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Classical Swine Fever Outbreak after Modified Live LOM Strain Vaccination in Naive Pigs, South Korea

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We report classical swine fever outbreaks occurring in naive pig herds on Jeju Island, South Korea, after the introduction of the LOM vaccine strain. Two isolates from sick pigs had >99% identity with the vaccine strain. LOM strain does not appear safe; its use in the vaccine should be reconsidered.

Classical swine fever is a highly contagious disease of pigs that tremendously affects the swine industry. Although several countries have become free from classical swine fever after eradication programs, sporadic outbreaks continue to occur in most major pig-producing countries, and classical swine fever is endemic to some countries in Asia. Vaccination is regarded as one of the most effective tools to prevent and control classical swine fever. Modified live vaccines (MLVs) mainly containing C-strain have been used widely because of their safety and provide complete protection against virus challenge (1,2).

Since 1974, the LOM strain has been the MLV strain for classical swine fever in South Korea. As a result of the government's classical swine fever eradication program, Jeju Island, South Korea, became a classical swine fever virus (CSFV)-free area, and vaccination efforts ceased there in 1999 (3). Strong prohibition of live pig trade has

also contributed to the maintenance of CSFV-naive herds on Jeju Island for over a decade, although sporadic classical swine fever outbreaks have occurred in mainland South Korea, despite mandatory vaccination with the LOM strain (4). This study describes classical swine fever outbreaks in naive pig herds on Jeju Island caused by the MLV.

Since 2014, multiple classical swine fever outbreaks have occurred on Jeju Island (online Technical Appendix Figure 1, <https://wwwnc.cdc.gov/EID/article/24/4/17-1319-Techapp1.pdf>). Clinical manifestation is characterized by reproductive problems (including stillbirth and fetus mummification), lethargy, cutaneous hyperemia, and cyanosis of the ear in young pigs. Pathologic examination showed typical classical swine fever lesions (Figure). Clinical samples from 2 nonvaccinated herds in 2016 were submitted for laboratory analysis.

PCR showed that these samples were positive for CSFV. Other viral pathogens involved in abortions (e.g., porcine reproductive and respiratory syndrome virus, Aujeszky disease virus, porcine parvovirus, Japanese encephalitis virus, and encephalomyocarditis virus) were not detected in any samples; however, lymph node, tonsil, lung, and brain fetal specimens and placenta specimens from farm A and lung specimens from farm B were weakly positive for porcine circovirus type 2, which is ubiquitous in South Korea (5). At farm B, serum samples from 20% of suckling piglets and 30% of weaned pigs were positive for CSFV. Although blood samples from growing and finishing pigs were not positive for CSFV, fecal samples were positive, indicating possible horizontal transmission in the field.

LOM isolates JJ-1601 (identified in a placenta sample from farm A) and JJ-1602 (in a spleen sample from farm B) shared 99.0% nucleotide identity with each other; and JJ-1601 shared 99.1% and JJ-1602 shared 99.5% nucleotide identity with the LOM strain. However, they shared low nucleotide identity (84%) with PC11WB, a virus isolated from a wild boar in South Korea (6). Phylogenetic analysis indicated that both viruses were classified within subgroup 1.1 (online Technical Appendix Figure 2). Compared with LOM, JJ-1601 contained 5 aa and JJ-1602 10 aa substitutions in the N^{pro}-E2 region; these substitutions are not critical for acquisition of pathogenicity (online Technical Appendix Table 2) (7).

In this study, we observed residual virulence of the LOM strain in naive herds. Since CSFV vaccine was accidentally introduced onto Jeju Island in 2014, continuous LOM outbreaks have occurred (online Technical Appendix Table 3), resulting in tremendous damage to pig farms on the island. In addition, the virus has persistently circulated and caused repeated problems within the infected herds. Given that accidental vaccination was limited in 2014, the

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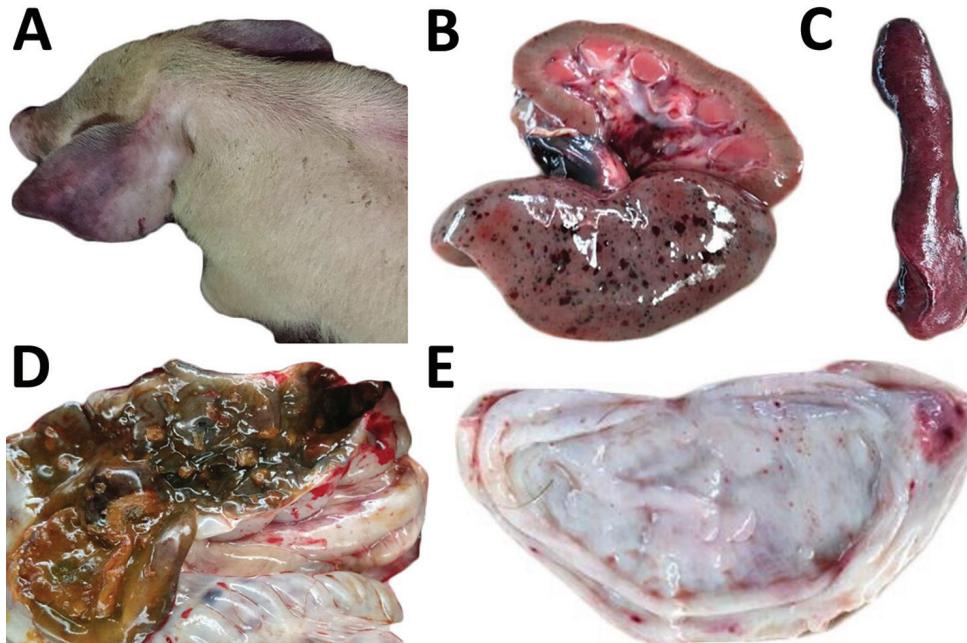


Figure. Clinical signs and pathologic lesions in naive pig infected with classical swine fever virus LOM vaccine strain, Jeju Island, South Korea. A) Cyanosis of ear. B) Hemorrhages in kidney. C) Marginal infarction of spleen. D) Button ulcers in large intestine. E) Hemorrhages in bladder.

continuous classical swine fever outbreaks, including those occurring on farms A and B, resulted from farm-to-farm transmission of the vaccine virus strain.

CSFV live vaccination should guarantee safety to host animals: safety in young pigs, safety in pregnant sows, non-transmissibility, and no reversion to virulence (8). The first problem with the LOM vaccine was that the virus spread beyond initially introduced herds. Our results indirectly support horizontal transmissibility of the LOM vaccine within the infected herd. Another factor is the capacity of LOM to cause clinical signs in both young pigs and pregnant sows. Although we could not make observations in 2014 when the vaccine strain was first introduced, viruses with 99% nucleotide identity with LOM were found in CSFV-infected pigs that exhibited clinical signs and typical pathologic lesions of classical swine fever. This virulence could have occurred because of insufficient attenuation or reversion to virulence (7,9). In a previous study, vaccination of naive pregnant sows with LOM induced stillbirth and fetus mummification (10). These results suggested that transplacental transmission and fetal death might be inherent features of the vaccine, which indicates insufficient attenuation during virus adaptation in vitro. Further study is needed to determine the basis for the virulence of the LOM strain in young pigs.

In conclusion, we must reconsider the use of LOM in the classical swine fever MLV and use a strain with experimental results satisfying safety requirements. Furthermore, control methods, including a marker vaccine for differentiating infected from vaccinated animals, are needed to stop the continuous damage and spread of LOM on Jeju Island.

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Imported Congenital Rubella Syndrome, United States, 2017

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Although transmission of rubella virus within the United States is rare, the risk for imported cases persists. We describe a rubella case in a newborn, conceived in Saudi Arabia, in Texas during 2017, highlighting the importance of active surveillance and early diagnosis of this disease.

A full-term male infant was born in Houston, Texas, USA, in early 2017 to a 29-year-old woman from Pakistan; this pregnancy was her first. Delivery was by emergent cesarean section because of fetal cardiac decelerations. The mother had lived in Saudi Arabia for 3 years before traveling to the United States in her third trimester of pregnancy.

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Early in pregnancy, while in Saudi Arabia, she had acute onset of fever and rash, then arthralgia. Symptoms resolved within a week without medical treatment. She reported prenatal care in Saudi Arabia but had no records with her; she knew of no ill contacts during pregnancy. At delivery, she had negative results for HIV and negative rapid plasma reagin but positive rubella IgG titers (>500 IU/ML; reference, positive >10 IU/mL).

The infant was transferred to The University of Texas Health Science Center (Houston, Texas, USA). Birthweight and head circumference were below the third percentile. Symptoms were respiratory distress, left leukocoria (Figure), systolic heart murmur, and depressed neonatal reflexes. Laboratory evaluation showed normal peripheral leukocyte count, hemoglobin, and liver enzymes and platelet count of 93,000/mm³. Because of suspected congenital rubella infection, we placed the patient on contact isolation. Tests for cytomegalovirus and toxoplasma were negative. We considered congenital Zika syndrome, but no testing was done. An ophthalmologic exam confirmed left cataract without retinal involvement. Chest radiograph showed clear lungs; echocardiogram showed supravulvar pulmonary stenosis and patent ductus arteriosus. Cerebrospinal spinal fluid (CSF) analysis showed normal leukocyte, glucose, and protein levels. Blood and CSF cultures were negative. On the fourth day of life, blood rubella IgG was >500 IU/mL (reference, immune ≥10 IU/mL), and blood rubella IgM was >400 AU/mL (reference range 20–24.9 AU/mL). Ultrasound examination of the brain was unremarkable. Radiographic evaluation of long bones showed diffuse coarse trabecular pattern, striated appearance of the metaphysis, and lucent linear areas. Audiometry brainstem response testing failed in the left ear. Thrombocytopenia self-resolved.

We reported the case to the local health department. We sent no clinical specimens for rubella virus detection. The patient was discharged on his tenth day of life and had uncomplicated pulmonary valvuloplasty and cataract removal surgery by 6 weeks of age. The infectious disease team last saw the patient at 2 months of age; at that time, he was developing well, but his growth was borderline. The patient and his family traveled to Pakistan 3 months after birth.

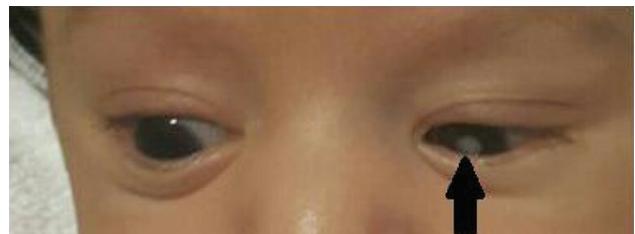


Figure. Left eye cataract (arrow) in case-patient with congenital rubella syndrome, Texas, USA, 2017. Patient was 4 weeks of age.

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Technical Appendix

Materials and Methods

A mummified fetus from farm A and a dead pig from farm B, both not vaccinated against classical swine fever virus (CSFV), were submitted (online Technical Appendix Figure 1). In addition, blood and fecal samples were also submitted from farm B for laboratory analysis. For farm A, we used spleen, lymph node, tonsil, lung, and brain samples from the mummified fetus and placenta for the detection of CSFV-specific RNA. For farm B, we used spleen, lymph node, tonsil, and lung tissue samples from a dead pig. We suspended tissue and fecal samples in Dulbecco's modified Eagle medium and homogenized. These homogenized samples, along with blood samples, were centrifuged at 3,000 rpm for 10 min. We processed the supernatant and serum fractions further.

Total RNA was extracted from 200 μ L of serum or supernatant using Qiazol Lysis Reagent (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions. With the extracted RNA, one-step reverse transcription PCR (RT-PCR) was performed using CSFV-specific primer sets with SuPrimeScript RT-PCR premix (GeNet Bio, Daejeon, South Korea) (online Technical Appendix Table 1). The structural genes of the CSFVs from both farms were amplified and sequenced using 2 sets of primers. We aligned the nucleotide sequences encoding the structural proteins with those of reference strains deposited in GenBank and constructed a phylogenetic tree using the maximum-likelihood method with 1,000 bootstrap replicates. The nucleotide sequences of the structural genes of the 2 vaccine-related strains were deposited in GenBank (accession nos. KX954607 and KX954608).

To determine if other pathogens besides CSFV that can also induce reproductive failures were present in the mummified fetus and dead pig, we performed PCR for porcine reproductive

and respiratory syndrome virus, porcine circovirus type 2, Aujeszky disease virus, porcine parvovirus, Japanese encephalitis virus, and encephalomyocarditis virus. In brief, viral DNA and RNA was extracted by using Viral Gene-Spin Kit (iNtRON Biotechnology Inc., Seongnam, South Korea) according to the manufacturer's instructions. To detect porcine reproductive and respiratory syndrome virus, we performed conventional RT-PCR using virus-specific primer sets and SuPrimeScript RT-PCR premix (GeNet Bio). To detect porcine circovirus type 2, we performed PCR assays using virus-specific primer sets and HS Prime Taq Premix (GeNet Bio) (online Technical Appendix Table 1). In addition, 2 commercially available kits were used to detect Aujeszky disease virus and porcine parvovirus (VDx Abortion MP PCR kit, MEDIAN Diagnostics Inc., Chuncheon, South Korea) and encephalomyocarditis virus and Japanese encephalitis virus (VDx Abortion MP RT-PCR II, MEDIAN Diagnostics Inc.).

Technical Appendix Table 1. Primer sequences used to detect viruses and sequence the structural genes of classical swine fever virus, Jeju Island, South Korea, 2016*

Purpose	Virus	Orientation	Sequence
Diagnosis	CSFV	Forward	5'-CTA GCC ATG CCC AYA GTA GG-3'
		Reverse	5'-CAG CTT CAR YGT TGA TTG T-3'
	PRRSV	Forward	5'-ATG GCC AGC CAG TCA ATC A-3'
		Reverse	5'-TCG CCC TAA TTG AAT AGG TG-3'
	PCV2	Forward	5'-CAC GGA TAT TGT AGT CCT GGT-3'
		Reverse	5'-CCG CAC CTT CGG ATA TAC TGT C-3'
Sequencing	CSFV-F1	Forward	5'-CTA GCC ATG CCC AYA GTA GG-3'
		Reverse	5'-CCG GGG TGC AGT TGT TWG T-3'
	CSFV-F2	Forward	5'-TGC CTA TGC CCT ATC ACC TTA-3'
		Reverse	5'-CTA ACA GTG CTA CTA CCA AG-3'

*CSFV, classical swine fever virus; PCV2, porcine circovirus type 2; PRRSV, porcine reproductive and respiratory syndrome virus.

Technical Appendix Table 2. Differences in amino acids between LOM strain and 2 viruses identified in field samples, Jeju Island, South Korea, 2016

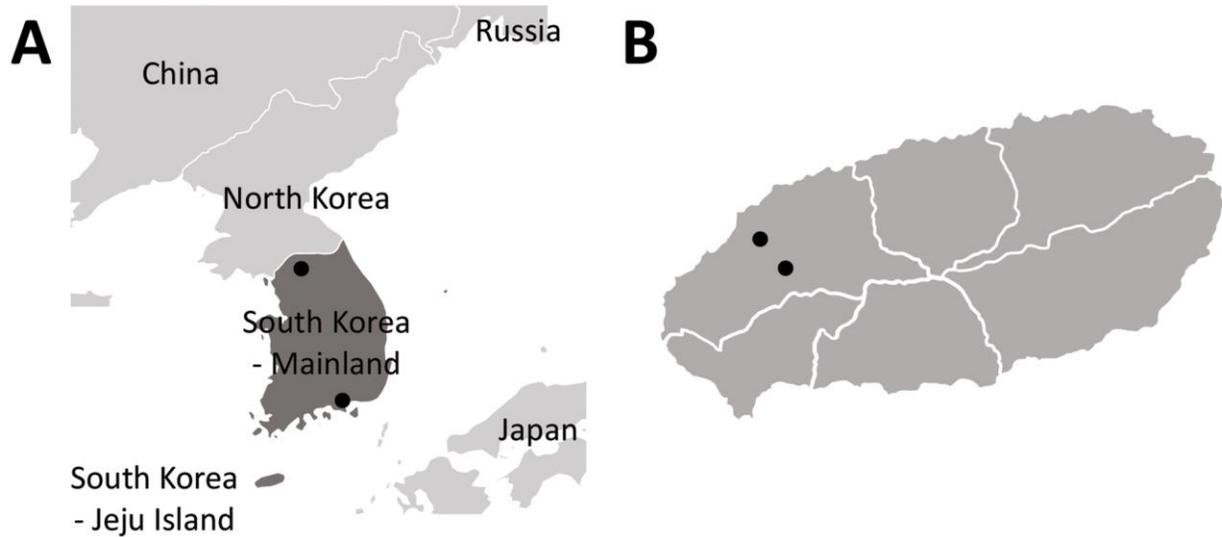
Virus	Amino acid position												Accession no.
	57	143	311	352	386*	476	539	581	584	870*	871*	933	
LOM	K	L	Y	Y	H	R	K	S	E	N	W	D	EU789580
JJ-1601	K	L	Y	Y	D	R	K	S	V	K	L	N	KX954607
JJ-1602	R	Q	H	H	D	S	R	P	E	K	L	D	KX954608

*These mutations were identical to those of vaccine strains bottled in all 5 commercial vaccines (Y.S. Lyoo, unpub. data).

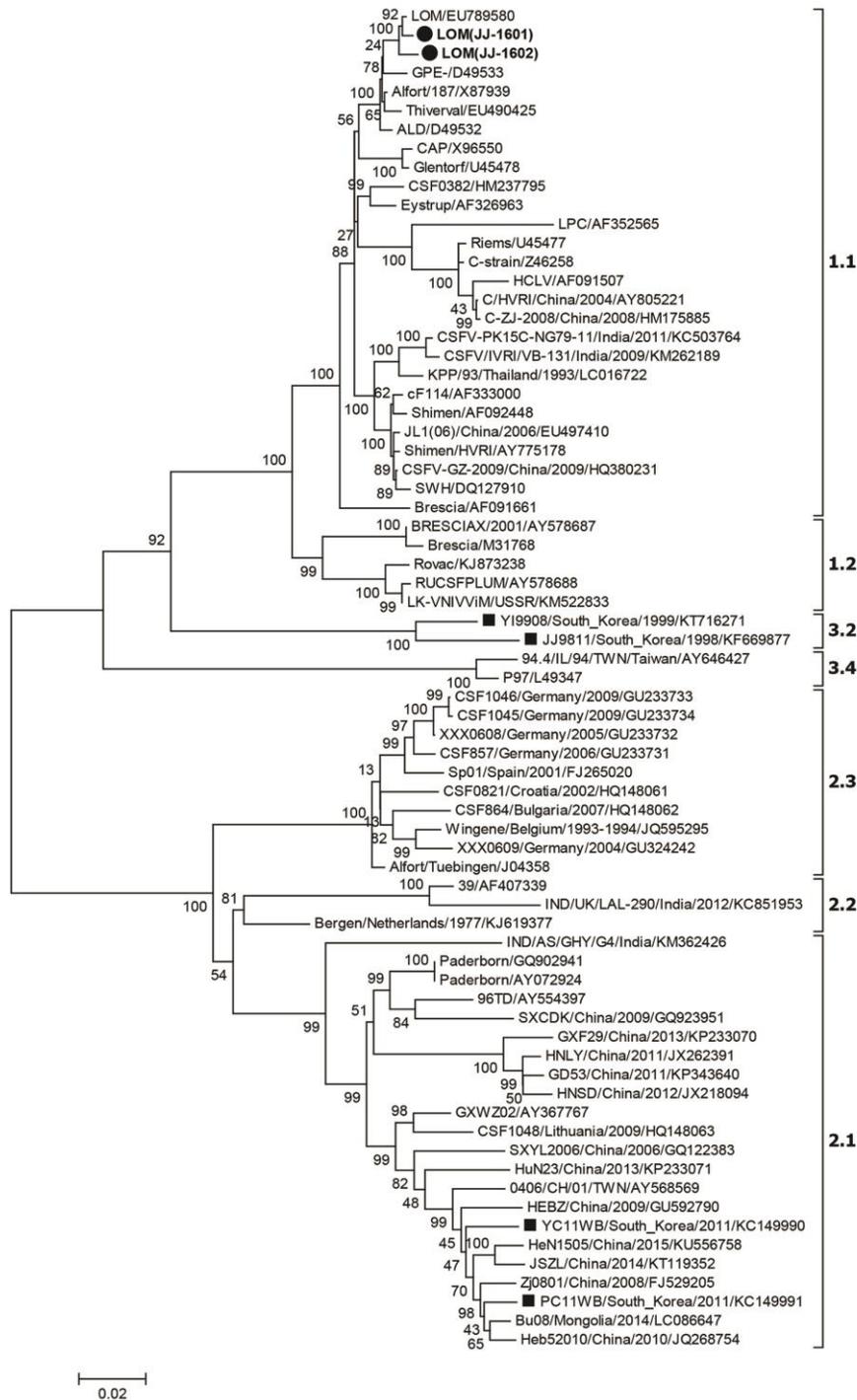
Technical Appendix Table 3. Farms with pigs infected with LOM strain, Jeju Island, South Korea, 2014–2017*

Farm type	2014	2015	2016	2017
Newly infected				
Accidental introduction			19	
LOM confirmed		1	16	20
Serology			6	6
Repeated detection from previous year			3	13
Total		20	25	39

*NA, not available.



Technical Appendix Figure 1. Geographic location of mainland and Jeju Island of South Korea. A) Jeju Island is 80-km away from the South Korea mainland at the closest. Although 2 classical swine fever outbreaks caused by field strains occurred in domestic pig farms on the South Korea mainland, Jeju Island had maintained CSFV-free status without vaccination for over a decade. Lower and upper dots indicate classical swine fever outbreaks in 2014 and 2016. B) Geographic location of farms A and B (black dots; 5 km apart) on Jeju Island.



Technical Appendix Figure 2. Phylogenetic trees based on the whole structural genes of classical swine fever virus. The tree was constructed by using the maximum-likelihood method based on the general time-reversible plus gamma distribution plus invariable site model and tested by using 1,000 bootstrapping values. Black circles indicate the 2 isolates from a classical swine fever virus outbreak caused by the vaccine strain. Black squares indicate other field isolates found in South Korea.