



**Guehwan Jang¹, Eun-Joo Kim²,
Seong-Cheol Cho², Sung-Up Moon²,
Byeong Soo Kim³, Jinhee Kim³,
Kyoung Ju Jeong³, Kyungok Song³,
Seong Hwan Mun³,
Won-Myoung Kang³, Jonghoo Lee⁴,
Changnam Park⁵,
Hyoung-Seok Yang⁵, Changhee Lee¹**

¹College of Veterinary Medicine and Virus Vaccine Research Center, Gyeongsang National University, Jinju; ²Animal Health Division, ³Veterinary Research Institute, ⁴Jeju-si Livestock Division, and ⁵Seogwipo-si Livestock Division, Jeju Special Self-Governing Province, Jeju, Korea

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Corresponding author: Changhee Lee, DVM, PhD
 College of Veterinary Medicine, Gyeongsang National University, 501 Jinju-daero, Jinju 52828, Korea
 Tel: +82-55-772-2345, Fax: +82-55-772-2349
 E-mail: changhee@gnu.ac.kr

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Field evaluation of the safety and immunogenicity of a classical swine fever virus E2 subunit vaccine in breeding and nursery animals on Jeju Island, South Korea

Purpose: Classical swine fever (CSF) reemerged on CSF-free Jeju Island where vaccination is not practiced by the unintentional injection of a live attenuated vaccine (modified live attenuated vaccines—low-virulence Miyagi [MLV-LOM]) in 2014. Since the Jeju provincial authority is considering adopting a voluntary immunization policy using a CSF-E2 subunit vaccine to combat LOM-derived CSF endemic, this study aimed to evaluate in Jeju herds.

Materials and Methods: Two vaccination trials using the Bayovac CSF-E2 vaccine licensed for use in South Korea assessed the safety and humoral immunity of the CSF-E2 vaccine in breeding (trial 1) and nursery animals (trial 2) under farm application conditions.

Results: Neither local nor systemic (including reproductive) adverse effects were objectively observed in pregnant sows and young piglets following a respective vaccination regime at pregnancy or weaning, respectively. Trial 1 showed that sows immunized with the CSF-E2 vaccine possessed high and consistent E2-specific and neutralizing antibody levels. The CSF-E2 vaccine-immunized pregnant sows subsequently conferred appropriate and steady passive immunity to their offspring. In trial 2, a double immunization scheme of the CSF-E2 vaccine in piglets at 40 and 60 days of age could elicit a consistent and long-lasting adequate antibody response. Additionally, the two trials detected no E^{ms}-specific antibody responses, indicating that CSF-E2 vaccine can differentiate infected from vaccinated animals (DIVA).

Conclusion: Our trial data collectively provide invaluable information on applying the CSF-E2 subunit vaccine to circumvent the possible drawbacks associated with the MLV-LOM concerning the safety, efficacy, and DIVA, in the LOM-endemic field farms and contribute to advanced CSF eradication on Jeju Island.

Keywords: Classical swine fever virus, MLV-LOM, Jeju Island, CSF-E2 subunit vaccine, Field application

Introduction

Classical swine fever (CSF) is considered one of the most devastating and transboundary viral diseases of swine worldwide. The disease has many typical or atypical clinical outcomes that can range from subclinical to fatal, depending on the age of the infected animal and the virulence of the virus [1,2]. CSF is caused by the classical swine fever virus (CSFV, currently re-designated as *Pestivirus C*) that is an enveloped, positive-

strand RNA virus belonging to the genus *Pestivirus* in the family *Flaviviridae* [3]. The CSFV genome possesses one large open reading frame of about 12.3-kb flanked by two untranslated regions at both ends. It encodes a single polyprotein precursor that undergoes co- and post-translational modifications with the help of cellular and viral proteases to manufacture four structural (C, E^{ns}, E1, and E2) and eight non-structural proteins (N^{pro}, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [1,4-6].

CSF outbreaks cause tremendous socioeconomic consequences because of impaired pig production, mass (pre-emptive) slaughter, and movement and trade restrictions on pigs and pork products. Because of this socioeconomic significance on the global swine industry, CSF is notifiable to the World Organization of Animal Health (OIE) [2]. Major intervention measures include stamping out of suspected and infected herds and/or vaccination to control epidemic outbreaks. Multiple efficacious modified live attenuated vaccines (MLV) paved the path for CSF removal during the past decades [7]. Although prophylactic vaccination is prohibited in CSF-free countries, except for emergency cases, vaccination strategies using MLV are still implemented in numerous countries with endemic CSF as part of national control programs [4].

The LOM strain, an attenuated virus of a low-virulence Miyagi (LOM) isolate from Japan, has been used as a live CSF vaccine (MLV-LOM) for a compulsory vaccination policy as part of a CSF-free project in South Korea [8]. Consequently, Jeju Province, the largest island of South Korea, declared CSF-free status and banned the CSF vaccine mandate in 1998 [9]. However, this provincial non-vaccination policy caused several CSF outbreaks on Jeju Island by the unintentional introduction or injection of the MLV-LOM into CSFV-naïve herds from the mainland of South Korea, drawing suspicion regarding the safety of this sole national commercial vaccine [8,10-12]. Despite the benefits of its use for protection and prevention, the MLV-LOM carries authentic disbenefits of using the live attenuated vaccine, which includes the possibility of reversion to virulence and adverse effects in the field [13,14]. More importantly, like conventional live vaccines, the MLV-LOM has the inherent disadvantage that it cannot serologically differentiate infected from vaccinated animals (DIVA), thereby hampering CSF eradication, especially in endemic areas [2,4]. Thus, it is desirable to develop alternative vaccines, including subunit, live attenuated marker, and viral vector vaccines, that satisfy safety, DIVA, and efficacy.

Alternative CSF vaccine progress introduced many subunit marker vaccines based on a single or fusion form of the immunogenic E2 glycoprotein synthesized in a baculovirus (Bayovac and Porcilis Pesti), lentivirus (Porvac), or plant (HERBAVAC) expression system [7,15,16]. Although these E2 subunit vaccines are licensed for market use, several weaknesses of these vaccines, including the constraints in protection against early infection phase or transplacental transmission despite a double vaccination regime, have caused their narrow use in field conditions [7,17,18]. Nevertheless, the E2 marker vaccines have advantages over conventional live attenuated vaccines concerning safety and DIVA. The former issue can be solved by removing all potentially dangerous factors from whole virus-based vaccines. The latter relies on antibody detection against the CSFV E^{ns} protein to identify infection with field virus [4]. Moreover, recent studies exhibited their efficacy on early protection and vertical transmission prevention against CSF infection under experimental or field conditions [19-22].

Jeju Island, an once CSF-free region, has faced an endemic circumstance since its last resurgence in 2014. The LOM-derived field strains continue to circulate in Jeju pig herds, causing sporadic outbreaks [13]. A vaccination strategy is demanded to impede the circulation of LOM field virus and to eliminate the disease, which needs vaccines, ideally with the safety, efficacy, and DIVA characteristics, such as CSFV E2 subunit marker vaccines. Before the E2 subunit vaccine against CSF is authorized for use in pigs on Jeju Island, evaluations of its safety and effectiveness are essential under field conditions without pre-existing CSF antibodies. Accordingly, we conducted field trials to assess the safety and humoral immunity of the E2 subunit vaccine in CSFV-naïve farms without previous exposure to the LOM vaccine on Jeju Island.

Materials and Methods

Cells, virus, and vaccine

LLC-PK1 cells (American Type Culture Collection CL-101) were cultured in alpha-minimum essential medium (Invitrogen, Carlsbad, CA, USA) with 5% fetal bovine serum (Invitrogen) and penicillin-streptomycin (100×; Invitrogen). The cells were maintained at 37°C in an atmosphere of humidified air containing 5% CO₂. The commercial CSFV MLV-LOM strain (GenBank accession number: MK121886) was acquired from ChoongAng Vaccine Laboratories (CAVAC, Daejeon, Korea) [11] and propagated in LLC-PK1 cells as de-

scribed previously [13].

Field trial design

CSF vaccine

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.

Farm selection

The study was conducted on three commercial farrow-to-finish swine farms (named Farm A, B, and C), with 1,500–2,000 heads per farm, located on Jeju Island. All pig farms have about five buildings at one site for breeding herds, farrowing sows, nursery, growing, and finishing pigs and have adopted a continuous flow production for marketing pigs. These farms had good health status and no CSF outbreak and vaccination history.

Field experiments

Trial 1 assessed the safety and efficacy of the CSF-E2 subunit vaccine on pregnant sows and the kinetics of E2-specific maternally derived antibodies (MDA) in their offspring. Six pregnant sows (parities 1–3) from each farm (total 18 sows from three different farms) were randomly assigned to two groups and vaccinated with three doses of Bayovac CSF-E2 (n=3) or placebo (n=3) at –10, –7, and –4 weeks post-parturition (WPP) (Fig. 1A). All sows were monitored daily for clinical signs throughout the trial following immunization. Blood was taken from vaccinated sows (n=18; six sows per farm) before each vaccination and at three and six WPP and from new-

born piglets (n=54; three piglets per litter) randomly selected from those of CSF-E2 vaccinated dams at 3 and 6 weeks after birth (Fig. 1A). Serum samples were centrifuged and used for CSFV serology.

Trial 2 evaluated CSF-E2 subunit vaccine-induced immune responses in field farm applications on naïve young pigs with a long-term observation from the weaning to the finishing stage. Twenty-four piglets from each farm (72 pigs from three different farms) were randomly allocated to two groups and immunized with two doses of Bayovac CSF-E2 (n=12) or placebo (n=12) at 40 and 60 days of age. All animals were monitored daily for clinical signs throughout the experiment. Blood was taken from vaccinated animals (n=72; 24 pigs per farm) before each vaccination and at 100, 130, and 160 days of age (Fig. 1B). Serum samples were centrifuged and tested for the level of CSFV E2-specific immunity.

Safety assessments

Safety was assessed by monitoring local reactions at the injection sites in all animals and measuring reproductive performance of female breeding pigs. All injection sites were observed in the vaccinated breeding and nursery animals for the presence or absence of reactions, including redness, swelling, necrosis, and pain during palpation at 2 hours post-vaccination. The general health of all vaccinated pigs was also monitored to investigate the presence of side effects, including abscess formation at the injection site, appetite, and digestion for 21 days after each vaccination. Post-mortem meat inspection of slaughtered pigs was performed to examine the presence or absence of visible lesions at the vaccine injection site in pork. The percentage of piglets born alive (PBA) per litter at farrowing was used as a benchmarking measurement to compare the reproductive performance be-

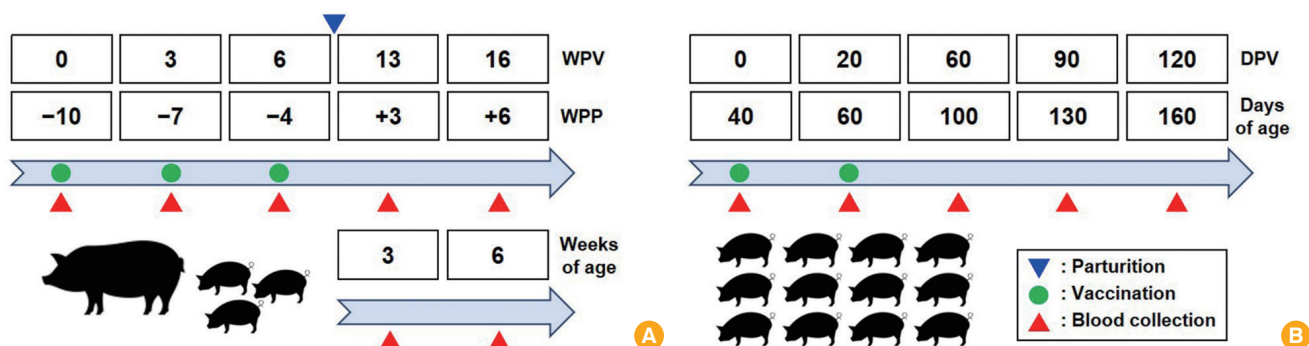


Fig. 1. Schematic diagram of the field experimental design in this study. The time points for parturition, vaccination, and blood collection from both trials were presented. (A) In trial 1, pregnant sows received three doses of classical swine fever (CSF)-E2 subunit vaccine injected intramuscularly at –10, –7, and –4 weeks parturition. (B) In trial 2, naïve young piglets received two doses of CSF-E2 subunit vaccine injected intramuscularly at 40 and 60 days of age. WPP, weeks post-parturition; WPV, weeks post-vaccination; DPV, days post-vaccination.

tween sows vaccinated with Bayovac CSF-E2 or placebo (control) from each farm. PBA percentage was calculated as follows: (number of PBA per sow/total number of piglets born per sow) × 100%.

Classical swine fever virus serology

The CSFV E2-specific antibody level in serum samples collected from pigs was analyzed using the CSFV antibody B-enzyme-linked immunosorbent assay (ELISA) kit (BIONOTE, Hwaseong, Korea) following the manufacturer's instructions. The results were expressed as the percent inhibition and a percent inhibition value equal to or greater than 40% was considered positive for the presence of CSFV E2 antibodies. Serum samples were also tested for the detection of CSFV E^{ms}-specific antibodies to evaluate the DIVA capability after immunization with the E2 subunit vaccine using the pigtype CSFV Erns Ab (Indical Bioscience, Leipzig, Germany), in accordance with the manufacturer's protocols. The 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^{ms} antibodies.

The CSFV-specific neutralizing antibody (NAb) level against the CSFV (LOM strain) was determined using neutralizing peroxidase-linked assay (NPLA) according to the standard manual of OIE [23]. The neutralizing endpoint titers were calculated as the reciprocal of the highest serum dilution that neutralized 100 TCID₅₀ (50% tissue culture infective dose) of the LOM vaccine strain in 50% of culture replicates. The NAb titers were transformed to a log₂ scale and the serum samples with neutralizing endpoint titers of ≥ 1:16 were considered positive for the presence of CSFV NABs.

Statistical analysis

All values are expressed as mean ± standard deviation of the mean difference. Statistical analyses were conducted using two-way analysis of variance, followed by Tukey's multiple-comparison test using the GraphPad Prism 8 software package (GraphPad Software, San Diego, CA, USA). All p-values below 0.05 were considered statistically significant.

Ethics statement

No ethical review and approval were required, as the vaccine used in this study is approved from 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 to use the animals in the experiment from the farm owner.

Results

Safety of the classical swine fever-E2 subunit vaccine in naïve breeding and nursery pigs

To evaluate the safety of the CSF-E2 subunit vaccine in pigs, local and systemic abnormalities were monitored in all animals after vaccination. Neither trial revealed injection site reactions in breeding and nursery animals vaccinated with the CSF-E2 vaccine during palpation at 2 hours post-vaccination. There were no differences between vaccinated animals and animals of the placebo (control) group (data not shown). Good health status also remained without obvious side effects for 3 weeks following each vaccination in all vaccinated sows (trial 1) and piglets (trial 2). It did not vary between the vaccinated and control groups (data not shown). Moreover, none of the vaccinated animals developed any objective clinical consequences, including mortality, related to vaccination in trial 1 or trial 2 throughout the experimental period compared with that in the control animals vaccinated with placebo (data not shown). However, subsequent post-mortem meat inspection of the trial 2 pigs at the slaughterhouse revealed a perceptible gross lesion comprising yellow-green creamy pus with clear margins at the vaccine injection site in one slaughtered pig immunized with the CSF-E2 vaccine.

Further, sow reproductive performance was investigated to assess the safety of the CSF-E2 subunit vaccine in pregnant sows by measuring the number and percentage of PBA from three individual farms in trial 1. There were two aborted and stillborn pigs in the control group; hence, the PBA proportion in pregnant sows vaccinated with placebo was 97.5%. By contrast, no reproductive challenges associated with the CSF vaccine, including abortion and stillbirth, occurred in pregnant sows immunized with the CSF-E2 vaccine. Thus, the PBA percentage in the vaccinated group was determined to be 100%.

Immunogenicity of the classical swine fever-E2 subunit vaccine in naïve pregnant sows

In trial 1, the immune response of the CSF-E2 subunit vaccine in sows was assessed. Eighteen pregnant sows from three individual farms were divided into two groups (nine sows in each group) and immunized with CSF-E2 subunit vaccine or placebo at 10, 7, and 4 weeks before parturition.

CSFV serological analyses, including E^{tns}- or E2-specific antibody ELISA and NPLA, were conducted using serum samples obtained from all sows at the time of each vaccination and at three and six WPP. Serology results from the control group were omitted, since all the animals vaccinated with placebo tested negative for CSFV-specific antibody responses at the 1st vaccination and remained seronegative to CSFV throughout the experiment. None of the vaccinated sows from three farms tested positive for the E2-specific antibody and NAb levels at the time of the 1st vaccination (−10 WPP), corroborating that they have not been exposed to CSFV or CSF vaccines (Fig. 2A, B). Seroconversion occurred in most sows (7/9), except two from each Farm A and B, at the 2nd vaccination (−7 WPP) or 3 weeks post-vaccination (WPV), as

evident using the E2-specific antibody ELISA and NPLA (Fig. 2A, B). The animals that were seronegative at −7 WPP, all seroconverted by the 3rd vaccination (−4 WPP or 6 WPV). Since then, all vaccinated sows remained positive for E2-specific antibody and NAb titers until the trial 1 ended (6 WPP or 16 WPV) (Fig. 2A, B). By contrast, the E^{tns}-specific antibody ELISA was negative for all sera collected from the immunized sows and their offspring throughout the trial (data not shown), demonstrating the ability of the CSF-E2 subunit vaccine for DIVA.

At −4 WPP (after the 2nd vaccination), the E2-specific antibody titers presented as percent inhibition values for the vaccinated group ranged from 81.97%–92.46% with a mean value of 91.01% ± 1.63% (Farm A), 87.77% ± 2.95% (Farm B), and

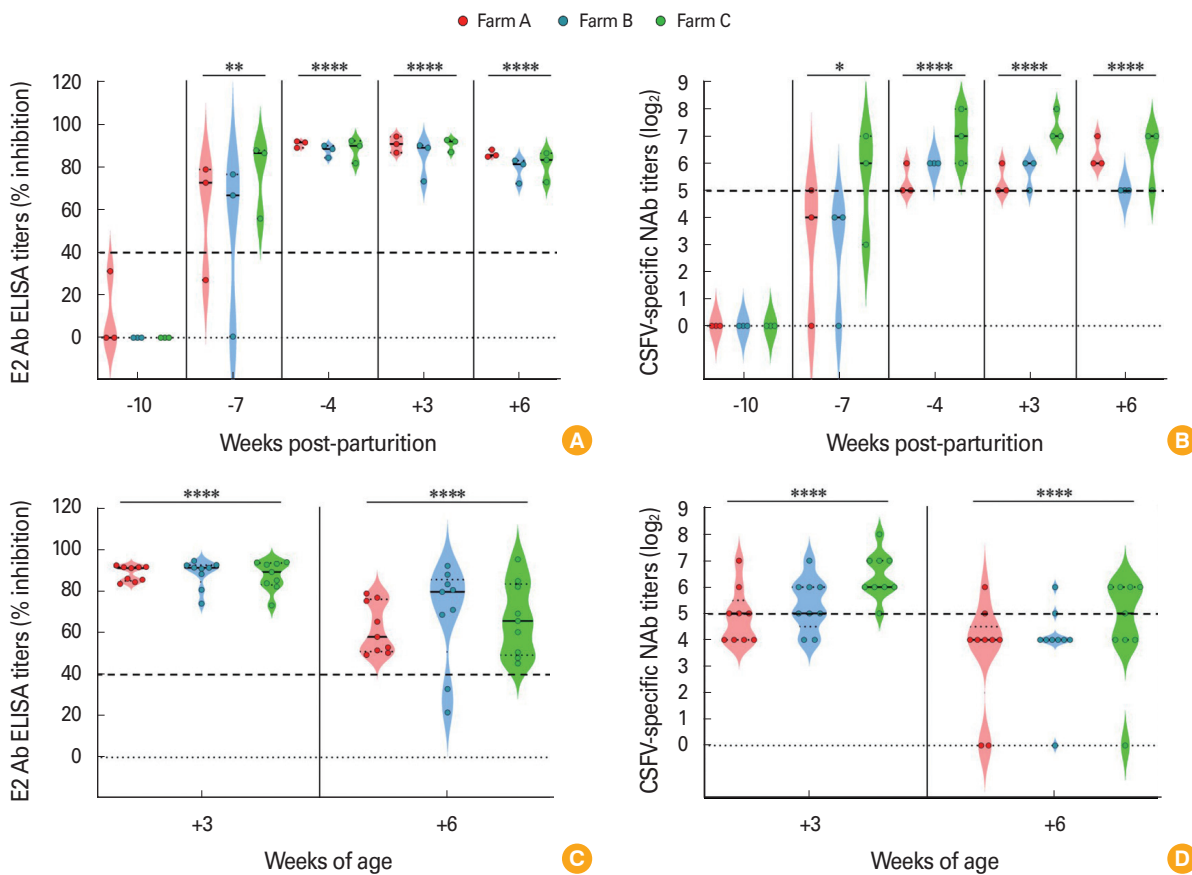


Fig. 2. Classical swine fever virus (CSFV)-specific antibody responses of pregnant sows (A, B) and newborn piglets (C, D) in field farm applications of CSF-E2 subunit vaccine. Pregnant sows were immunized with CSF-E2 vaccine at −10, −7, and −4 weeks post-parturition. The serum samples were collected from pregnant sows and their representing offspring at the indicated time points and subjected to CSFV E2-specific antibody enzyme-linked immunosorbent assay (ELISA) (A, C) and neutralizing peroxidase-linked assay (B, D). CSFV E2-specific antibody titers were expressed as the percent inhibition. Individual samples are considered positive for the presence of CSFV E2 antibodies if the percent inhibition value is equal to or greater than 40% indicated as the dashed line. CSFV-specific neutralizing antibody (NAb) titers for individual samples were presented as a log₂ scale. The dashed line indicated the protective neutralizing antibody level (1:32). Error bars represent the standard deviation of the mean difference. All p-values were calculated by comparing the value of antibody titers between the time before and after vaccination by using two-way analysis of variance with Tukey’s multiple comparison test in GraphPad Prism software (GraphPad Software, San Diego, CA, USA). *p<0.05. **p<0.01. ***p<0.0001.

88.18%±5.51% (Farm C). These high levels for the E2-specific antibody were further maintained until 6 WPP (16 WPV), albeit the values tended to decline slightly at that time point (Fig. 2A). Likewise, the CSFV-specific NAb titers from all CSF-E2 vaccine-immunized sows were measured to be equal to or greater than the hypothetical protective NAb level ($5 \log_2$ or 1:32) against CSFV and ranged from 5–8 \log_2 with a mean titer of $5.33 \log_2 \pm 0.58$ (Farm A), $6.00 \log_2 \pm 0.00$ (Farm B), and $7.00 \log_2 \pm 1.00$ (Farm C) at –4 WPP (6 WPV). Despite a reduction in a mean NAb titer of Farm B at 6 WPP (16 WPV), those protective NAb levels of sows persisted until the termination of trial 1 (Fig. 2B).

Next, the passive immunity of suckling piglets acquired from their dams immunized with the CSF-E2 subunit vaccine was evaluated. The serum samples were collected from three newborn piglets per vaccinated sow (27 piglets from nine sows) at 3 and 6 weeks of age and subjected to serology to measure the kinetics of MDA. At 3 weeks of age, all animals (offspring from the CSF-E2 vaccinated dams) possessed a high E2-specific antibody level, ranging from 73.38%–94.82% with a mean value of $88.85\% \pm 3.66\%$ (Farm A), $88.65\% \pm 6.73\%$ (Farm B), and $87.70\% \pm 7.05\%$ (Farm C) (Fig. 2C). Although the MDA level of those piglets decreased to $66.20\% \pm 12.25\%$ (Farm A), $68.82\% \pm 24.79\%$ (Farm B), and $66.96\% \pm 17.76\%$ (Farm C) at 6 weeks of age, the majority of offspring (25/27), except two in Farm B, maintained the E2-specific antibody ELISA values surpassing a positive antibody response (40% cutoff) (Fig. 2C). Similarly, the CSF-E2 vaccination in pregnant sows caused a passive transfer of the CSFV-specific NAb to their suckling piglets, as they ranged 4–8 \log_2 with a mean titer of $4.89 \pm 1.05 \log_2$ (Farm A), $5.33 \pm 1.00 \log_2$ (Farm B), and $6.44 \pm 0.88 \log_2$ (Farm C) in neonates at 3 weeks of age (Fig. 2D). The passive NAb lasted in the piglets at 6 weeks of age, but the level reduced to $3.44 \pm 2.07 \log_2$ (Farm A), $3.89 \pm 1.61 \log_2$ (Farm B), and $4.56 \pm 1.94 \log_2$ (Farm C), which included four NAb-negative piglets (2/9, 1/9, and 1/9 from Farm A, B, and C, respectively) (Fig. 2D).

Immunogenicity of the classical swine fever-E2 subunit vaccine in naïve nursery pigs

In parallel with trial 1, another field application trial (trial 2) was independently carried out to assess the immune response of the CSF-E2 subunit vaccine in nursery piglets with long-term observation from weaning to finishing. Seventy-two animals from three commercial farms were allocated to two groups (36 piglets in each group) and immunized with

CSF-E2 subunit vaccine or placebo at 40 and 60 days of age. The serum samples were collected at each vaccination and at 100, 130, and 160 days of age and used to detect seroconversion. As expected, no CSFV-specific antibodies were detected in control animals vaccinated with placebo. Moreover, the E2-specific antibody and NAb levels were tested negative in all vaccinated piglets ($n=36$) from three farms at the time of the 1st vaccination (40 days of age), verifying that the farms selected in this study had not been exposed to CSF vaccine or infection (Fig. 3A, B). The E2-specific antibody ELISA showed that nearly all piglets (33/36), excluding three animals (2/12 and 1/12 from Farm A and B, respectively), seroconverted at the 2nd vaccination (60 days of age) or 20 days post-vaccination (DPV), as determined using the E2-specific antibody ELISA (Fig. 3A). However, the NPLA data indicated that eight piglets (3/12, 3/12, and 2/12 from Farm A, B, and C, respectively), including those three animals that were E2 antibody ELISA-negative, did not develop the detectable NAb level after the 1st vaccination (Fig. 3B). Nevertheless, those piglets that were seronegative at 60 days of age all seroconverted by 100 days of age (60 DPV). All vaccinated piglets further remained positive for E2-specific antibody and NAb titers by 130 days of age (90 DPV) (Fig. 3A, B). At 160 days of age (120 DPV) before slaughter, most vaccinated pigs retained consistent antibody levels exceeding a positive antibody response, even though one (1/12 from Farm A) or four (1/12, 2/12, and 1/12 from Farm A, B, and C, respectively) pigs reverted to negative to the E2 antibody or NAb, respectively (Fig. 3A, B). Alternatively, all vaccinated piglets tested negative for the E^{ms}-specific antibody ELISA throughout the trial, thereby confirming the DIVA capacity of the CSF-E2 subunit vaccine in weaning piglets.

After the 1st vaccination, a mean value of the E2-specific antibody production in the vaccinated piglets at 60 days of age (20 DPV) was determined to be $64.36\% \pm 18.82\%$ (Farm A), $62.22\% \pm 11.61\%$ (Farm B), and $74.13\% \pm 17.18\%$ (Farm C). These levels markedly increased, with a mean value of $92.46\% \pm 2.38\%$ (Farm A), $91.73\% \pm 6.03\%$ (Farm B), and $91.26\% \pm 4.65\%$ (Farm C) at 100 days of age (60 DPV) following the 2nd vaccination, and then slowly but marginally declined after that (Fig. 3A). Similarly, the CSFV-specific NAb titers were greatly boosted in all CSF-E2 vaccinated piglets from three farms after the 2nd vaccination (60 DPV), with a mean titer of $7.25 \pm 1.60 \log_2$ (Farm A), $6.50 \pm 1.24 \log_2$ (Farm B), and $6.50 \pm 1.44 \log_2$ (Farm C) exceeding the protective NAb level against CSFV (Fig. 3B). At 130 days of age (90 DPV),

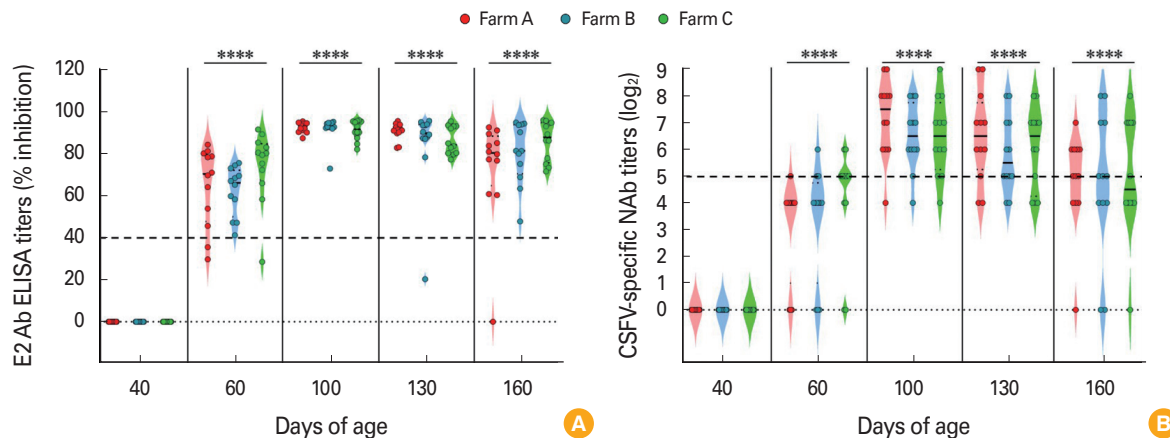


Fig. 3. Classical swine fever virus (CSFV)-specific antibody responses of nursery piglets in field farm applications of CSF-E2 subunit vaccine. Piglets were immunized with CSF-E2 subunit vaccine at 40 and 60 days of age. The serum samples were collected from 12 piglets per farm at the indicated time points and subjected to CSFV E2-specific antibody enzyme-linked immunosorbent assay (ELISA) (A) and neutralizing peroxidase-linked assay (B). CSFV E2-specific antibody titers were expressed as the percent inhibition. Individual samples are considered positive for the presence of CSFV E2 antibodies if the percent inhibition value is equal to or greater than 40% indicated as the dashed line. CSFV-specific neutralizing antibody (NAb) titers for individual samples were presented as a log₂ scale. The dashed line indicated the protective neutralizing antibody level (1:32). Error bars represent the standard deviation of the mean difference. All p-values were calculated by comparing the value of antibody titers between the time before and after vaccination by using two-way analysis of variance with Tukey's multiple comparison test in GraphPad Prism software (GraphPad Software, San Diego, CA, USA). ****p<0.0001.

each mean NAb titer from all three farms decreased slightly but still was above the protective level. Subsequently, overall mean NAb titers in the vaccinated piglets at 160 days of age (120 DPV) further declined, with a mean titer of 4.83 ± 1.80 log₂ (Farm A), 4.75 ± 2.67 log₂ (Farm B), and 5.08 ± 2.23 log₂ (Farm C). Presently, more than 40% of the vaccinated piglets (15/36) from three farms (4/12 at Farm A, 5/12 at Farm B, and 6/12 at Farm C) failed to maintain the protective NAb level (Fig. 3B).

Discussion

Vaccination is one of the most effective strategies for CSF control in endemic regions. Considering several circumstances, including the disease epidemiology, animals affected, and economic state, the vaccination policy can differ, and the vaccine to be administered can vary [4]. The LOM strain is a commonly used MLV in South Korea and plays a crucial role in combating the CSF epidemic in domestic pigs [8,24]. The South Korean government has continued mandatory immunization with the MLV-LOM to control CSF on the mainland; however, Jeju Island discontinued CSF vaccination after the announcement of CSF-free status by the provincial authority in 1998 [9]. Although Jeju Island strictly prohibits the trade of live pigs from the mainland, this conflicting policy may contain the risk of CSF reemergence in unvac-

inated CSF-naïve herds through introduction of the LOM vaccine strain from the mainland. Indeed, during the last 2 decades, with the implementation of a non-vaccination policy against CSF, Jeju pig farms have repeatedly suffered sporadic resurging incursions through unintentional exposure of naïve pigs to the LOM vaccine. In 2014, CSF recurred on Jeju Island after accidental vaccination with the MLV-LOM, and the LOM strain has since persistently circulated in Jeju herds, causing considerable socioeconomical losses to the provincial swine industry [11-13]. Thus, the Jeju provincial authority decided to adopt a vaccination policy to control CSF outbreaks sparked by the LOM strain in 2019. Under the endemic circumstance of LOM-derived field virus, the safety and efficacy of the MLV-LOM may be influenced by multiple factors, including the vaccinee (pregnant sows and immunosuppressive or weak piglets) or concurrent infection with viral or/and bacterial pathogens during vaccination, and thus, significant obscurities may occur [25,26]. In addition, recent studies showed that the MLV-induced immunity might not inhibit the circulation of medium and low virulence CSFV strains under field conditions [27,28]. Moreover, given that the MLV-LOM cannot be DIVA, using this vaccine to control and eradicate CSF is problematic. Considering these issues, Jeju Island necessitates an effective alternative vaccine with a safety guarantee and DIVA capacity, such as Bayovac CSF-E2 vaccine approved for use in South Korea. Therefore, this

study conducted two trials concurrently in CSF-naïve commercial farrow-to-finish pig farms on Jeju Island. The trials involved (trial 1) assessing the safety and immunogenicity of the licensed Bayovac CSF-E2 subunit vaccine in pregnant sows and passive immunity in their offspring and (trial 2) evaluating the safety and immunogenicity of Bayovac CSF-E2 in young piglets.

Recent studies indicated the safety and efficacy of various CSF-E2 subunit protein-based non-infectious marker vaccines on breeding pigs under field or experimental conditions [19,20-22]. Likewise, in trial 1 of this study, high safety and immunity were obtained by the application of Bayovac CSF-E2 vaccine on pregnant sows in field farms. Local and systemic inspections revealed that sows immunized at 4, 7, and 10 weeks before parturition had no adverse reactions. Furthermore, despite two abortions or stillbirth cases in one control sow, all vaccinated groups from three farms had a perfect reproductive performance with 100% of PBA. These data verify that an antepartum immunization program comprising three doses of Bayovac CSF-E2 3 weeks apart is harmless in pregnant sows and thus applicable at any pregnancy stage. More importantly, pregnant sows with a triple vaccination scheme exhibited high and consistent humoral immunity levels against CSFV. The 1st vaccination evoked a CSFV E2-specific antibody response in nearly all vaccinated sows (7/9), and the remaining sows seroconverted thereafter. After the 2nd vaccination at -4 WPP, the NAb levels of all vaccinated sows were over 5 log₂ (1:32), which was sufficient to protect individual sows and their fetus and prevent virus transmission in the population [7,19]. These E2-specific antibodies and protective NAb levels lasted until the end of the trial (6 WPP or 16 WPV) in all vaccinated sows, suggesting that the sow vaccination can elicit humoral immunity levels ample to prevent the risk of vertical and horizontal transmission to fetuses or piglets.

Immunization of pregnant sows stimulates lactogenic immunity that confers passive protection in neonatal piglets against infectious diseases through colostrum and milk. Thus, the sows with a higher antibody level before or after parturition can transfer a more adequate passive immunity (MDA) against CSFV to their offspring. The high and long-lasting MDA is ultimately critical for piglets to protect against infection during the suckling to the weaning phase before primary vaccination. In this study, monitoring MDA dynamics showed that the offspring received high and long-lasting E2-specific and neutralizing antibodies from the CSF-E2 vac-

cine-immunized dams. Although few piglets (2/27) possessed an insufficient E2-specific antibody response (percent inhibition 33.01% and 21.70%) at 6 weeks of age, the vaccinated sows can reliably provide adequate passive E2-specific humoral immunity to piglets until the end of the trial. At 6 weeks of age, a majority of suckling piglets (23/27) retained detectable maternal NAb levels (4 log₂ or 1: 16), which included 21/27 (3-week-old) and 9/27 (6-week-old) piglets with the protective NAb level of 5 log₂. Interference with MDA in piglet vaccination could be another concern in fields, leading to variant vaccination efficacy. However, Chen et al. [19] in 2021 showed that the high MDA level has no effect on the CSF-E2 vaccine-induced immune response in piglets. For safety concerns, the application of the MLV-LOM in sows is recommended only before insemination to prevent transplacental transmission in South Korea. However, individual sows vaccinated pre-breeding would develop fluctuant antibody titers, which may cause variable and inconsistent passive immunity levels to their litters during the nursing to the weaning stage [19]. By contrast, the safety and immunogenicity properties of Bayovac CSF-E2 vaccine proved in trial 1 will enable sows to be vaccinated at any stage of gestation. It will provoke a more robust humoral immunity in sows and subsequently allow the immunized sows to transfer more adequate and consistent protective MDA levels in piglets until CSF primary vaccination at 40 days of age.

For the further long-term evaluation of the safety and humoral immunity on the CSF-E2 vaccine application in field farms during the nursery phase, trial 2 was designed to immunize piglets without pre-existing CSFV antibodies with the Bayovac CSF-E2 at 40 and 60 days of age. Like trial 1, a double immunization regime with the CSF-E2 vaccine showed significant safety and antibody responses on nursery piglets in field farms. Although there were no clinical abnormalities associated with vaccination in CSF-E2-vaccinated pigs throughout trial 2, a macroscopic pus-like lesion appeared at the vaccine injection site of one slaughtered pig vaccinated with the CSF-E2 vaccine during the slaughter inspection. Such injury is uncharacterized because of a possible link between administration of different pig vaccines or antibiotics against pleuropneumonia. Excepting three animals with a low E2 antibody level (percent inhibition 35.55%, 29.70%, 28.51%), all piglets (33/36) immunized with CSF-E2 vaccine developed sufficient E2-specific humoral immunity. Virtually all vaccinated piglets maintained high and sufficient E2-specific antibody levels during the weaning to finishing stage, albeit one

piglet had a scanty or no antibody level at 130 and 160 days of age, respectively. Furthermore, all piglets produced a protective NAb level after the 2nd immunization. Although some pigs (4/36) had no detectable NAb level at 160 days of age, the mean NAb titer of finishing pigs was still around the mean of 5 log₂. Altogether, these results indicated that the Bayovac CSF-E2 vaccine induced adequate protective humoral immunity in nursery piglets throughout the whole trial period to marketing. Additionally, both trials showed the DIVA capability of Bayovac CSF-E2, as no E^{ms}-specific antibody responses were evident in any vaccinated sows, their offspring, and immunized piglets. Since the LOM-derived field virus has become endemic in Jeju pig farms, the application of the CSF-E2 subunit vaccine rather than the MLV-LOM in field farms is approved to discriminate vaccinated pigs from LOM-infected animals.

Two trials were conducted in this study to assess the safety and humoral immunity of the CSF-E2 vaccine in pregnant sows and nursery piglets from conventional pig farms on Jeju Island, where the LOM strain has ravaged the provincial herds and undergone adaptive genetic drift. Our data showed that Bayovac CSF-E2 provides high safety, adequate immune response, and DIVA, and is thus appropriate for regions that experience reemergence of the virus, such as Jeju Island, mainly circulated with the vaccine-derived LOM virus. Routine immunization programs are encouraged among populations of sows or piglets at pregnancy or 4–6 weeks of age; however, a three- or two-dose CSF vaccination schedule in the same phase may enhance numerous stress factors and aggravate additional burden on pig farmers. Nevertheless, the application of the CSF-E2 subunit vaccine remains the most promising and effective way to protect against losses because of persistent or chronic infection under the endemic disease circumstance on Jeju Island.

ORCID

Guehwan Jang <https://orcid.org/0000-0003-3108-8087>

Eun-Joo Kim <https://orcid.org/0000-0002-6424-3904>

Seong-Cheol Cho <https://orcid.org/0000-0003-3366-990X>

Sung-Up Moon <https://orcid.org/0000-0002-7035-4360>

Byeong Soo Kim <https://orcid.org/0000-0003-3704-9521>

Jinhee Kim <https://orcid.org/0000-0001-6481-0481>

Kyoung Ju Jeong <https://orcid.org/0000-0003-4454-3368>

Kyungok Song <https://orcid.org/0000-0003-2195-5060>

Seong Hwan Mun <https://orcid.org/0000-0003-0638-0983>

Won-Myoung Kang <https://orcid.org/0000-0002-5769-0618>

Jonghoo Lee <https://orcid.org/0000-0003-1542-356X>

Changnam Park <https://orcid.org/0000-0001-7105-969X>

Hyoung-Seok Yang <https://orcid.org/0000-0003-4500-8826>

Changhee Lee <https://orcid.org/0000-0002-5930-5461>

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