Pathogenicity of a novel classical swine fever LOM vaccine-derived virus isolated on Jeju Island, South Korea

Guehwan Jang1 | Joo-Ah Kim2 | Changnam Park3 | Kyungok Song3
Won-Myoung Kang3 | Kyungsu Yang4 | Changhee Lee1

1College of Veterinary Medicine and Virus Vaccine Research Center, Gyeongsang National University, Jinju, Republic of Korea
2Livestock Affairs Division, Jeju Special Self-Governing Province, Jeju, Republic of Korea
3Veterinary Research Institute, Jeju Special Self-Governing Province, Jeju, Republic of Korea
4Farm & Pharm Veterinary Hospital, Jeju, Republic of Korea

Correspondence
Changhee Lee, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Republic of Korea. Email: changhee@gnu.ac.kr

Funding information
Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry, Grant/Award Number: 119081-5

Abstract

Background: Reemergent local outbreaks of classical swine fever (CSF) occurred simultaneously in multiple pig farms on CSF-free Jeju Island, South Korea, in 2014 because of inadvertent injection of a commercial CSF (LOM) vaccine into pregnant sows. The LOM virus has since spread across the island and has become endemic in Jeju herds, raising concern about possible reversion to the virulence of the LOM vaccine. We previously isolated LOM-derived field CSF virus (CSFV) strains with unique insertion-deletion (INDEL) mutations in the 3′-untranslated region (UTR), designated LOM-derived Jeju 3′-UTR INDEL variants, from CSF-recurrent swine farms on Jeju Island in 2019.

Methods: The present study conducted animal experiments to investigate whether a 2019 emergent LOM 3′-UTR INDEL variant, KNU-1905, has reverted to a pathogenic form in conventional pigs (n=10).

Results: Experimental animal infection showed that pigs inoculated with the commercial LOM vaccine strain developed no adverse effects compared to the sham-infected pigs. However, KNU-1905 displayed pathogenic characteristics in pigs, including clinical symptoms (e.g., lethargy, conjunctivitis, nasal discharge, and diarrhea), weight loss, and gross lesions. Moreover, viremia, virus shedding in faeces and nasal fluids, and viral loads in various tissues of all the KNU-1905-infected pigs were highly significant, in contrast to those of the LOM-infected group in which CSFV RNA was detected only in the serum, nasal, and tonsil samples of one identical pig.

Conclusions: Overall, the LOM-derived field isolate with molecular variations induced clinical adverse events in pigs, which commonly shed considerable amounts of CSFV. This study provides evidence that the genetic evolution of the LOM-derived CSFV circulating on Jeju Island might have allowed the LOM vaccine to recover its primary prototype and that these variants might have induced chronic or persistent infection in pigs that can shed CSFV in field farms leading to a risk of transmission among pigs or farms in this former CSF-free region.
1 | INTRODUCTION

Classical swine fever (CSF) is a highly contagious multisystemic viral disease of domestic pigs and wild boars with an enormous socioeconomic impact on animal health and production (Blome et al., 2017). The CSF virus (CSFV), a causative agent of the disease, is a small, enveloped, single-stranded, (+) sense RNA virus of the genus Pestivirus in the family Flaviviridae, and recently, this species was re-designated as Pestivirus C (Smith et al., 2017). The CSFV genome is approximately 12.3-kb long and contains one large open reading frame (ORF) flanked by two untranslated regions (UTRs) at both ends, the uncapped 5′-UTR with an internal ribosome entry site and the uridine-rich 3′-UTR. The single ORF encodes one precursor polyprotein that undergoes co- and post-translational processing by viral and cellular proteases to produce 12 mature proteins: four structural (C, E\(^{TM}\), E1, and E2) and eight non-structural proteins (N\(^{Pr}\), p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Blome et al., 2017; Ganges et al., 2020; Ji et al., 2015; Tautz et al., 2015).

CSF can be divided into acute (lethal), chronic, and persistent forms of the disease. The acute pattern is characterized by high fever, gastrointestinal symptoms, neurological signs, skin haemorrhages or cyanosis, and high mortality depending on the virulence of the virus strain and the age of the animal (Blome et al., 2017; Moennig et al., 2003; Petrov et al., 2014). Chronic CSF occurs in infected pigs that fail to mount an effective immune response to clear the virus from circulation. Although the outcome of the chronic disease is always fatal, affected animals can live for months and constantly shed large amounts of CSFV (Blome et al., 2017). The persistent course of CSF usually occurs when the virus infects pregnant sows, leading to vertical transmission to the fetus. Unlike the infected sows, which show mild clinical signs, CSFV infection results in fetal mummification, abortions, or stillbirth, depending on the strain and the gestation time. However, infection at 50–70 days of gestation can induce an immunotolerance phenomenon, leading to the birth of persistently infected (PI) offspring. The PI piglets appear to clinically normal but die due to the so-called late-onset CSF after several months. During this period, they can shed viral loads sufficient for transmission, and thus together with chronically infected pigs act as virus reservoirs (Bohórquez et al., 2020; Frey et al., 1980; Hermanns et al., 1981; Kaden et al., 2005; Meyer et al., 1981; Richter-Reichhelm et al., 1980; Stewart et al., 1973; Vannier et al., 1981; von Benten et al., 1980).

Strict intervention strategies, including quarantine and stamping out of affected herds with or without vaccination, allowed several countries to gain CSF-free status. Nevertheless, CSF remains endemic in South and Central America, Eastern Europe, and Asia (Blome et al., 2017; Ganges et al., 2020). Highly efficacious live-attenuated vaccines have existed for many decades and paved the road to CSF eradication (van Oirschot, 2003). These vaccines have been compulsory implemented for effective CSF control in endemic regions (Ji et al., 2015), and in 2019, 25 countries officially enforced mandatory vaccination campaigns (World Animal Health Information Database (WAHIS) Interface, 2022). Likewise, South Korea has maintained a nationwide mandatory immunization policy using a CSF-modified live vaccine (MLV) based on an attenuated form of a low-virulence strain of the Miyagi isolate (LOM) from Japan (MLV-LOM) (Kim et al., 2008). Owing to its CSF-free status, Jeju Province, the largest island of South Korea, where vaccination was banned in 1998, has been exempted from this mandate (Song et al., 2013). However, since the provincial no-vaccination policy, Jeju Island has experienced five outbreaks because of the unintentional introduction of the MLV-LOM into CSFV-naïve pigs via unexpected routes from mainland South Korea (Choe et al., 2019; Kim et al., 2008).

The most recent reemergence of CSFV on Jeju Island began in 2014 by the accidental inoculation of naïve sows with a commercial CSF LOM-swine erysipelas combined live vaccine (Jang et al., 2019; Je et al., 2018). Although vaccination was halted immediately, the LOM vaccine strain has spread via farm-to-farm transmission and affected more than 100 pig farms. At present, the LOM-derived CSFV strain is endemic in the western region of Jeju Island and undergoing substantial genetic drift (Jang et al., 2019). Furthermore, our previous study reported LOM-derived field CSFV variants with unique insertion-deletion (INDEL) mutations in the 3′-UTR responsible for current sporadic outbreaks on Jeju Island (Jang et al., 2020). Due to suspicion regarding the safety and reversion-to-virulence of the commercial LOM vaccine strain, this study aimed to investigate pathogenic traits of an LOM Jeju variant with 3′-UTR INDEL in vivo.

2 | MATERIALS AND METHODS

2.1 | Cells and viruses

LLC-PK1 cells (ATCC CL-101) were cultured in alpha-minimum essential medium (a-MEM; Invitrogen, Carlsbad, CA, USA) with 5% fetal bovine serum (FBS; 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 obtained from ChoongAng Vaccine Laboratories (CAVAC; Daejeon, South Korea) (Jang et al., 2019). The LOM-derived Jeju strain KNU-1905 (GenBank accession number: MN399380) was isolated and maintained in our laboratory (Jang et al., 2020). The viruses were independently propagated in LLC-PK1 cells, as described previously (Jang et al., 2020). Individual viral stocks were prepared from each fifth-passage cell
culture (LOM-P5 and KNU-1905-P5) and used as the challenge virus in this study.

2.2 | Pig infection experiments

Ten 4-week-old crossbred pigs (Great Yorkshire × Dutch Landrace) were obtained from a commercial farrow-to-finish farm with good health status and no previous CSFV outbreak or vaccination history in Jeju Province and were tested to confirm that they were not infected with CSFV. All animals were also determined to be negative for porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus 2 (PCV2) by virus-specific RT-PCR, as described previously (Jang et al., 2021; Park et al., 2020). Pigs were randomly assigned to three experimental groups: LOM-derivative Jeju isolate KNU-1905-inoculated group 1 (n = 4), LOM vaccine-inoculated group 2 (n = 4), and sham-inoculated control group 3 (n = 2). After 5-day acclimatization, the pigs in groups 1 and 2 were challenged intranasally (1 ml per nostril) with a 2-ml dose of LOM vaccine or KNU-1905 virus at a 50% tissue culture infectious dose per millilitre (TCID50/ml) of 10^4.0, respectively. The pigs from group 3 were sham-inoculated with cell culture media as a placebo. Following inoculation, all animals were monitored daily for clinical signs throughout the experiments. Rectal temperature and body weight were recorded at 0, 5, 7, 14, 21, and 28 days post-inoculation (DPI). Blood was taken from pigs in all groups at 0, 5, 7, 10, 14, 21, and 28 DPI, and serum samples were centrifuged. Nasal and faecal samples from pigs in all groups were also collected with 16-inch cotton-tipped swabs at the same interval and were prepared as 10% (wt/vol) suspensions, as described previously (Jang et al., 2021; Lee et al., 2015). All pigs from the virus-infected and sham-infected groups were euthanized and necropsied at 28 DPI. Various organ specimens (submandibular lymph node, mesenteric lymph node, inguinal lymph node, tonsil, lung, liver, spleen, kidney, duodenum, jejunum, ileum, and colon) were collected at necropsy and prepared as 10% (wt/vol) homogenates, as described previously (Lee et al., 2015).

2.3 | Clinical examinations

A clinical significance score (CSS) was determined using the previously established scoring system (Mittelholzer et al., 2000) with minor modifications to define the virulence of CSFV strains under identical experimental conditions. The determination of CSS values was based on seven CSF-relevant clinical manifestations, including lethargy (i.e., anorexia or depression), fever (pyrexia), skin haemorrhage (or cyanosis), conjunctivitis, respiratory symptoms (e.g., dyspnoea, sneezing, and nasal discharge), diarrhoea (or constipation), and neurological symptoms (e.g., tremors and ataxia). Each clinical presentation was judged as 1 point, and scores from each manifestation were added up, resulting in the total CSS of individual animals ranging from 0 to 7.

2.4 | Quantitative real-time RT-PCR

RNA isolation from serum, nasal, faecal, and tissue 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 Nanotech), following the manufacturer’s recommendations. CSFV 5′-UTR-based quantitative real-time RT-PCR (qRT-PCR) was performed using a VDx CSFq RT-PCR kit (Median Diagnostics, Chuncheon, South Korea), in accordance with the manufacturer’s instructions. The reaction was performed using a Thermal Cycler Dice Real-Time System (Takara, Otsu, Japan) according to the manufacturer’s protocols under the following conditions: 1 cycle of 50°C for 30 min, 1 cycle of 95°C for 15 min, and 42 cycles of 95°C for 10 s and 60°C for 1 min. The results were analyzed using an automatic baseline, as described previously (Lee et al., 2017; Sagong & Lee, 2011). A CSFV strain (LOM vaccine) with a known infectivity titre was 10-fold serially diluted to generate a standard curve for each PCR plate. The virus concentrations (genomic copies/ml) in the samples were calculated based on this standard curve. The mean cycle threshold (Ct) values were calculated based on PCR-positive samples, and the mean virus titres were calculated based on all pigs within the group.

2.5 | CSFV serology

CSFV E2-specific antibodies in serum samples collected from pigs experimentally inoculated with each virus or sham-inoculated were detected by the commercially available CSFV antibody B-ELISA kit (BioNote, Hwaseong, South Korea) according to 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 antibodies.

2.6 | Statistical analysis

All values are expressed as mean ± standard deviation of the mean difference (SDM). Statistical analyses were conducted using the GraphPad Prism 7 software package (GraphPad Software, San Diego, CA, USA). p-Values below 0.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Pathogenicity of the LOM vaccine and LOM-derived field isolate KNU-1905 strains in pigs

To assess the potential for reversion to or increase in virulence of the commercial LOM vaccine in target animals, the pathogenicity of the LOM and its field derivative KNU-1905 strains were characterized in
pigs. Ten pigs, divided into three groups of four animals each, were challenged intranasally with KNU-1905 (group 1) or LOM (group 2), and the remaining two pigs in a control group were sham inoculated with cell culture media (group 3). Clinical signs were recorded daily before and after the challenge for the duration of the study. During acclimation, all animals were active and showed no clinical manifestations. Following the challenge, none of the control pigs in group 3 developed clinical abnormalities associated with CSFV infection. Although two or three of the pigs infected with the LOM strain showed mild respiratory signs, including nasal discharge or sneezing, at 5–7 DPI (mean CSS of 0.5–0.75), all the animals in group 2 maintained a good health condition without remarked clinical presentation, similar to the control group (Figure 1a). By contrast, KNU-1905-challenged pigs (group 1) exhibited multiple clinical signs accompanied by lethargy, conjunctivitis, nasal discharge, and diarrhoea at 5–21 DPI and had significant CSS values (mean CSS of 0.75–2.50) compared to the LOM-inoculated pigs (group 2). In addition, the virus-infected pigs in groups 1 and 2 underwent neither CSF-specific nervous symptoms nor death.

None of the animals in all groups had a high fever (>41°C) throughout the experiment. Overall, the virus-infected groups 1 and 2 had a febrile response similar to the sham-infected control group and within the normal range, despite slightly higher mean rectal temperatures in these groups than in group 3 at 5 and 7 DPI (Figure 1b). All pigs were weighed at a 7-day interval, and the average body weight in each group was plotted at the indicated time points (Figure 1c). There was no noteworthy difference between the average weight gains of the LOM-inoculated and sham-inoculated groups at the indicated period; the pigs in groups 2 and 3 gained average weights of 11.29 and 10.65 kg, respectively, during the observation period (28 days). By contrast, as shown in Figure 1c, pigs in group 1 infected with KNU-1905 gained significantly less body weight than those in groups 2 and 3, achieving an average weight of 8.47 kg throughout the experimental period.

The serum samples collected from the pigs were used for the detection of CSFV RNA (viremia) and seroconversion. CSFV RNA was not detected by qRT-PCR in any of the sham-infected pigs (group 3), and the pigs remained CSFV-negative throughout the study (Figure 2a). In group 2, viral RNA was detected from the sera of two animals (2/4) inoculated with the LOM vaccine strain (Table 1); one pig was intermittently viraemic at 7 and 14 DPI with a viral RNA load of $10^{0.75}$ and $10^{2.22}$ copies/ml, respectively, and another pig was transiently viraemic at 10 DPI with a viral RNA load of $10^{3.56}$ copies/ml (Figure 2a). However, CSFV RNA was detected in the KNU-1905 strain-infected pigs (4/4) from five DPI and continuously present in their sera until 21 DPI, except for one that was viraemic up to 14 DPI (Table 1). The mean viral RNA loads in these pigs ranged from $10^{3.60}$ to $10^{7.62}$ copies/ml (at 21 and 10 DPI, respectively), values greater than in the LOM-inoculated pigs (Figure 2a). Detection of the presence of CSFV E2-specific antibodies by ELISA showed that the placebo-infected control pigs remained seronegative to CSFV throughout the trial. Interestingly, only two pigs (2/4) in the group inoculated with the LOM vaccine strain seroconverted by 21 DPI, whereas the remaining animals were seronegative until the end of the experiment. Conversely, all pigs infected with KNU-1905 in group 1 seroconverted by 21 DPI (seroconversion occurred in two pigs by 14 DPI) (Figure 2b). The antibody titres presented as percent inhibition values for the KNU-1905-infected group continued to rise gradually and were higher than those for the LOM-infected group.

All animals were euthanized and necropsied at the end of the study for post-mortem assessments to evaluate the presence of pathological symptoms in different organs and tissues. No lesions were observed in the organs collected from the LOM-inoculated pigs in group 2.
FIGURE 2  Viremia and seroconversion in pigs from three experimental groups: KNU-1905-inoculated group 1 (G1), LOM vaccine-inoculated group 2 (G2), and sham-inoculated control group 3 (G3). (a) Classical swine fever virus (CSFV) antigen levels of pigs. Quantification of CSFV genomic RNA in serum samples at each time point was determined using real-time RT-PCR analysis. The virus titres were expressed as genomic copies/ml. (b) CSFV E2-specific antibody response of pigs as measured by ELISA. Samples are considered positive for antibodies to CSFV if the percent inhibition value is equal to or greater than 40 indicated as the dashed line. Error bars represent the SDM. p-Values were calculated by comparing results from the KNU-1905-inoculated group 1 and LOM-inoculated group 2 using GraphPad Prism software. *p < 0.05; **p < 0.001

TABLE 1  Detection of classical swine fever virus (CSFV) RNA in pigs inoculated with the LOM vaccine or LOM-derived Jeju isolate

| Inoculum strain | No. of pigs | Sample | No. of CSFV positive pigs/No. of pigs tested |
|-----------------|-------------|--------|---------------------------------------------|
|                 |             |        | 0   | 5  | 7   | 10  | 14  | 21  | 28  |
| Group 1         |             |        |     |    |     |     |     |     |     |
| KNU-1905        | 4           | Serum  | 0/4 | 4/4| 4/4 | 4/4 | 4/4 | 3/4 | 0/4 |
|                 |             | Nasal  | 0/4 | 3/4| 4/4 | 4/4 | 4/4 | 1/4 | 0/4 |
|                 |             | Faecal | 0/4 | 2/4| 3/4 | 4/4 | 3/4 | 0/4 | 0/4 |
| Group 2         |             | Serum  | 0/4 | 0/4| 1/4 | 1/4 | 1/4 | 0/4 | 0/4 |
| LOM             | 4           | Nasal  | 0/4 | 0/4| 0/4 | 1/4 | 0/4 | 0/4 | 0/4 |
|                 |             | Faecal | 0/4 | 0/4| 0/4 | 0/4 | 0/4 | 0/4 | 0/4 |
| Group 3         |             | Serum  | 0/2 | 0/2| 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| Sham            | 2           | Nasal  | 0/2 | 0/2| 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |
|                 |             | Faecal | 0/2 | 0/2| 0/2 | 0/2 | 0/2 | 0/2 | 0/2 |

*Days post-inoculation.

which were macroscopically comparable to the sham-infected control group (Figure 3, middle and bottom panels). However, from a macroscopic pathology perspective, all the pigs infected with the LOM-derived KNU-1905 strain (group 1) presented mild-to-moderate CSF-specific pathological changes, particularly in immune organs, including enlargement and haemorrhage of the lymph nodes and haemorrhagic infarction around the edge of the spleen (Figure 3, top panels).

3.2  CSFV shedding and tissue distribution in pigs infected with the LOM vaccine or KNU-1905 strain

Nasal and rectal swabs were collected at the indicated time points and subjected to qRT-PCR to quantify viral RNA for viral shedding analysis. CSFV RNA was not detected in the nasal fluid and faeces of any negative-control pigs tested throughout the experimental period. Similarly, except for one pig (1/4) with viral shedding in the nasal fluid at 10 DPI, most animals inoculated with the LOM vaccine strain in group 2 had no detectable CSFV-shedding in the nasal fluid and faeces during the study (Table 1). However, in the KNU-1905-infected group, CSFV RNA was identified in 3/4 nasal secretions at 5 DPI, 4/4 at 7–14 DPI, and 1/4 at 21 DPI (Table 1), with a mean viral shedding of 10^{0.91–10^{5.06}} genomic copies/ml during 5–21 DPI (Figure 4a). Viral RNA was also detected in 2/4 faecal samples at 5 DPI, 3/4 at 7 DPI, 4/4 at 10 DPI, and 3/4 at 14 DPI, with a mean viral shedding of 10^{0.89–10^{4.71}} genomic copies/ml during 5–14 DPI (Figure 4b).

In addition, each tissue sample collected from the pigs euthanatized at 28 DPI was tested for the presence of CSFV RNA by qRT-PCR. As expected, there was no detectable CSFV RNA in organs from sham-infected pigs in group 3, whereas viral RNA was present only in tonsil collected from one pig infected with LOM in group 2 (Figure 4c). However, the KNU-1905-infected pigs had considerably high viral loads in all tissue samples tested, including lymph nodes, tonsil, lung, liver, spleen, kidney, and intestines. In particular, CSFV RNA was detectable
in 100% (4/4) of lymph node (mesenteric and inguinal), tonsil, lung, liver, and intestinal (duodenum, jejunum, ileum, and colon) samples taken from all of the pigs in group 1. Individual viral loads in various tissues sampled after necropsy for pigs inoculated with the LOM or KNU-1905 strain are further summarized in Table 2.

4 | DISCUSSION

Since its first reported outbreak in 1947, CSF has been regarded as one of the most devastating diseases affecting intensive pig production in South Korea. The MLV-LOM was introduced in 1974 and has since been used nationwide to combat CSF in South Korea. In 1996, the implementation of a national CSF eradication programme, including compulsory vaccination, was launched by the South Korean government, leading to a gradual reduction in the number of CSF outbreaks. As a result, the government declared in December 2001 that the country was CSF-free, and CSF vaccination was stopped (Kim et al., 2008; Wee et al., 2005). Shortly afterwards, recurrences of CSF occurred, with 13 (regional-scale) and 72 (national-scale) outbreaks reported in 2002 and 2003, respectively (Kim et al., 2008). Subsequent government policy changes have mandated vaccination and quarantine across the country since 2009 (Kim et al., 2008). However, Jeju Province was exempt because the provincial authority declared a CSF-free status and discontinued CSF vaccination in 1998 (Song et al., 2013). Although this region is considered free of the disease without vaccination, it may remain under a constant treat of the reintroduction if CSF-vulnerable pigs are exposed to the virus or even to the vaccine. This risk has become a reality, leading to various degrees of CSF resurgence in unvaccinated swine herds on Jeju Island for the past two decades through accidental exposure (or vaccination) of naïve pigs to the LOM vaccine. In particular, the MLV-LOM causing the last reemergence in 2014 continues to ravage the provincial swine industry and has continued to undergo substantial genetic variations in the field, which may contribute to reversion to an original low virulence phenotype of the commercial LOM vaccine strain (Jang et al., 2019, 2020). Therefore, we attempted to analyze the pathogenicity of the LOM-derived CSFV variant (KNU-1905) isolated in a CSF-recurrent pig farm on Jeju Island (Jang et al., 2020).

Adverse effects of the MLV-LOM have been reported in CSFV-naïve pregnant sows and immunosuppressed pigs co-infected with both PRRSV and PCV2 after LOM vaccination; the former case caused abortion (Choe et al., 2020; Lim, Jeoung, et al., 2016), while the latter was associated with a prolonged duration of viral shedding (Lim, Song, et al., 2016). Nevertheless, a recent safety study revealed that despite the presence of CSFV RNA in some blood and organ specimens, specific pathogen-free (SPF) pigs inoculated with the LOM vaccine strain remained asymptomatic without CSFV RNA shedding in the stool or saliva during the experimental period (Choe et al., 2020). The present study also confirmed that LOM vaccination had no harmful influence on commercial pigs exhibiting the overall CSS, fever response, weight gain, and pathological lesions akin to unvaccinated control pigs; albeit some vaccinated pigs developed only mild and transient clinical signs. Although CSFV RNA was detected in serum and nasal samples from the same animal in group 2, the viral RNA detection outcomes in the blood, faeces, or nasal fluid from the LOM-inoculated pigs were comparable to those previously reported (Choe et al., 2020). Unlike the previous study showing viral antigen detection in multiple organs, including tonsil, lymph nodes, spleen, and ileum, the present experiment detected CSFV RNA only in tonsil from one LOM-inoculated pig, whose blood and nasal secretion also contained viral antigen. Choe et al. (2020) also reported that seroconversion occurred appreciably at 14 DPI in all animals following LOM inoculation; however, only 50% of the LOM-inoculated pigs seroconverted at 21 DPI under the current experimental condition. This dissimilarity may be explained by differences in the inoculation route of the LOM vaccine used in the experiments;
FIGURE 4  Classical swine fever virus (CSFV) RNA detection in nasal (a), faecal (b), and tissue (c) samples of pigs from three experimental groups: KNU-1905-inoculated group 1 (G1), LOM vaccine-inoculated group 2 (G2), and sham-inoculated control group 3 (G3). Virus shedding in nasal and rectal swabs and viral loads in the indicated tissues were determined using real-time RT-PCR analysis. The virus titres were expressed as genomic copies/ml. Error bars represent the SDM. p-Values were calculated by comparing results from the KNU-1905-inoculated group 1 and LOM-inoculated group 2 using GraphPad Prism software. *p < 0.05; **p < 0.001

the former study applied intramuscular (IM) inoculation that is identical to a common administration route of the MLV-LOM specified by the vaccine manufacturers, whereas the current study used intranasal (IN) inoculation, which is a natural infection and transmission route in the field. It is thus reasonable that the mucosal IN route might elicit a lower immune response in the host than the IM vaccination route.

A previous safety study also assessed the pathogenic characteristics of LOM-derived field (designated Jeju LOM) strains isolated in 2016. The consequences of experimental infection with the Jeju LOM strains were similar to those of the MLV-LOM infection, resulting in adverse effects on the fetuses of pregnant sows or no pathogenicity in SPF pigs (Choe et al., 2020). By contrast, an LOM-derived Jeju strain, KNU-1905, tested in the current study, caused clinical illness in infected young pigs, evident by notably higher CSS and less weight gain than LOM-vaccinated and control animals. Although previous work and the present findings commonly confirmed viremia in pigs during the course of infection, KNU-1905 induced a higher magnitude and longer duration viremia than the Jeju LOM viruses described by Choe et al. (2020). Similarly, the tissue distribution of CSFV RNA was comparable in each study, but the viral loads in immune organs, including lymph nodes and spleen, with macroscopic lesions determined in this study were greater than those in organs without pathological changes in the previous study. These results further suggest that CSFV titre in individual organs would be positively associated with the severity of tissue injury. More interestingly, the viral-shedding pattern observed under the current experimental condition showed considerable durations and amounts of viral shedding in nasal secretions and faeces of KNU-1905-infected pigs, in contrast to the previous data in which viral shedding in the saliva or faeces of infected SPF pigs was absent (Choe et al., 2020). These clinically altered outcomes might be due to a discrepancy between the pig breeds (i.e., CSFV-naïve conventional versus SPF pigs) used in individual studies. Moreover, the virus strain used cannot be excluded because the LOM-derived field strain (KNU-1905) possesses unique genotypic features, including the INDEL and point mutations in the 3′-UTR or/and at other hotspots, which were absent in the Jeju LOM strains. In particular, genetic drift, including the INDEL and substitutions in the 3′-UTR, of the LOM field isolates might be associated with a reversion to the primary pathogenicity of LOM (Jang et al., 2020). Despite its unimportance for viral replication, the 3′-UTR in the CSFV plays an important role in viral pathogenesis (Li et al., 2014). Furthermore, the polyuridine insertion was reported in the 3′-UTR of several attenuated vaccine or low virulence strains of CSFV, indicating its function in virulence (Coronado et al., 2017; Fan et al., 2008; Wang et al., 2019; Wu et al., 2001). Thus, gain-of-function research using reverse genetics is needed to investigate whether clinical and pathogenic characteristics of KNU-1905 described in this study stem from its genetic evolution in hotspots, including the 3′-UTR, or are identical to those of the LOM itself.

CSFV infection results in a failure in the immune system, such as lymphopenia, accompanied by a so-called cytokine storm due to aberrant upregulation of inflammatory cytokines and chemokines, which is incompetent to control disease progression (Ganges et al., 2020). Although we did not measure leukocyte and cytokine concentrations in this study, SPF pigs infected with the Jeju LOM strains have temporarily shown lymphopenia with elevated interleukin-10 levels related to general immunosuppression (Choe et al., 2020). This situation may be connected to secondary bacterial infection in the LOM-affected pig farms, which can aggravate damage in the field. Another concern could be a practical co-infection scenario of the LOM virus with...
TABLE 2  Tissue distribution of classical swine fever virus (CSFV) RNA in pigs inoculated with the LOM vaccine or LOM-derived Jeju isolate

| Inoculum strain | No. of pigs | SL | ML | IL | To | Lu | Li | Sp | Ki | Du | Je | Il | Co |
|-----------------|-------------|----|----|----|----|----|----|----|----|----|----|----|----|
| Group 1         |             |    |    |    |    |    |    |    |    |    |    |    |    |
| KNU-1905        | 1           | 4.2| 1.5| 3.4| 5.5| 5.7| 3.9| 4.6| -  | 4.6| 4.4| 5.5| 6.1|
|                 | 2           | 0  | 3.3| 3.8| 4.2| 5.1| 3.6| -  | 4.6| 5.2| 4.7| 5.7|    |
|                 | 3           | 3.5| 4.3| 3.7| 4.7| 2.9| 1.3| 3.9| -  | 4.8| 4.8| 5.1| 5.1|
|                 | 4           | 3.1| 3.4| 2.3| 3.0| 4.4| 2.5| 3.7| 2.4| 2.3| 1.7| 0.5| 2.3|
| Group 2         |             |    |    |    |    |    |    |    |    |    |    |    |    |
| LOM             | 1           | -  | -  | -  | -  | 4.0| -  | -  | -  | -  | -  | -  | -  |
|                 | 2           | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|                 | 3           | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|                 | 4           | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Group 3         |             |    |    |    |    |    |    |    |    |    |    |    |    |
| Sham            | 1           | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
|                 | 2           | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |

Abbreviations: Co, colon; Du, duodenum; IL, inguinal lymph node; Il, ileum; Je, jejunum; Ki, kidney; Lu, lung; Li, liver; ML, mesenteric lymph node; SL, submandibular lymph node; Sp, spleen; To, tonsil.

Immunosuppressive pathogens, such as PRRSV and PCV2, despite infection with multiple viruses (MLV-LOM, PRRSV, and PCV2) shown to be irrelevant to any adverse effects in pigs (Lim, Jeoung, et al., 2016). However, the MLV-LOM can remain in the immunosuppressed pigs for a relatively long time, amplifying the risk of virus shedding and transmission (Lim, Jeoung, et al., 2016). Furthermore, due to the continuing circulation of various PRRSV and PCV2 strains across the province, the viruses are endemic in Jeju swine populations (Jang et al., 2021). Considering these circumstances, the possibility that co-infection of LOM virus with PRRSV or PCV2 or both may occur can increase and, consequently, may lead to serious clinical outcomes in the field at any time. More importantly, the pigs infected with the LOM-derived KNU-1905 strain could shed large amounts of the virus in their nasal fluids and faeces for more than a week after infection, which serve as a critical source for horizontal transmission. Indeed, most contemporary CSFV detection and isolation cases in Jeju herds have been identified on farms with recurrent LOM infections (Jang et al., 2020). In addition, we detected a high viral load in the tonsils of all the pigs infected with KNU-1905 or even in that of the LOM-inoculated pig at 28 DPI. This result indicates that the tonsil is a persistent infection site for the LOM strain, which may be relevant to viral shedding in oral fluids. Given these conditions, animals that have been LOM-infected but survived can remain asymptomatic or mild-symptomatic and become PI pigs that can exude an infective quantity of the virus in their saliva, nasal secretions, and faeces and act as dangerous virus reservoirs to spread the virus via pig-to-pig and farm-to-farm transmissions. Therefore, the diagnosis and subsequent pre-emptive stamping out of PI shedders from affected farms must be proactively practiced in parallel with the efforts to regain the former CSFV-free status on Jeju Island.

The present study provide evidence that the LOM-derived field virus is a pathogenic revertant of the MLV-LOM, likely resulting from phenotypic changes involved in viral fitness due to substantial genetic changes acquired during field adaptation (since 2014). It is presumed that the LOM variants are capable of establishing persistent or chronic infection under the endemic disease circumstance, without including severe clinical manifestations that would allow easy detection and elimination in field farms. In conclusion, the LOM-derived CSFV appears to evolve genetically and clinically in Jeju herds, leading to its phenotype shift from the vaccine itself to the disease-causing virus. To this end, continuous monitoring and surveillance of the LOM virus are indispensable practice to clarify the characteristics of the LOM variants in the field, and customized control strategies based on individual farm circumstances, including biosecurity practices, vaccination, PI pig removal, and sow replacement (if possible), should be established and implemented to control CSF on this LOM-contaminated island.

ACKNOWLEDGEMENTS
This research was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Animal Disease Management Technology Development Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (119081-5).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS
Formal analysis, investigation, methodology, software, visualization, and writing—original draft: Guehwon Jang. Conceptualization, investigation, resources, and writing—original draft: Joo-Ah Kim. Investigation and resources: Changnam Park. Resources: Won-Myoung Kang and Kyungsu Yang. Conceptualization, data curation, funding acquisition, supervision, validation, and writing—review and editing: Changhee Lee.

ETHICS STATEMENT
The authors confirm that the ethical policies of the journals, as noted on the journal’s author guideline page, have been adhere to. The
animal experiments described here were carried out in accordance with the guidelines established by the Institutional Animal Care and Use Committee.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are included in this published article.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/vms.903.

ORCID
Guehwan Jang https://orcid.org/0000-0003-3108-8087
Changhee Lee https://orcid.org/0000-0002-5930-5461

REFERENCES
Bohórquez, J. A., Muñoz-González, S., Pérez-Simó, M., Muñoz, I., Rosell, R., Coronado, L., Domingo, M., & Ganges, L. (2020). Foetal immune response activation and high replication rate during generation of classical swine fever congenital infection. Pathogens, 9, 285.
Blome, S., Staubach, C., Henke, J., Carlson, J., & Beer, M. (2017). Classical swine fever—an updated review. Viruses, 9, 86.
Choe, S., Kim, J. H., Kim, K. S., Song, S., Cha, R. M., Kang, W. C., Kim, H. J., Park, G. N., Shin, J., Jo, H. N., Cho, I. S., Hyun, B. H., Park, B. K., & An, D. J. (2020). Adverse effects of classical swine fever virus LOM vaccine and Jeju LOM strains in pregnant sows and specific pathogen-free pigs. Pathogens, 9, 18.
Choe, S., Kim, J. H., Kim, K. S., Song, S., Kang, W. C., Kim, H. J., Park, G. N., Cha, R. M., Cho, I. S., Hyun, B. H., Park, B. K., & An, D. J. (2019). Impact of a live attenuated classical swine fever virus introduced to Jeju Island, a CSF-free area. Pathogens, 8, 251.
Coronado, L., Lingier, M., Muñoz-González, S., Postel, A., Perez, L. J., Perez-Simó, M., Perea, C. L., Frias, M. T., Rosell, R., Grundhoff, A., Indenbirken, D., Alawi, M., Fischer, N., Becher, P., Ruggli, N., & Ganges, L. (2017). Novel poly-uridine insertion in the 3′ UTR and E2 amino acid substitutions in a low virulent classical swine fever virus. Veterinary Microbiology, 201, 103–112.
Fan, Y., Zhao, Q., Zhao, Y., Wang, Q., Ning, Y., & Zhang, Z. (2008). Complete genome sequence of attenuated low-temperature Thiverval strain of classical swine fever virus. Virus Genes, 36, 531–538.
Frey, H. R., Liess, B., Richter-Reichhelm, H. B., von Benten, K., & Trautwein, G. (1980). Experimental transplacental transmission of hog cholera virus in pigs. I. Virological and serological studies. Zentralbl Veterinärmed B, 27, 154–164.
Ganges, L., Crooke, H. R., Bohórquez, J. A., Postel, A., Sakoda, Y., Becher, P., & Ruggli, N. (2020). Classical swine fever virus: The past, present and future. Virus Research, 289, 181518.
Hermanns, W., Trautwein, G., Meyer, H., & Liess, B. (1981). Experimental transplacental transmission of hog cholera virus in pigs. V. Immunopathological findings in newborn pigs. Zentralbl Veterinärmed B, 28, 669–683.
Jang, G., Kim, J. A., Kang, W. M., Yang, H. S., Park, C., Jeong, K., Moon, S. U., Park, C. K., Lyoo, Y. S., & Lee, C. (2019). Endemic outbreaks due to the re-emergence of classical swine fever after accidental introduction of modified live LOM vaccine on Jeju Island, South Korea. Transboundary and Emerging Diseases, 66, 634–639.
Jang, G., Kim, J. A., Yoo, H., Kang, Y., Yang, H. S., Park, C., Jeong, K., Park, C. K., Lyoo, Y. S., & Lee, C. (2020). Genomic characterization of classical swine fever virus LOM variants with 3′-UTR INDELs from pigs on Jeju Island, South Korea. Archives of Virology, 165, 1691–1696.
Jang, G., Yoo, H., Kim, Y., Yang, K., & Lee, C. (2021). Genetic and phylogenetic analysis of porcine circovirus type 2 on Jeju Island, South Korea, 2019–2020: Evidence of a novel intergenotypic recombinant. Archives of Virology, 166, 1093–1102.
Je, S. H., Kwon, T., Yoo, S. J., Lee, D. U., Lee, S., Rich, J. A., & Lyoo, Y. S. (2018). Classical swine fever outbreak after modified live LOM strain vaccination in naive pigs, South Korea. Emerging Infectious Diseases, 24, 798–800.
Ji, W., Guo, Z., Ding, N. Z., & He, C. Q. (2015). Studying classical swine fever virus: Making the best of a bad virus. Virus Research, 197, 35–47.
Kaden, V., Steyer, H., Schnabel, J., & Bruer, W. (2005). Classical swine fever (CSF) in wild boar: The role of the transplacental infection in the perpetuation of CSF. Journal of Veterinary Medicine, Series B, 52, 161–164.
Kim, B., Song, J. Y., Tark, D. S., Lim, S. I., Choi, E. J., Kim, J., Park, C. K., Lee, B. Y., Wee, S. H., Bae, Y. C., Lee, O. S., Kwon, J. H., Kang, W. C., Kim, T. Y., Kim, J. H., Lee, J. H., & Kang, M. I. (2008). Feed contaminated with classical swine fever vaccine virus (LOM strain) can induce antibodies to the virus in pigs. Veterinary Record, 162, 12–17.
Lee, S., Kim, Y., & Lee, C. (2015). Isolation and characterization of a Korean porcine epidemic diarrhoea virus strain KNU-141112. Virus Research, 208, 215–224.
Lee, S., Son, K. Y., Noh, Y. H., Lee, S. C., Choi, H. W., Yoon, I. J., & Lee, C. (2017). Genetic characteristics, pathogenicity, and immunogenicity associated with cell adaptation of a virulent genotype 2b porcine epidemic diarrhoea virus. Veterinary Microbiology, 207, 248–258.
Li, C., Li, Y., Shen, L., Huang, J., Sun, Y., Luo, Y., Zhao, B., Wang, C., Yuan, J., & Qiu, H. J. (2014). The role of noncoding regions of classical swine fever virus C-strain in its adaptation to the rabbit. Virus Research, 183, 117–122.
Lim, S. I., Jeoung, H. Y., Kim, B., Song, J. Y., Kim, J., Kim, H. Y., Cho, I. S., Woo, G. H., Lee, J. B., & An, D. J. (2016). Impact of porcine reproductive and respiratory syndrome virus and porcine circovirus-2 infection on the potency of the classical swine fever vaccine (LOM strain). Veterinary Microbiology, 193, 36–41.
Lim, S. I., Song, J. Y., Kim, J., Hyun, B. H., Kim, H. Y., Cho, I. S., Kim, B., Woo, G. H., Lee, J. B., & An, D. J. (2016). Safety of classical swine fever virus vaccine strain LOM in pregnant sows and their offspring. Vaccine, 34, 2021–2026.
Meyer, H., Liess, B., Frey, H. R., Hermanns, W., & Trautwein, G. (1981). Experimental transplacental transmission of hog cholera virus in pigs. IV. Virological and serological studies in newborn piglets. Zentralbl Veterinärmed B, 28, 659–698.
Mittelholzer1, C., Moser2, C., Tratschin, J. D., & Hofmann, M. A. (2000). Analysis of classical swine fever virus replication kinetics allows differentiation of highly virulent from avirulent strains. Veterinary Microbiology, 74, 293–308.
Moennig, V., Flogel-Niesmann, G., & Greiser-Wilke, I. (2003). Clinical signs and epidemiology of classical swine fever: A review of new knowledge. The Veterinary Journal, 165, 11–20.
Park, J., Choi, S., Jeon, J. H., Lee, K. W., & Lee, C. (2020). Novel lineage 1 recombinants of porcine reproductive and respiratory syndrome virus isolated from vaccinated herds: Genome sequences and cytokine production profiles. Archives of Virology, 165, 2259–2277.
Petrov, A., Blohm, U., Beer, M., Pietschmann, J., & Blome, S. (2014). Comparative analyses of host responses upon infection with moderately virulent classical swine fever virus in domestic pigs and wild boar. Virology Journal, 11, 134.
Richter-Reichhelm, H. B., Trautwein, G., von Benten, K., Liess, B., & Frey, H. R. (1980). Experimental transplacental transmission of hog cholera virus in pigs. II. Immunopathological findings in the fetus. Zentralbl Veterinärmed B, 27, 243–252.
Sagong, M., & Lee, C. (2011). Porcine reproductive and respiratory syndrome virus nucleocapsid protein modulates interferon-β production by inhibiting IRF3 activation in immortalized porcine alveolar macrophages. Archives of Virology, 156, 2187–2195.
Smith, D. B., Meyers, G., Bukh, J., Gould, E. A., Monath, T., Muerhoff, A. S., Pletnev, A., Rico-Hesse, R., Stapleton, J. T., Simmonds, P., & Becher, P. (2017). Proposed revision to the taxonomy of the genus Pestivirus, family Flaviviridae. *Journal of General Virology*, 98, 2106–2112.

Song, J. Y., Lim, S. I., Jeoung, H. Y., Choi, E. J., Hyun, B. H., Kim, B., Kim, J., Shin, Y. K., dela Pena, R. C., Kim, J. B., Joo, H., & An, D. I. (2013). Prevalence of classical swine fever virus in domestic pigs in South Korea: 1999–2011. *Transboundary and Emerging Diseases*, 60, 546–551.

Stewart, W. C., Carbrey, E. A., & Kresse, J. I. (1973). Transplacental hog cholera infection in susceptible sows. *American Journal of Veterinary Research*, 34, 637–640.

Tautz, N., Tews, B. A., & Meyers, G. (2015). The molecular biology of pestiviruses. *Advances in Virus Research*, 93, 47–160.

Vannier, P., Plateau, E., & Tillon, J. P. (1981). Congenital tremor in pigs farrowed from sows given hog cholera virus during pregnancy. *American Journal of Veterinary Research*, 42, 135–137.

van Oirschot, J. T. (2003). Vaccinology of classical swine fever: From lab to field. *Veterinary Microbiology*, 96, 367–384.

von Benten, K., Trautwein, G., Richter-Reichhelm, H. B., Liess, B., & Frey, H. R. (1980). Experimental transplacental transmission of hog cholera virus in pigs. III. Histopathological findings in the fetus. *Zentralbl Veterinarmed B*, 27, 714–724.

Wang, M., Liniger, M., Muñoz-González, S., Bohórquez, J. A., Hinojosa, Y., Gerber, M., López-Soria, S., Rosell, R., Ruggli, N., & Ganges, L. (2019). A polyuridine insertion in the 3′ untranslated region of classical swine fever virus activates immunity and reduces viral virulence in piglets. *Journal of Virology*, 94, e01214–19.

Woo, S. H., Park, C. K., Jeong, J. M., Kim, C. H., Hwang, I. J., Kim, S. J., Yoon, H., Lee, E. S., Nam, H. M., Park, J. Y., & Moon, Q. K. (2005). Outbreaks of classical swine fever in the Republic of Korea in 2003. *Veterinary Record*, 157, 113–115.

World Animal Health Information Database (WAHIS) Interface. (2022). [https://wahis.oie.int/#/dashboards/control-measure-dashboard](https://wahis.oie.int/#/dashboards/control-measure-dashboard)

Wu, H. X., Wang, J. F., Zhang, C. Y., Fu, L. Z., Pan, Z. S., Wang, N., Zhang, P. W., & Zhao, W. G. (2001). Attenuated lapinized chinese strain of classical swine fever virus: Complete nucleotide sequence and character of 30-nonocding region. *Virus Genes*, 23, 69–76.

**How to cite this article:** Jang, G., Kim, J.-A., Park, C., Song, K., Kang, W.-M., Yang, K., & Lee, C. (2022). Pathogenicity of a novel classical swine fever LOM vaccine-derived virus isolated on Jeju Island, South Korea. *Veterinary Medicine and Science*, 8, 2434–2443. [https://doi.org/10.1002/vms3.903](https://doi.org/10.1002/vms3.903)