Purpose: Accidental vaccination with a live attenuated low-virulence strain of Miyagi (LOM) vaccine led to the reemergence of classical swine fever virus (CSFV) in Jeju province, South Korea in 2014. To control the continual outbreaks of LOM-derived CSFV, the provincial government launched a provincial mass vaccination project using a CSF-E2 subunit vaccine. We conducted this study to assess the herd immunity level and outcomes of E2 vaccine-based immunization in breeding and growing herds on Jeju Island during 2020–2021.
Materials and Methods: A large-scale vaccination trial using the Bayovac CSF-E2 vaccine investigated its efficacy in breeding and growing herds under farm application conditions (10 CSFV-affected and three CSFV-naïve swine farms).
Results: The level of herd immunity in each farm was classified into three (S1–S3) and six (G1–G6) profiles in breeding and growing herds, respectively. Immunity monitoring revealed a remarkable improvement in the herd immunity status in all farms. The majority (10/13) of farms, including CSFV-free farms, showed the S1G1 immunity profile in 2021, indicating the appropriate implementation of the advised vaccination regime. Moreover, there were significant decreases in E^{rns} seropositivity from 100% to 50% and 25.9% to 4.3% at farm and pig levels, respectively. In particular, all farms were confirmed as CSFV free in the growing-finishing herds.
Conclusion: Our large-scale trial demonstrated the effectiveness of the E2 subunit vaccine in establishing herd immunity stabilization and eliminating CSFV circulation in the affected farms and highlighted the need for a provincial vaccination policy to regain the CSF-free status on Jeju Island.

Keywords: Classical swine fever virus, CSF-E2 subunit vaccine, Herd immunity stabilization, Jeju Island, Provincial vaccination

Introduction

Classical swine fever (CSF) is a highly contagious, multisystemic viral disease affecting the members of the *Suidae* family, such as domestic pigs and wild boars [1,2]. Because CSF outbreaks cause negative social and financial repercussions, including ethical issues following mass slaughter, direct or indirect losses to pig production, and intense constraints on trade and movement of pigs and their products, the World Organization for Animal Health (WOAH) has listed CSF as a notifiable disease [3]. CSF virus (CSFV, recently redesignated as *Pestivirus C*), the causative agent of CSF, is a small enveloped, positive-sense, single-stranded RNA virus that belongs to the *Pestivirus* genus

within the *Flaviviridae* family [4]. The genome of CSFV is approximately 12.3 kb in length and consists of one large open reading frame, flanked by 5′- and 3′-untranslated regions at both ends, which encodes a single polyprotein of 3,898 amino acids. The polypeptide precursor is then cotranslationally and posttranslationally cleaved by viral and cellular proteases to form four structural (C, E^{rns}, E1, and E2) and eight nonstructural proteins (N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2,5-7]. CSFV strains can be phylogenetically divided into three genotypes (1, 2, and 3), each comprising three to four subgenotypes [8,9].

The WOAH currently lists 38 CSF-free countries located in North America, part of South America, Oceania, and the European Union; however, CSF still remains endemic and reemerging disease in most parts of the world with large pork production, including Central and South America, Eastern Europe, and Asia [10,11]. Therefore, depending on the epidemiological conditions of the affected geographical area, the two primary strategies for CSF control are non-vaccination stamping-out and systematic prophylactic vaccination. The former is appropriate for CSF-free zones or where eradication is in progress, and the latter is effective in CSF-endemic countries [12,13]. Several conventional live attenuated vaccines (LAVs) obtained after serial passages of CSFV in cell cultures or rabbits confer effective and rapid immune protection against CSFV infection [1]. Although LAVs lack a serological notion of differentiating infected from vaccinated animals (DIVA), massive vaccination using LAVs has been implemented to fight CSF, and together with additional biosecurity measures, has paved the way to CSF elimination in numerous endemic regions as a national compulsory control program [2,10].

In South Korea, a live attenuated low-virulence strain of Miyagi (LOM) vaccine (LAV-LOM) has been extensively applied for a mandatory vaccination policy throughout the country to eradicate CSF since 1974, indicating its safety and high immunogenicity in pigs [14]. However, despite the national vaccination strategy, sporadic CSF outbreaks have continued to occur across mainland South Korea [14-16]. Moreover, there has been an uncertainty regarding a potential reversion of LAM-LOM to virulence, although it has been considered genetically stable through continuous propagation in bovine or porcine kidney cells. In fact, the unintentional vaccination of LAV-LOM in CSFV-naïve sow herds in 2014 resulted in the reemergence of CSFV in Jeju province, the largest island of South Korea, which had achieved a CSF-free status and then prohibited prophylactic vaccination in 1998 [17-19]. Subsequent animal studies have revealed clinical adverse effects of the used LAV-LOM in pigs [17,20]. Since its reemergence, there have been frequent reports of pig farms affected by LAV-LOM-derived CSFV on Jeju Island (Fig. 1), indicating the establishment of endemic CSF that aggravates socioeconomic losses to the provincial pig industry [20,21].

For the control and eradication of CSF on Jeju Island, the provincial government considered switching to a vaccination policy using desirable vaccines that fulfill the requirements, including safety and the DIVA concept, such as CSFV E2 subunit vaccines. Like the LOM vaccine, the LOM-derived field strains circulating in Jeju swine populations are grouped within the genotype 1.1 clade [17,18,21]. Considering these issues, Jeju Island necessitates an effective alternative vaccine

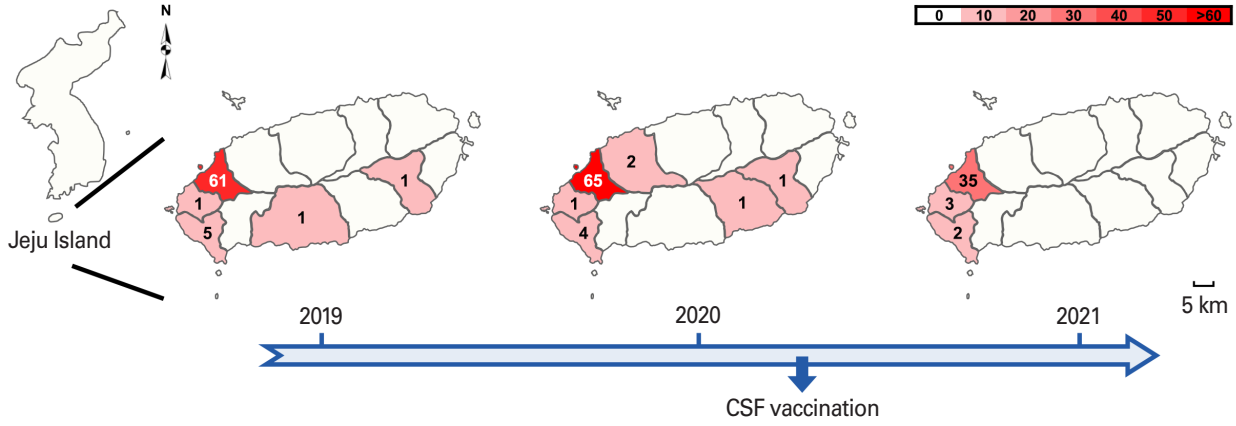

Fig. 1. Number of classical swine fever (CSF) cases on Jeju Island, South Korea, during 2019–2021. Heat maps (upper panel) of 12 districts on Jeju Island (located southwest of the mainland) illustrate the distribution of CSF cases in Jeju province by year (2019–2021), with the legend from red to white, representing most to least. A thick (azure) arrow (lower panel) represents the timeline indicating the implementation of provincial CSF immunization with the E2 subunit vaccine on Jeju Island.

with a safety guarantee and DIVA capacity, such as Bayovac CSF-E2 vaccine approved for use in South Korea. The CSFV E2 subunit vaccine was thus selected for the provincial mass vaccination project on Jeju Island since the E2 antigen was originated from a homologous CSFV Brescia strain (genotype 1.2) [22]. In addition, the CSF-E2 vaccine was demonstrated to confer effective protection against multiple genotypes 1.1, 1.2, and 2.1 of CSFV [22-25]. Our field trials further confirmed the safety and immunogenicity of the E2 subunit vaccine in CSFV-naïve farms without previous exposure to the LOM vaccine on Jeju Island [20]. Subsequently, the E2 subunit vaccine was permitted for use in provincial pig populations in 2020. In the present study, we investigated herd immunity and outcomes following provincial CSF immunization implementation with the E2 subunit vaccine on Jeju Island, South Korea, during 2020–2021.

Materials and Methods

CSF vaccination

The licensed CSFV E2 subunit vaccine (Bayovac CSF-E2 vaccine; Bayer Taiwan Co. Ltd., Taipei, Taiwan) from commercial batches was used in this study. For immunization with the Bayovac CSF-E2 vaccine, pigs were injected intramuscularly at the neck musculature behind the ear at an administration volume of 2 mL per dose according to the manufacturer's instructions. Sows were vaccinated with three doses of Bayovac CSF-E2 at 10, 7, and 4 weeks antepartum, whereas weaning pigs received two doses of the E2 vaccine at 40 and 60 days of age.

Farm selection

A total of 13 farrow-to-finish (FTF) commercial herds (Farm A–M) were selected across the province based on the presence (Farms A–J) or absence (Farms K–M) of a past history of CSFV outbreak as determined in a previous field survey. These farms were chosen to conduct serological monitoring of different pig groups before (May 2020) and after (September 2020 and March 2021) CSFV E2 vaccination during 2020–2021.

Blood sampling

Blood was collected from sows (n=10 per farm), 20-day-old suckling piglets (n=10 per farm) randomly selected from those of vaccinated dams, 40-day-old weaning pigs (n=11 per farm), 70-day-old (n=5 per farm) and 100-day-old (n=5 per farm) growing pigs, and 130-day-old finishing pigs (n=5 per farm). Blood samples were centrifuged, and serum was stored at -20°C until CSFV serology.

Cells and viruses

LLC-PK1 cells (American Type Culture Collection cell lines-101) were cultured in alpha-minimum essential medium (Invitrogen, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (Invitrogen) and penicillin-streptomycin (100×; Invitrogen). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. The commercial CSFV LAV-LOM strain (GenBank accession number: MK121886) was obtained from ChoongAng Vaccine Laboratories (CAVAC, Daejeon, Korea) [18] and was propagated in LLC-PK1 cells as described previously [21].

CSFV serology

The CSFV E2-specific antibody levels in serum samples collected from pigs were analyzed using a CSFV antibody B-enzyme-linked immunosorbent assay (ELISA) kit (BIONOTE, Hwaseong, Korea) according to the manufacturer's protocols. Results were expressed as percent inhibition (PI), and a PI value equal to or greater than 40% was considered positive for the presence of CSFV E2 antibodies.

The CSFV-specific neutralizing antibody (NAb) level against CSFV (LOM strain) was determined using the neutralizing peroxidase-linked assay (NPLA) according to the standard WOAH manual [26]. The neutralizing endpoint titers were calculated as the reciprocal of the highest serum dilution that neutralized 100 $TCID_{50}$ (50% tissue culture infective dose) of the LOM vaccine strain in 50% of culture replicates. The NAb titers were transformed into a log2 scale, and serum samples with neutralizing endpoint titers of ≥1:16 were considered positive for CSFV NAbs.

Serum samples were also evaluated for the detection of CSFV E^{rns}-specific antibodies to determine DIVA capability after immunization with the E2 subunit vaccine using the pig-type CSFV E^{rns} Ab (Indical Bioscience, Leipzig, Germany), in accordance with the manufacturer's instructions. Results were expressed as the sample-to-positive (S/P) ratio, and an S/P value equal to or greater than 0.5 was considered positive for the presence of CSFV E^{rns} antibodies.

Quantitative real-time RT-PCR

RNA isolation from serum samples was performed automatically using an SLA-E13200 TANBead Nucleic Acid Extraction System (Taiwan Advanced Nanotech, Taoyuan, Taiwan) with a TANBead Nucleic Acid Extraction Kit (Taiwan Advanced

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Nanotech), following the manufacturer's recommendations. CSFV 5′-UTR-based real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was conducted using a VDx CSFV qRT-PCR kit (MEDIAN Diagnostic, Chuncheon, Korea), according to the manufacturer's instructions. The reaction was performed using a CronoSTAR 96 Real-Time PCR System (Clontech, Mountain View, CA, USA) according to the manufacturer's protocols as described previously [20].

Ethics statement

No ethical review and approval were required, because the vaccine used in this study was approved by the South Korean Government for commercial use, and this waiver was approved by the Institutional Animal Care and Use Committee of Jeju Veterinary Research Institute. The authors obtained written informed consent from the farm owner to use the animals in the experiment.

Results

Evaluation of herd immunity

We monitored transversal serum profiles from different pig groups to explore the status and trend of immunization after the implementation of the provincial vaccination policy. A total of 598 serum samples collected from 13 different pig farms, including 10 CSFV-positive and three CSFV-negative farms, were used for serology to profile the herd immunity level (i.e., seropositivity) of each farm during 2020–2021. In addition, sera collected from the pigs were used for the detection of viral RNA using qRT-PCR. CSFV RNA was undetectable by qRT-PCR in any of the pigs from the 13 farms throughout the study. Based on the serological (CSFV E2 ELISA and NPLA) and virological (qRT-PCR) statuses, breeding (sow and 20-day-old piglets) and growing (40-, 70-, 100-, and 130-day-old pigs) herds were categorized into three (S1–S3) and six (G1–G6) profiles of the immune response to CSFV, respectively (Table 1). The seroprevalence from different pig groups per farm was plotted, and the vaccination status (or infection status before vaccination) of each farm was expressed as a combination of each antibody profile corresponding to the breeding and growing pigs (Fig. 2). The percentage of each herd immunity profile during 2020–2021 is depicted in Fig. 3.

Before the implementation of the provincial vaccination in May 2020, 10 farms (Farms A–J) with previous exposure to CSFV tested seropositive in both E2 ELISA and NPLA (Fig. 2). Among these, six farms (Farms D, F, G, H, I, and J) contained either seropositive sows or piglets or both in breeding herds but seronegative animals in growing herds, which corresponded to an S2G5 profile. The remaining four farms (Farms A, B, C, and E) exhibited seropositivity in both breeding and growing herds. In latter case, some pigs at 70 days of age tested seropositive, corresponding to the S2G3 category. However, three CSFV-free farms (Farms K–M) confirmed seronegativity in both breeding and growing herds.

In September 2020 (3 months after vaccination), 30.8% (4/13) of the farms (Farms B, C, E, and L) were classified into an S1G1 profile, indicating the strict implementation of the recommended vaccination program for breeding and growing herds (Fig. 3B). An identical number of farms was determined in the S1G2 (2/13) and S1G3 (1/13) categories that adequately implemented sow vaccination but missed 1st or 2nd vaccination in grow-

Table 1. Herd immunity profiles based on the serological and virological statuses

	Profile	Serology status	qRT-PCR status	Interpretation
Sow herds	S1	≥80% of sows and piglets are seropositive	Negative	Outstanding vaccination
	S2	≤50% of sows and piglets are seronegative	Negative	Inadequate vaccination (low MDA)
	S3	No seroconversion in sow and piglets	Negative	Omitting vaccination
Growing herds	G1	≥80% of 70- to 130-day-old pigs are seropositive	Negative	Outstanding vaccination
	G2	Pigs are seronegative at 70 days of age, but seroconverted at 100 days of age	Negative	Omitting 1st vaccination
	G3	Pigs are seropositive at 70 days of age, but seronegative at 100 days of age	Negative	Omitting 2nd vaccination
	G4	Pigs are seronegative at 70 to 100 days of age, but seroconverted at 130 days of age	Negative	Delayed vaccination
	G5	No seroconversion in all growing herds	Negative	Omitting vaccination
	G6	Mean PI and CV in growing pig herds are ≥90% and ≥60%	Positive or negative	Infection suspected

qRT-PCR, quantitative reverse transcription-polymerase chain reaction; MDA, maternally-derived antibody; PI, percent inhibition; CV, coefficient of variation.

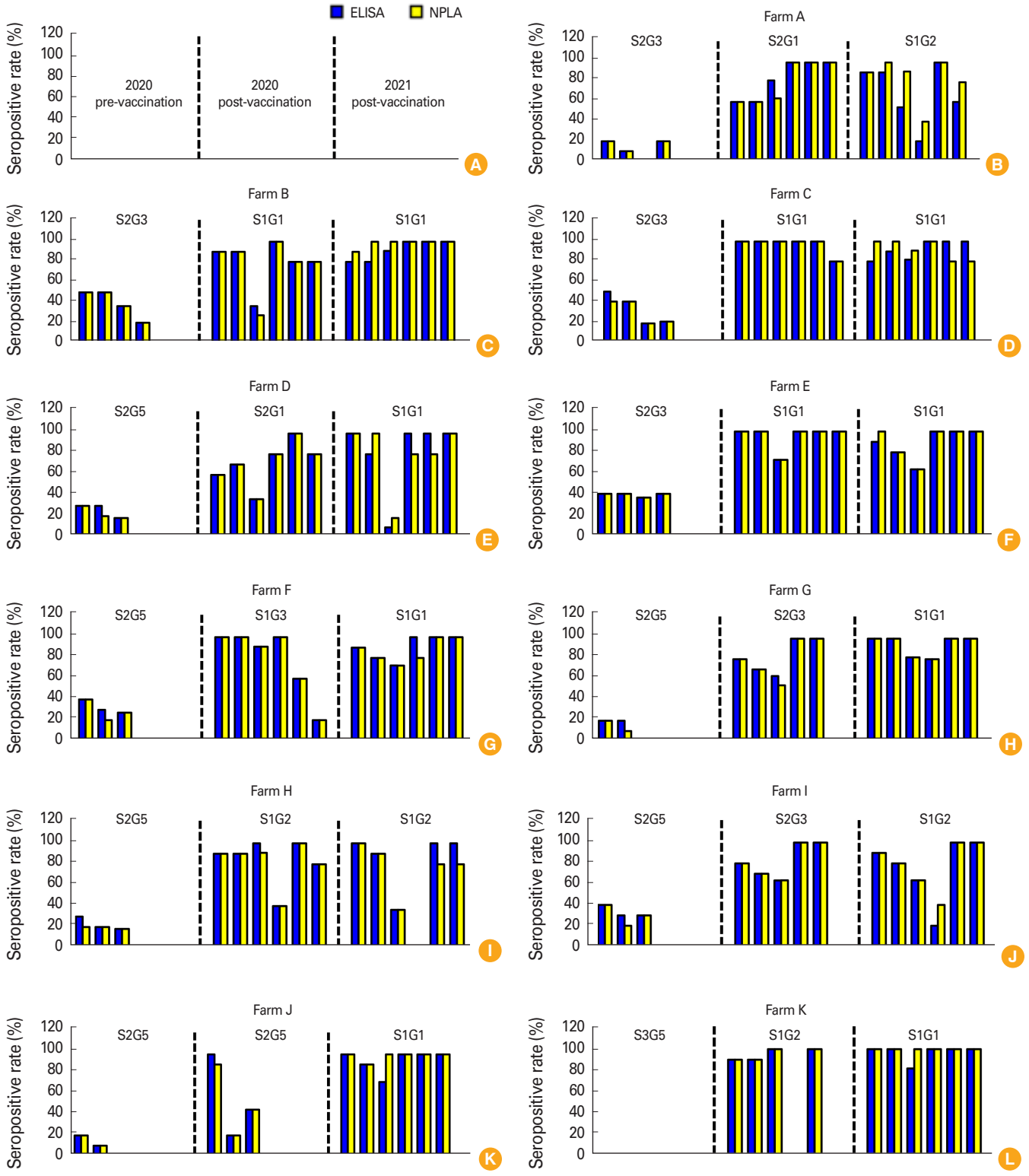

Fig. 2. Herd immunity profiles of individual pig farms based on classical swine fever virus (CSFV)-specific antibody responses to breeding and growing herds in the field farm application of the CSFV-E2 subunit vaccine. Serum samples were collected from breeding (sow and 20-day-old piglets) and growing-finishing (40-, 70-, 100-, and 130-day-old pigs) herds before and after vaccination and subjected to CSFV E2-specific antibody enzyme-linked immunosorbent assay (ELISA) and neutralizing peroxidase-linked assay (NPLA) to determine herd immunity profiles (Table 1). **(A)** A blank graph in the upper left represents the vaccination status and monitoring year: 2020 pre-vaccination (left bars), 2020 post-vaccination (middle bars), and 2021 post-vaccination (right bars). **(B–N)** Herd immunity profiles are indicated at the top of each graph. (Continued on next page.)

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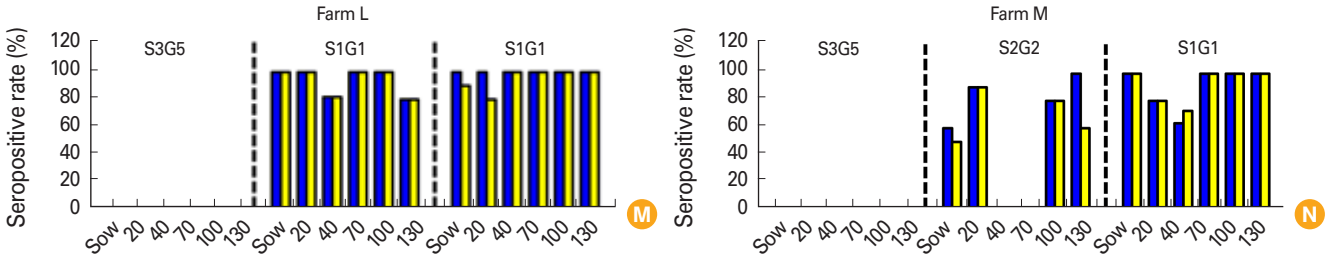

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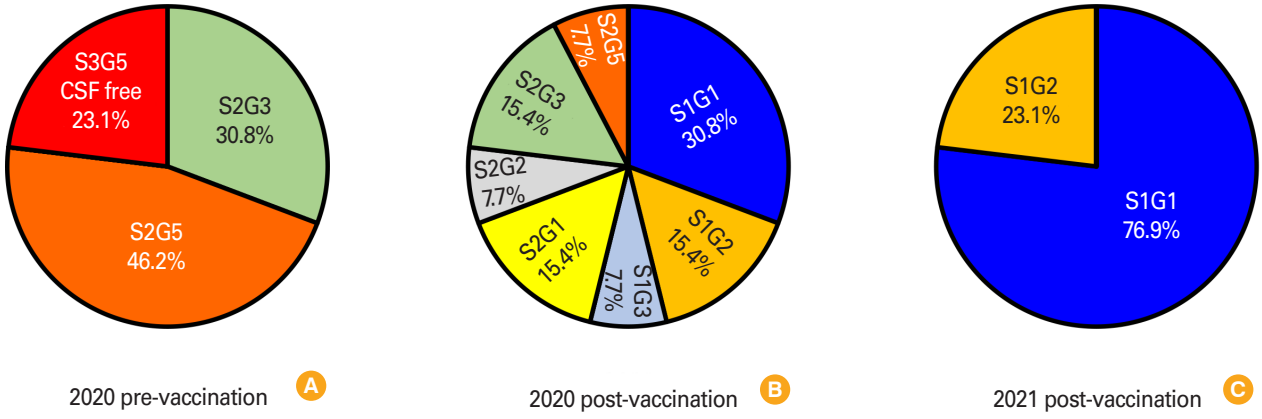

Fig. 3. (A–C) Percentage of herd immunity profiles during 2020–2021. Thirteen different pig farms were categorized into three (S1–S3) and six (G1–G6) immune response profiles according to their serological and virological statuses (Table 1). CSF, classical swine fever.

ing herds, respectively. Furthermore, two farms (15.4%) were categorized into S2G1 and demonstrated unstable sow herd immunity with low maternally-derived antibody (MDA) levels but stable immunity level in the growing herd. The remaining farms incompletely fulfilled the vaccination program in both breeding and growing herds and thus belonged to S2G2 (1/13), S2G3 (2/13), or S2G5 (1/13). In particular, Farm J (S2G5) employed a sow vaccination regime but absolutely omitted the immunization regime in growing herds. In contrast, the herd immunity status was significantly improved in 2021 (Fig. 3C); 10 farms (76.9%) belonged to S1G1, whereas the remaining three farms (23.1%) belonged to S1G2.

Presence of CSFV E^{rns}-specific antibodies

Next, serum samples from 13 pig farms were used to test DIVA by assessing the level of anti-CSFV E^{rns} antibodies in pigs using the pig-type CSFV E_{rns} Ab. The number of E^{rns} antibody-positive pigs (i.e., CSFV-infected animals) from all age groups during 2020–2021 is summarized in Table 2. In September 2020, all farms (10/10), excluding three CSFV-negative farms (Farms K–M), tested seropositive in the E^{rns}-specific prototype ELISA as detected in May 2020 (pre-vaccination); however, the rate of CSFV E^{rns} seropositivity was diminished at the pig level compared with that in May 2020 (Fig. 4). The results showed that 21.3% (98/460) of the tested pigs had a clear presence of CSFV E^{rns} antibodies, and 37.0% (37/100) and 42.0% (42/100) of the sows and piglets tested seropositive, respectively. The E^{rns}-specific antibody was detected in 16.4% (18/110) and 0.7% (1/150) of the pigs at 40 and 70–130 days of age, respectively.

E^{rns} seropositivity was remarkably reduced at both farm and pig levels in 2021 (Fig. 4). Five swine farms (50%) still continued to exhibit E^{rns} antibodies, whereas the remaining farms (5/10,

Table 2. Number of CSF E^{rns} antibody-positive pigs in 10 CSFV-positive farms during 2020–2021

Pig	No. of CSFV E^{rns} positive pigs/no. of pigs tested (positive rate %)		
	2020 pre-vaccination	2020 post-vaccination	2021 post-vaccination
Sow	46/100 (100)	37/100 (37)	7/100 (7)
20-day-old	41/100 (41)	42/100 (42)	4/100 (4)
40-day-old	28/110 (25.5)	18/110 (16.4)	9/110 (8.2)
70-day-old	2/50 (4)	1/50 (2)	0/50 (0)
100-day-old	1/50 (2)	0/50 (0)	0/50 (0)
130-day-old	1/50 (2)	0/50 (0)	0/50 (0)
Total	119/460 (25.9)	98/460 (21.3)	20/460 (4.3)

CSF, classical swine fever; CSFV, CSF virus.

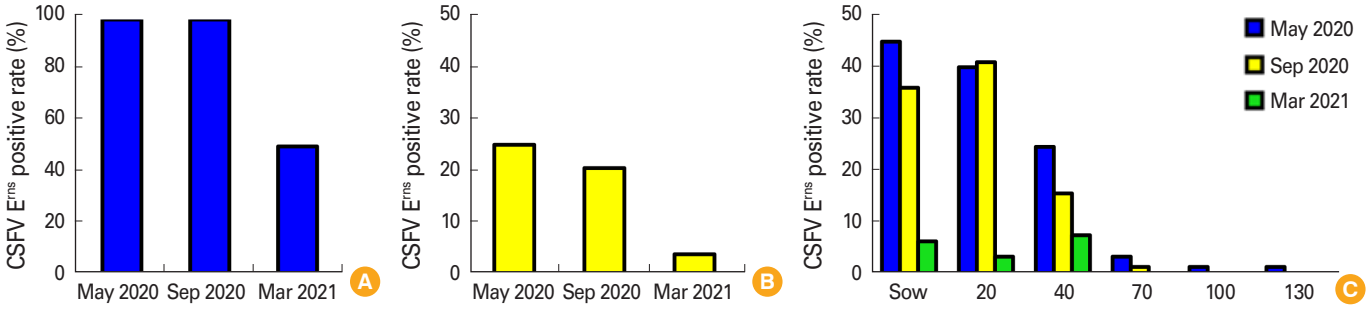

Fig. 4. Positive rates of classical swine fever virus (CSFV) E^{rns}-specific antibodies at farm **(A)**, pig **(B)**, and age **(C)** levels. Serum samples collected from 10 CSFV-positive farms at the indicated sampling times or ages were tested for detecting CSFV E^{rns}-specific antibodies.

50%) converted into the CSFV-free status (i.e., the absence of CSFV E^{rns} antibodies). Three CSFV-negative farms (Farms K–M) maintained the disease-free status throughout the study. In the breeding herds, the E^{rns}-specific antibody was confirmed in 7.0% (7/100) and 4.0% (4/100) of sows and piglets, respectively. At 40 days of age, 8.2% (9/110) of pigs were E^{rns}-seropositive. Interestingly, none of the growing-finishing (70–130 days of age) pigs from the 13 pig farms tested seropositive in the E^{rns} ELISA. Based on the DIVA test, we could certify that the farms (10/10, 100%) tested were CSFV-free in the growing herds. Altogether, our results indicate that the implementation of provincial immunization with the E2 subunit vaccine alleviated CSFV circulation and contributed to the recovery of the virus-free status in Jeju pig herds.

Discussion

Considering that vaccination is one of the most powerful tools in veterinary medicine to prevent infectious diseases and protect animal health, massive vaccination using various LAVs has been utilized in numerous CSF-endemic countries to control the disease and safeguard the swine industry. Similarly, the South Korean government launched a mandatory LAV-LOM vaccination program to combat CSF in 1996. Although CSF vaccination was discontinued in the 2000s, regional- and national-scale recurrences of CSF resulted in the maintenance of a national eradication policy that enforces compulsory vaccination and quarantine across the country, excluding Jeju province [14,27]. After the declaration of a provincial CSF-free status in 1998, Jeju Island pursued a non-vaccination policy against CSF [15,20]. Nevertheless, Jeju swine herds have experienced several CSF incidents over the past two decades through involuntary exposure of naïve animals to the LOM vaccine. In 2014, accidental LOM vaccination led to the reemergence of CSF on Jeju Island, causing the persistent circulation of LOM-derived CSFV in the provincial pig population [18,19]. To fight the endemic CSF spreading LOM virus, Jeju province authorized the implementation of a vaccination policy in 2019 [20]. Considering safety guarantee and DIVA strategy, the CSFV E2 subunit vaccine was approved for provincial use on Jeju Island. Shortly after, we conducted two trials to evaluate the safety and immunogenicity of the approved Bayovac CSF-E2 subunit vaccine in breeding and finishing pigs from three CSF-naïve commercial FTF farms on Jeju Island. Although the previous study was limited by the group size, it demonstrated the safety and efficacy of the E2 subunit vaccine under field conditions [20]. The present study was extended to a large-scale field trial of the E2 subunit vaccine in 13 FTF farms, including the three CSF-naïve herds, which implemented routine parenteral immunization with a three- or two-dose Bayovac CSF-E2 vaccine in pregnant sows and young pigs, respectively.

Infection monitoring in May 2020 confirmed the circulation of CSFV in breeding and/or growing herds. Subsequently, the first immunity monitoring in 3 months after mass vaccination indicated that herd immunity statuses varied among the farms. On the basis of herd immunity profiles, almost 70% of farms inadequately followed the vaccination regime in breeding and/or growing herds, which led to unstable immunity levels in sows with low MDA levels and/or growing pigs. The unsatisfactory implementation of vaccination might represent the continual presence of CSFV in all affected farms; however, it exerted a palliative effect on the number of CSFV-E^{rns}-positive animals in the farms. The second immunity monitoring in March 2021 revealed that most farms precisely applied the recommended CSF E2 vaccination schedule, resulting in 70% (7/10) affected and 100% (3/3) naïve farms with herd immunity stabilization. This stable immunity in breeding and growing herds correlated with a significant decline in CSFV-E^{rns} positivity at both farm and pig

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levels. The DIVA test confirmed that five of the 10 affected farms recovered their CSFV-free status. The immunity level among growing herds in some farms (3/10) was classified as G2, resulting from the application of a single-shot vaccination rather than an advised two-dose regimen in growing pigs. However, the growing and finishing pigs from all the affected farms tested CSFV negative, indicating the elimination of CSFV in at least growing herds. In contrast, the status of breeding herds in all farms, including the three CSFV-naïve farms, exhibited the S1 profile. Despite immunity stabilization, the vaccination failed to remove the circulation of CSFV in breeding herds, as the E^{rns}-specific antibody was detected in sows and 20- and 40-day-old pigs from five farms. This status may reflect the limitation of a pre-farrow immunization regime with three doses of the E2 subunit vaccine in pregnant sows, which may not produce sufficient amounts of protective immunity in sows that can confer passive protection to their offspring and last for at least 40 days.

The CSFV vaccination project started in 2019 was launched to investigate the safety and immunogenicity of the E2 subunit vaccine in CSF-naïve farms and further evaluate its efficacy in CSFV-affected farms. The present study demonstrated the effect of mass immunization with the E2 vaccine on CSFV-affected farms. Our results indicated that the E2 vaccine-based immunization strategy followed by routine immunity monitoring assisted in the removal of CSFV circulation in breeding and growing herds. To maximize vaccine effectiveness, the vaccination policy should be combined with other control measures such as stringent biosecurity performance. In particular, CSFV-positive farms with vaccination should strictly follow biosecurity/disinfection practices and monitor coinfection with other viral pathogens, such as porcine reproductive and respiratory syndrome virus and porcine circovirus 2, to prevent the aggravation of economic losses. Our data emphasize the necessity of an active provincial vaccination campaign that can provide insights into establishing a CSFV eradication policy to recover the disease-free status on Jeju Island.

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