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Safety of classical swine fever virus vaccine strain LOM in pregnant sows and their offspring

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ABSTRACT

The present study aimed to evaluate the safety of the classical swine fever virus (CSFV) vaccine strain LOM in pregnant sows. Pregnant sows with free CSFV antibody were inoculated with a commercial LOM vaccine during early pregnancy (day 38; $n = 3$) or mid-pregnancy (days 49–59; $n = 11$). In pregnant sows vaccinated during the early stages of gestation, abortion (day 109) was observed in one case, with two stillbirths and seven mummified fetuses. The viability of live-born piglets was 34.9% in sows vaccinated during mid-pregnancy compared with 81.8% in the control group. Post-mortem examination of the organs of the sows and piglets did not reveal any pathological lesions caused by CSFV; however, CSFV RNA was detected in the organs of several vaccinated sows and their litters. The LOM strain was transmitted from sows with free CSFV antibody to their fetus, but did not appear to induce immune tolerance in the offspring from vaccinated pregnant sows. Side effects were not observed in pregnant sows with antibody to the LOM strain: transmission from sow to their litters and stillbirth or mummified fetuses. The LOM strain may induce sterile immunity and provide rapid, long-lasting, and complete protection against CSFV; however, it should be contraindicated in pregnant sows due to potential adverse effects in pregnant sows with free CSFV antibody.

1. Introduction

Q3 Classical swine fever (CSF) is a highly contagious, multisystemic, hemorrhagic, viral disease of pigs that may be manifested as an acute, subacute, chronic, or late-onset disease [1]. The causative agent, classical swine fever virus (CSFV), is a member of the genus *Pestivirus* of the family *Flaviviridae*.

Modified live vaccines against CSFV are inexpensive and may induce complete protection against virulent CSFV, and thus are widely used in CSF endemic areas such as Asia, and Central and South America [2–5].

Low virulence strains of CSFV may be transmitted via the placenta from an infected sow to the fetus, causing fetal death in the form of mummification or stillbirth, or malformations. Furthermore, transplacental transmission of low virulence CSFV results in the development of persistently infected piglets [6–9]. Transplacental transmission of CSFV was first described after vaccination of pregnant sows with insufficiently attenuated or modified vaccines, or after vaccination and challenge exposure [10,11]. Attenuated CSFV has been associated with fetal malformations and other abnormalities in immune and non-immune pregnant sows that were exposed up to 30 days after mating. The highest fetal mortality rates were found in pregnant sows infected between the 65–67th and 85th day of gestation [12]. Fetal susceptibility was lowest on the last day (day 94) of gestation and serum neutralization tests revealed that only 10% of fetuses were immunocompetent. Offspring exhibited growth retardation and most died after weaning [10]. Pigs infected *in utero* with a low virulence CSFV field strain may develop persistent infection characterized by persistent viremia, continuous virus excretion, late-onset disease, and death 2–11 months after birth, with no CSFV-specific humoral immune response [8].

Since 1974, a live attenuated vaccine (LOM strain) has been used to control CSF. In December 2001, the Korean government declared the country to be CSF-free and CSFV vaccination was banned. In 2003, CSF outbreaks were reported throughout Korea, prompting a nationwide CSF vaccination campaign. Since then, anecdotal

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evidence from pig farmers and veterinarians has suggested that the LOM vaccine strain may cause serious side effects such as abortion, stillbirth, and reproductive failure in vaccinated pregnant sows. To date, the safety of the LOM strain in susceptible pregnant sows in Korea has not yet been reported. The aim of the present study was to evaluate the safety and efficacy of the LOM vaccine strain in pregnant sows and their offspring.

2. Materials and methods

2.1. Virus, vaccine, and animals

The porcine kidney cell line PK-15 (ATCC CCL33, USA) was used for virus propagation and isolation, and for the detection of antibodies against CSFV using a virus neutralization test. Fetal calf serum was tested and found to be free of antigens and antibodies against bovine viral diarrhea virus (BVDV). The virus neutralization test was performed using the LOM vaccine strain (GenBank accession number EU789580). The LOM vaccine strain was purchased from the Korean Animal Vaccine Company and a viral titer of $10^{3.5}$ $TCID_{50}$/ml was used for animal inoculation in 16 pregnant sows from Jeju island. All 16 pregnant sows were confirmed to be free of CSFV, BVDV, and porcine reproductive and respiratory syndrome (PRRSV). Four pregnant sows with antibodies specific to the CSFV LOM vaccine were also obtained from Chungnam province.

2.2. Experimental design

Intramuscular inoculation with a commercial LOM vaccine ($10^{3.5}$ $TCID_{50}$/ml) was performed. The pregnant sows were divided into five groups. Group I sows comprised three pregnant sows without antibodies to CSFV and were inoculated at day 38 of gestation (early gestation). Group II comprised 11 pregnant sows without antibodies to CSFV and were inoculated between days 49 and 59 of gestation (mid-gestation). Group III group comprised two pregnant sows with antibodies to CSFV and were inoculated at day 38 of gestation (early gestation). Group IV group comprised two pregnant sows with antibodies to CSFV and were inoculated between days 55 and 58 of gestation (mid-gestation). Two unvaccinated pregnant sows were inoculated at day 49 of gestation (middle gestation) in group V and were used as a control group.

Animals were observed until farrowing to determine the effects on gestation, time of parturition, and number and condition of the piglets born. Three weeks following vaccination, necropsies were performed on three sows without antibodies to CSFV that were vaccinated during mid-gestation to determine whether the LOM virus was transmitted to the fetus. The remaining sows (vaccinated and control groups) were euthanized and a necropsy was performed to identify any pathological changes. Several newborn piglets were sacrificed for the detection of CSFV antigen and antibodies. Newborn piglets born under normal conditions were vaccinated with the LOM vaccine at 65 days of age to evaluate immune tolerance.

2.3. Viral and serological evaluations

Tissue samples from sacrificed sows and piglets were examined for the presence of CSFV RNA by RT-PCR. Total RNA was extracted from all samples using a micro-column-based QIAamp Viral RNA Mini Kit (Qiagen, Germany). RT-PCR was used for the quantification of a sequence in the 5′ non-coding region (5′ NCR) of CSFV [13]. Amplified products were separated by 1.5% agarose gel electrophoresis and visualized with 10 mg/ml ethidium bromide. Virus isolation was performed in PK-15 cells. Tissue samples from the tonsil, lymph nodes, lung, heart, liver, kidney, ileum, cecum, spleen, and brain were collected for viral and pathological evaluation. For virus isolation, PK-15 cells, grown to 80% confluence in 24-well plates, were inoculated with 10% α-minimum essential medium suspensions of tissue samples followed by incubation for 72 h at 37 °C. An indirect immunofluorescence assay (IFA) using a CSFV-specific E2 monoclonal antibody (Animal and Plant Quarantine Agency (QIA), South Korea) was used for the detection of the LOM strain. In all sows, CSFV-specific neutralizing antibodies were analyzed at week 3 or week 4 post-vaccination and after farrowing. For newborn piglets born under normal conditions, CSFV-specific neutralizing antibodies were analyzed 1–3 weeks after birth at 7 day intervals. For the detection of CSFV-specific neutralizing antibodies, a neutralizing peroxidase-linked assay was used in accordance with the standard manual of the Office International des Epizooties (OIE) [13,14].

2.4. Post-mortem examination

Post-mortem examination of all sows and piglets were performed to determine the presence of pathological lesions in different organs and tissues. Tissue samples (tonsil, lymph node, lung, heart, liver, kidney, ileum, cecum, spleen, and brain) were collected and fixed in 10% neutral buffered formalin, followed by sample processing procedures for microscopic evaluation using a double-blind protocol. Immunohistochemistry was performed using a CSFV-specific E2 monoclonal antibody (QIA).

2.5. Statistical analysis

Results are expressed as means ± standard error of the mean (SEM) and were analyzed using the GraphPad Prism software (version 5.0).

3. Results

3.1. Clinical findings and fetal mortality

No clinical signs of CSF were observed in the vaccinated sows or the control sows during the course of the study. Local reactions after vaccination were not found in the pregnant sows. The mean gestational period of the vaccinated sows in groups I and II was 113.7 ± 1.8 days compared with 114.0 ± 0.0 days in the control group (Table 1). There was no significant difference between the mean gestational period of the sows vaccinated in early gestation (112.7 ± 3.2 days) in group I and that of pregnant sows in group II that were vaccinated mid-gestation (114.1 ± 0.9 days). The three pregnant sows in group I farrowed a total of 14 live piglets, two stillborn piglets, and seven mummified piglets. One sow farrowed two stillborn piglets and seven mummified piglets, and the other two sows farrowed nine and five live piglets, respectively (Table 2). The eight pregnant sows vaccinated during mid-gestation (group II) farrowed 29 live piglets, 13 stillborn piglets, and 41 mummified piglets. Pregnant sows vaccinated in mid-gestation farrowed an average of 3.6 ± 3.3 live piglets, 1.6 ± 2.3 stillborn piglets, and 5.1 ± 5.8 mummified piglets per litter (Table 1). The control unvaccinated sows farrowed 27 live piglets, five stillborn piglets, and one mummified piglet with an average of 13.5 ± 4.9 live piglets, 2.5 ± 3.5 stillborn piglets, and 0.5 ± 0.7 mummified piglets per litter. Pregnant sows in group III (early gestation) and group IV (mid-gestation) with antibodies to CSFV antibody farrowed a similar number of piglets as control unvaccinated sows (58 live piglets and two stillborn piglets; Table 2). Three sows vaccinated in group II (mid-gestation) carried litters of 16, 10, and 14 piglets, respectively. Crown-rump lengths are summarized in Table 2. The crown-to-rump lengths for all of the litters were observed to be within the normal range (>21 cm) with the exception of the mummified piglets (crown-to-rump length, 12–15 cm).

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