



Experimental infections using the foot-and-mouth disease virus O/JPN/2010 in animals administered a vaccine preserved for emergency use in Japan

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ABSTRACT. The effectiveness of a vaccine preserved for emergency use in Japan was analyzed under experimental conditions using cows and pigs in order to retrospectively evaluate the effectiveness of the emergency vaccination performed in the 2010 epidemic in Japan. Cows and pigs were administered a vaccine preserved for emergency use in Japan at 3 or 30 days before virus infection (dbv) and were subsequently infected with the foot-and-mouth disease virus (FMDV) O/JPN/2010, which was isolated in the 2010 epidemic in Japan. All animals vaccinated at 30 dbv and one of three pigs vaccinated at 3 dbv showed no vesicular lesions during the experimental period. The virus titers and viral RNA loads obtained from clinical samples were lower in the vaccinated cows than in the non-vaccinated cows. The viral excretion periods were shorter in the vaccinated cows than in the non-vaccinated cows. In contrast, in the vaccinated pigs, the virus titers and viral RNA loads obtained from the samples, except for those obtained from sera, were not decreased significantly, and the viral excretion periods were not sufficiently shortened. These results suggest that the vaccine can protect against clinical signs of infection by the FMDV O/JPN/2010 in animals; however, it should be noted that in vaccinated and infected animals, especially pigs, clinical samples, such as saliva and nasal swabs, may contain excreted viruses, even if no clinical signs were exhibited.

KEY WORDS: cow, foot-and-mouth disease virus, pig, vaccine

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Foot-and-mouth disease (FMD) is the most contagious disease in cloven-hoofed animals, such as cattle, pigs, sheep and goats [7]. FMD is caused by the FMD virus (FMDV), which belongs to the genus *Aphthovirus* within the family *Picornaviridae*. Japan is currently an FMD-free country where routine vaccination is not practiced. FMD is endemic in neighboring Asian countries, such as China and South Korea [2]. Japan therefore has a significantly high risk of invasion by FMD.

FMDV is divided into seven serotypes: A, O, C, SAT1, SAT2, SAT3 and Asia1 [7]. There are also several topotypes in each serotype. The topotypes are classified based on comparisons of their VP1-coding sequences [16]. Serotype O is the most predominant among the serotypes around the world [2]. Serotype O is divided into 11 topotypes: ME-SA, SEA, Cathay, ISA-1, ISA-2, EURO-SA, WA, EA-1, EA-2, EA-3 and EA-4 [2]. The ME-SA, SEA and Cathay topotypes are endemic in Southeast Asian and East Asian countries [2].

An outbreak of FMD occurred in Japan from April to July 2010 [20]. It was the first epidemic in Japan since 2000. A total of 292 cases were confirmed during this period. Approximately 0.3 million animals including those that were affected by, came into contact with or were administered a vaccine against FMD were destroyed as a control measure.

Immediately sacrificing the affected animals and all animals that have come into contact with the affected animals and controlling the movement of livestock inside the affected regions are generally applied as control measures when an FMD outbreak occurs in FMD-free countries where vaccination is not practiced. Emergency vaccination can also be applied. Emergency vaccination was carried out as one of the control measures in the 2010 epidemic in Japan and was mentioned [20].

The selection of an adequate vaccine is necessary when implementing an emergency vaccination regimen in order for the vaccination to have maximum effectiveness. An adequate vaccine is generally selected by analyzing the serological relationship

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between a vaccine strain and the field strain involved in an outbreak. The serological relationship between the strains is analyzed by a virus neutralization test (VNT) or by ELISA using sera collected from cattle that have been administered the vaccine [3]. The vaccine is judged to be effective in controlling the field strain, if the ratio between an antibody titer of antibodies to the vaccine strain in the sera of the vaccinated cattle and an antibody titer of antibodies to the field strain in the sera of the vaccinated cattle is more than 0.3 as determined by the VNT or more than 0.4 as determined by the ELISA. The ratio is called the r_1 value.

In the 2010 epidemic in Japan, the number of reported cases decreased gradually after the emergency vaccination [20]. The vaccination was reported to be effective for reducing the intensity of the clinical signs of infection, especially in pigs [24]. The effectiveness of the vaccine preserved for emergency use in Japan, however, has never been examined using the FMDV O/JPN/2010 isolated from the 2010 epidemic in Japan in animals that have received the vaccine under any experimental conditions. The objective of this study was to retrospectively evaluate the effectiveness of the emergency vaccination administered in the 2010 epidemic in Japan by means of experimental infections. For this purpose, cows and pigs were administered the vaccine at 3 or 30 days before virus infection (dbv) and then were infected with the FMDV O/JPN/2010 in this study. Their clinical signs, virus excretion and antibody responses were compared with those of infected animals that had not received the vaccination.

MATERIALS AND METHODS

Ethics

The Animal Care and Use Committee of the National Institute of Animal Health (NIAH) approved all animal procedures prior to the initiation of this study (authorization numbers: 763, 812, 12-027, 13-024, 13-054, 14-009 and 14-080). All experimental infections using live viruses were performed in a high-containment facility at the NIAH. The high-containment facility is compliant with a containment level for group 4 pathogens described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2014 [4].

Cells and viruses

IB-RS-2 and LFBK- α , β_6 cells [17] were used in this study. The IB-RS-2 cells were maintained using Eagle's minimum essential medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 2.4 g/l of tryptose phosphate broth, 1% of 7.5% NaHCO₃, 1% of 2.92% of L-glutamine and 5% of neonatal calf serum. The LFBK- α , β_6 cells were maintained using Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM; Life Technologies, Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum (FBS). The FMDV O/JPN/2010-1/14C was isolated from the first reported case in the 2010 epidemic in Japan [10]. It was initially isolated using primary bovine kidney cells and subsequently passed two (for experimental infections) or three (for VNT) times using BHK-21 cells. The FMDV O Manisa was passed 11 times using BHK-21 cells.

Antisera

Antisera were collected from vaccinated animals as follows. Four 6-month-old Japanese Black cows and four 3-month-old Holstein cows were administered an FMDV vaccine (six 50% protection doses, serotype O, O Manisa strain, Aftpor, Merial, Lyon, France) once intramuscularly. Sera were collected from the cows at 21 days after the vaccination.

Experimental infections

Seven 3-month-old Holstein cows and six 2-month old pigs were intramuscularly administered a single dose of the above-mentioned FMDV vaccine. At 3 or 30 days post-vaccination, the vaccinated cows and pigs were inoculated with 1 ml of 10⁶ TCID₅₀ (titrated using IB-RS-2 cells) of the FMDV O/JPN/2010-1/14C by intradermal and intraoral routes, respectively, as described previously [12, 21]. Basically, the vaccines preserved for emergency use in Japan are used urgently in an outbreak. The number of days when animals are administered the vaccine and when they are subsequently infected with an FMDV is therefore expected to be varied. Therefore, in this study, the virus infection was performed at both early and late time points after the vaccination. The animals were observed for approximately 2 weeks to 1 month after the infection. The clinical signs, virus excretion and antibody responses of the infected animals that had received the vaccination were compared with those of infected animals that had not received the vaccination reported previously [11, 12, 21]. Four 3-month-old Holstein cows were additionally inoculated with 1 ml of 10⁶ TCID₅₀ of the FMDV O/JPN/2010-1/14C by the intradermal route as described previously [21] and were observed for approximately 2 weeks. Their clinical signs, virus excretion and antibody responses were also compared with those of the infected cows that had received the vaccination.

Cows 130, 141, 146, 133, 137, 142 and 149 were designated as cows 0, 1, 6, 3, 7, 2 and 9, respectively, in our previous report [12]. Cows 121 and 122 were designated as cows 1 and 2, respectively, in our previous report [21]. Pigs 141–146 were designated as pigs 1–6, respectively, in our previous report [11].

Sera were collected from the animals' cervical veins using a vacuum blood collection tube (Venoject II, Terumo Corporation, Tokyo, Japan). Saliva was collected from the animals' oral cavities using a roll-shaped synthetic saliva collector (Salivette, Sarstedt KK, Tokyo, Japan) and forceps. Nasal swabs were collected from the animals' nasal cavities using a cotton swab (Men-tip, JCB Industry Limited, Tokyo, Japan). Esophageal-pharyngeal fluid and throat swabs were collected from 3 (cows 130, 133 and 137) of the vaccinated cows and all of the vaccinated pigs using a probang cup and the cotton swab, respectively. Collection of the clinical samples except for the esophageal-pharyngeal fluid and throat swabs was performed daily until 10 days post-infection (dpi) and at 3- to 4-day intervals after that. The esophageal-pharyngeal fluid and throat swabs were collected at 0 dpi and twice a week after 18 dpi.

Virus isolation and titration

The LFBK- $\alpha_v\beta_6$ cells were prepared using the DMEM supplemented with 10% FBS in 24-well plates at 1 day before virus isolation was carried out. Ten-fold dilutions of the clinical samples were serially prepared in tubes in order to determine the virus titers in the samples. After the cells were washed once, a 150 μ l volume of each dilution of the clinical samples was transferred to 4 wells of the 24-well plates and incubated at 37°C for 1 hr. The cells were washed again and added to the DMEM supplemented with 10% FBS. The cells were incubated at 37°C for 72 hr in 5% CO₂ and observed microscopically for the appearance of a cytopathic effect (CPE). The specificity of the CPE was confirmed by a monoclonal antibody-based sandwich ELISA for FMDV detection [19]. Virus isolation and titration were performed on the day when each clinical sample was obtained in order to minimize any decrease of the virus titers during chilled storage or due to the freezing and thawing processes. Virus titers were calculated by the Reed–Muench method [22].

RNA extraction, RT-PCR and real-time RT-PCR assays

Viral RNAs were extracted from the clinical samples using the High Pure Viral RNA kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. An RT-PCR assay was performed as previously described using primers FM8 and FM9 [23]. A real-time RT-PCR assay was performed using primers and a probe described elsewhere [3].

VNT

A VNT was performed using the LFBK- $\alpha_v\beta_6$ cells as previously described [3]. The FMDV O/JPN/2010-1/14C and O Manisa were used as antigens in the VNT in order to determine the r_1 values of the O/JPN/2010-1/14C to the O Manisa and antibody responses to both the FMDVs in the vaccinated animals. The VNT was performed simultaneously using both the FMDVs in order to minimize the variation of the serum dilution among the VNT trials. The r_1 values were determined as follows: the antibody titer of an antiserum to the O/JPN/2010-1/14C was divided by the antibody titer of the same antiserum to the O Manisa [3]. The VNT was repeated at least three times. Antibody titers were expressed as the mean values of the results of the VNT trials.

Liquid phase blocking ELISA (LPBE)

LPBE (Biological Diagnostic Supplies Limited, Ayrshire, UK) was performed according to the manufacturer's instructions. The O Manisa strain was used as an antigen in the LPBE. The antibody titer was expressed as the reciprocal of the dilution of each serum sample.

RESULTS

r_1 values

Supplementary Table 1 shows the r_1 values of the antisera. The r_1 values of the O/JPN/2010 to the O Manisa were between 0.36 and >1.

Clinical signs

Supplementary Tables 2 and 3 show the dpi when vesicular development was initially observed in the infected animals with and without vaccination. The animals vaccinated at 30 dbv did not show any vesicular lesions except for vesicular lesions at the virus inoculation sites in the cows during the experimental period. The animals vaccinated at 3 dbv showed vesicular development between 1 and 6 dpi and between 4 and 11 dpi in the cows and pigs, respectively; however, there were several sites where vesicular development was not observed during the experimental period. In addition, pig 144 did not show any vesicular lesions during the experimental period. The clinical signs of the vaccinated animals were apparently mitigated. Vesicular development in the infected animals without vaccination was observed earlier and at more sites than that in the infected animals with vaccination.

Virus excretion

Figures 1–3 and Supplementary Table 4 show virus excretion from the cows with and without vaccination. In 2 of 3 cows vaccinated at 30 dbv, viruses were obtained from the serum and saliva at 2 dpi and between 1 and 4 dpi, respectively. Virus titers in the serum and saliva were 10^{1.6}, and between 10^{2.6} and 10^{4.8} TCID₅₀/ml, respectively. Viruses were not obtained from the nasal swabs of the 2 cows nor from any of the clinical samples of cow 141 during the experimental period.

In the cows vaccinated at 3 dbv, viruses were obtained from the sera, saliva and nasal swabs at 1 dpi, between 1 and 4 dpi, and between 1 and 4 dpi, respectively. The virus titers in the sera, saliva and nasal swabs were between 10^{1.6} and 10^{2.3}, between 10^{2.6} and 10^{5.3}, and between 10^{2.6} and 10^{7.8} TCID₅₀/ml, respectively. Viruses were not isolated from the esophageal-pharyngeal fluid during the experimental period (data not shown).

In the cows that had not been vaccinated, viruses were obtained from the sera, saliva and nasal swabs between 1 and 3 dpi, between 1 and 7 dpi, and between 1 and 7 dpi, respectively. The virus titers in the sera, saliva and nasal swabs were between 10^{3.1} and 10^{4.8}, between 10^{1.3} and 10^{6.6}, and between 10^{2.3} and 10^{7.5} TCID₅₀/ml, respectively.

Figures 4–6 and Supplementary Table 5 show virus excretion in the pigs with and without vaccination. In the pigs vaccinated at 30 dbv, viruses were obtained from the saliva and nasal swabs between 1 and 5 dpi and between 1 and 5 dpi, respectively. The virus titers in the saliva and nasal swabs were between 10^{2.6} and 10^{6.8} and between 10^{2.6} and 10^{5.1} TCID₅₀/ml, respectively. Viruses were not obtained from the sera during the experimental period.

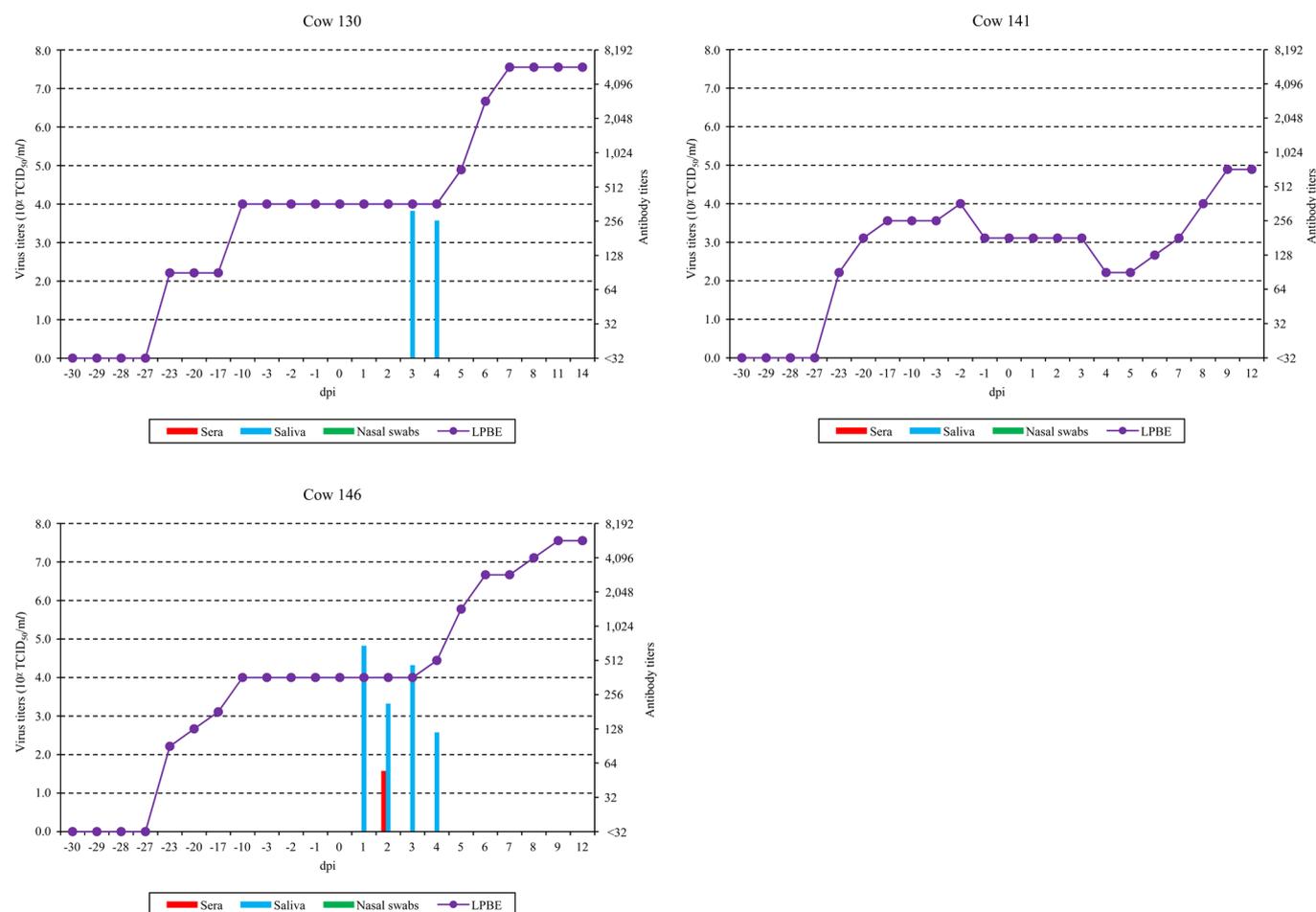


Fig. 1. Virus titers of the clinical samples collected from cows vaccinated at 30 days before the virus infection.

In 2 of 3 pigs vaccinated at 3 dbv, viruses were obtained from the sera between 6 and 7 dpi and between 3 and 5 dpi, respectively. However, viruses were not obtained from the sera of pig 144 during the experimental period. Viruses were obtained from the saliva and nasal swabs between 1 and 8 dpi and between 1 and 8 dpi, respectively. The virus titers in the sera, saliva and nasal swabs were between $10^{1.8}$ and $10^{3.8}$, between $10^{2.6}$ and $10^{7.3}$, and between $10^{2.6}$ and $10^{5.6}$ TCID₅₀/ml, respectively. Viruses were not isolated from the throat swabs during the experimental period (data not shown).

In the pigs that did not receive vaccinations, viruses were obtained from the sera, saliva and nasal swabs between 1 and 4 dpi, between 1 and 5 dpi, and between 1 and 5 dpi, respectively. Virus titers were between $10^{1.8}$ and $10^{6.8}$, between $10^{3.6}$ and $10^{7.3}$, and between $10^{3.6}$ and $10^{7.8}$ TCID₅₀/ml, respectively.

Detection and loads of viral RNAs

Supplementary Table 4 shows the viral RNA loads in the clinical samples collected from the cows with and without vaccination. In cow 130, which was vaccinated at 30 dbv, viral RNAs were detected from the sera, saliva and nasal swabs between 2 and 4 dpi, between 2 and 6 dpi, and at 2 dpi, respectively. The viral RNA loads in the clinical samples were between $10^{6.6}$ and $10^{7.5}$, between $10^{6.7}$ and $10^{10.0}$, and $10^{6.9}$ copies/ml, respectively. In the other cows, viral RNAs were detected only from the saliva between 1 and 6 dpi. The viral RNA loads in the saliva were between $10^{7.8}$ and $10^{10.9}$ copies/ml.

In the cows vaccinated at 3 dbv, viral RNAs were detected from the sera, saliva and nasal swabs between 1 and 3 dpi, between 1 and 7 dpi, and between 1 and 6 dpi, respectively. The viral RNA loads were between $10^{5.9}$ and $10^{7.8}$, between $10^{6.6}$ and $10^{10.6}$, and between $10^{6.8}$ and $10^{11.6}$ copies/ml, respectively. Viral RNAs were not detected from the esophageal-pharyngeal fluid during the experimental period (data not shown).

In the cows that were not vaccinated, viral RNAs were detected from the sera, saliva and nasal swabs between 1 and 7 dpi, between 1 and 9 dpi, and between 1 and 9 dpi, respectively. The viral RNA loads were between $10^{2.1}$ and $10^{9.4}$, between $10^{2.7}$ and $10^{10.0}$, and between $10^{5.6}$ and $10^{10.4}$ copies/ml, respectively.

Supplementary Table 5 shows the viral RNA loads in the clinical samples collected from the pigs with and without vaccination. In pig 142, which was vaccinated at 30 dbv, viral RNAs were detected from the sera, saliva and nasal swabs at 2 dpi, between 1 and 6 dpi, and between 1 and 11 dpi, respectively. The viral RNA loads in the clinical samples were $10^{6.9}$, between $10^{8.5}$ and $10^{10.9}$,

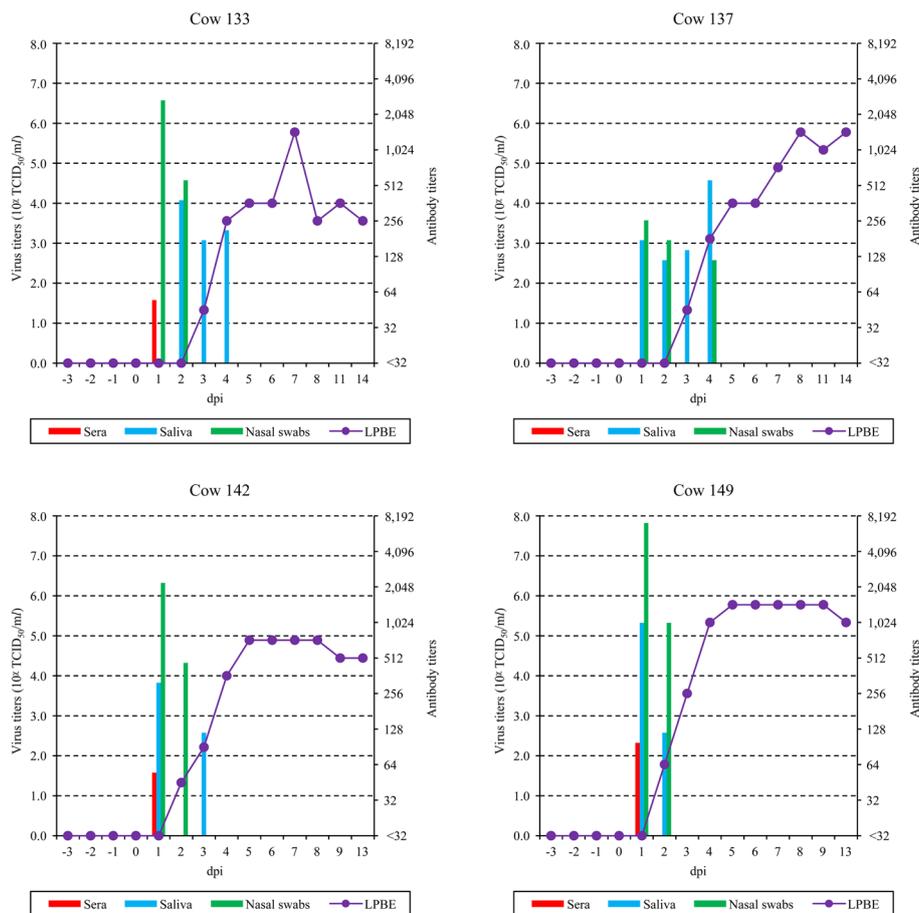


Fig. 2. Virus titers of the clinical samples collected from cows vaccinated at 3 days before the virus infection.

and between $10^{7.7}$ and $10^{10.0}$ copies/ml, respectively. In the other pigs, viral RNAs were detected only from the saliva and nasal swabs between 1 and 7 dpi and between 1 and 5 dpi, respectively. The viral RNA loads in the clinical samples were between $10^{7.2}$ and $10^{10.2}$, and between $10^{7.4}$ and $10^{9.4}$ copies/ml, respectively.

In the pigs vaccinated at 3 dbv, viral RNAs were detected from the sera, saliva and nasal swabs between 2 and 14 dpi, between 1 and 21 dpi, and between 1 and 18 dpi, respectively. The viral RNA loads were between $10^{6.3}$ and $10^{9.4}$, between $10^{7.1}$ and $10^{11.4}$, and between $10^{7.3}$ and $10^{9.6}$ copies/ml, respectively. Viral RNAs were not detected from the throat swabs during the experimental period (data not shown).

In the pigs that were not vaccinated, viral RNAs were detected from the sera, saliva and nasal swabs between 1 and 5 dpi, between 1 and 10 dpi, and between 1 and 10 dpi, respectively. The viral RNA loads in the clinical samples were between $10^{5.5}$ and $10^{10.7}$, between $10^{6.9}$ and $10^{10.1}$, and between $10^{6.4}$ and $10^{10.5}$ copies/ml, respectively.

The results of detection of the viral RNAs by the RT-PCR assay were almost comparable with those of the real-time RT-PCR assay (data not shown). Some of the results obtained by the RT-PCR assay have already been reported in previous papers [11, 12].

Antibody responses

The antibody responses in the infected animals with and without vaccination, except for cows 143, 147, 152 and 154, have been reported previously [11, 12, 21]. Briefly, in the cows vaccinated at 30 dbv, cows vaccinated at 3 dbv and cows without vaccination, antibodies were detected in the LPBE from 23 dbv, 2 dpi and 4 dpi, respectively (Figs. 1–3 and Supplementary Table 4). The antibody titers were between 90 and 5,792, between 45 and 1,448, and between 32 and 1,448, respectively.

In pigs 141, 142 and 143 vaccinated at 30 dbv, antibodies were detected in the LPBE from 3, 20 and 17 dbv; however, antibodies were not detected at 17, 10 and 2 dbv in pig 142, nor between 3 dbv and 4 dpi in pig 143, respectively (Fig. 4 and Supplementary Table 5). The antibody titers were between 45 and 2,048.

In the pigs vaccinated at 3 dbv and the pigs that were not vaccinated, antibodies were detected in the LPBE from 4 and 6 dpi, respectively (Figs. 5, 6 and Supplementary Table 5). The antibody titers were between 32 and 724, and between 45 and 724, respectively.

In contrast, antibody responses to the O/JPN/2010 and O Manisa were similar in the VNT (data not shown).

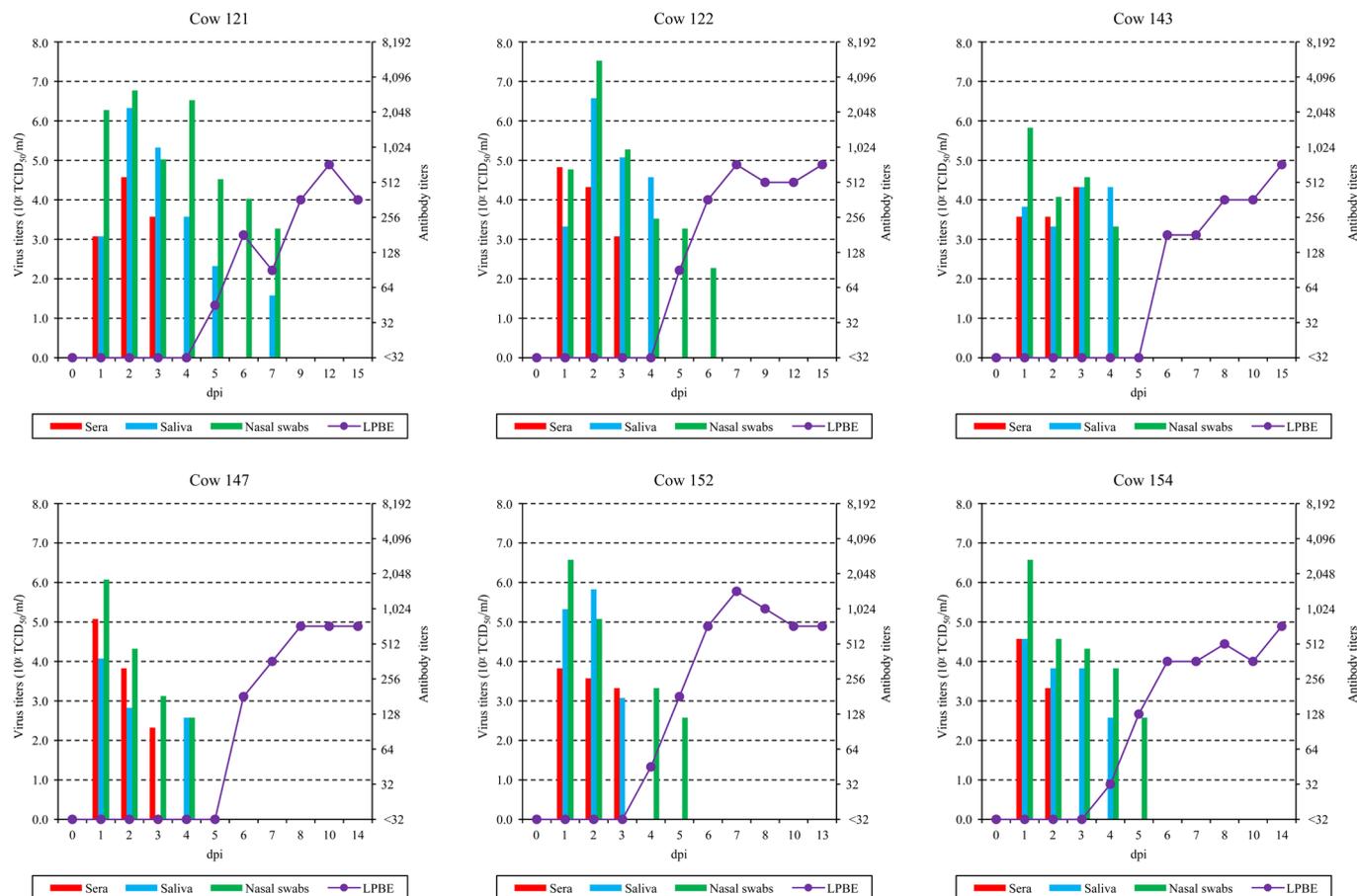


Fig. 3. Virus titers of the clinical samples collected from infected cows that did not receive the vaccination.

DISCUSSION

The effectiveness of FMD vaccines has been mainly analyzed using a homologous FMDV strain [5, 8, 9, 26]. In this study, the effectiveness of the O Manisa vaccine was analyzed using the heterologous O/JPN/2010 in order to retrospectively evaluate the effectiveness of the emergency vaccination used in the 2010 epidemic in Japan. The clinical signs of the animals vaccinated at 30 dbv were completely eliminated in this study. In addition, the clinical signs of pig 144, which was vaccinated at 3 dbv, were also completely eliminated. Similarly, the O Manisa vaccine prevented the appearance of any clinical signs in sheep and pigs infected with a heterologous FMDV isolate, which belongs to the same Mya-98 lineage of the SEA toptotype of serotype O as the O/JPN/2010 [14, 27]. Commercially available vaccines are produced using a limited number of strains [2]. Performing routine experimental infections using recent heterologous field strains on animals that have been administered vaccines preserved for emergency use is therefore crucial in order to evaluate the effectiveness of the preserved vaccines.

In general, the r_1 values of a field strain to a vaccine strain are less than 1, because the antibody titers of sera collected from vaccinated animals in response to a homologous vaccine strain are higher than those in response to a heterologous field strain [25]. However, the r_1 values of O/JPN/2010 to O Manisa were more than 1 in 4 of 8 sera used in this study (Supplementary Table 1). The reasons that the r_1 values of O/JPN/2010 to O Manisa were more than 1 in the sera are unknown; however, similar results were also reported previously [25]. The O Manisa used in this study was kindly provided by the World Reference Laboratory for FMD and was not the same virus stock as the O Manisa used by the manufacturer to produce the vaccine. For example, the passage histories of the O Manisa used in this study must be different from those of the O Manisa used for producing of the vaccine by the manufacturer, although origin of the O Manisa used in this study is the same with that of the O Manisa used for production of the vaccine by the manufacturer [personal communication]. Therefore, the reason that the r_1 values of O/JPN/2010 to O Manisa were more than 1 in the sera might be attributed to the difference in the passage histories. In addition, the O/JPN/2010 is thought to be related serologically with the O Manisa.

In this study, as mentioned above, the r_1 values were used as a measurement of the antigenic relationship between the O/JPN/2010 and O Manisa, because the objective of this study was to retrospectively evaluate the effectiveness of the emergency vaccination in the 2010 epidemic in Japan under experimental conditions. Previous studies have shown that there is a strong relationship between antibody responses and the mitigation of clinical signs in vaccinated and subsequently infected animals [13,

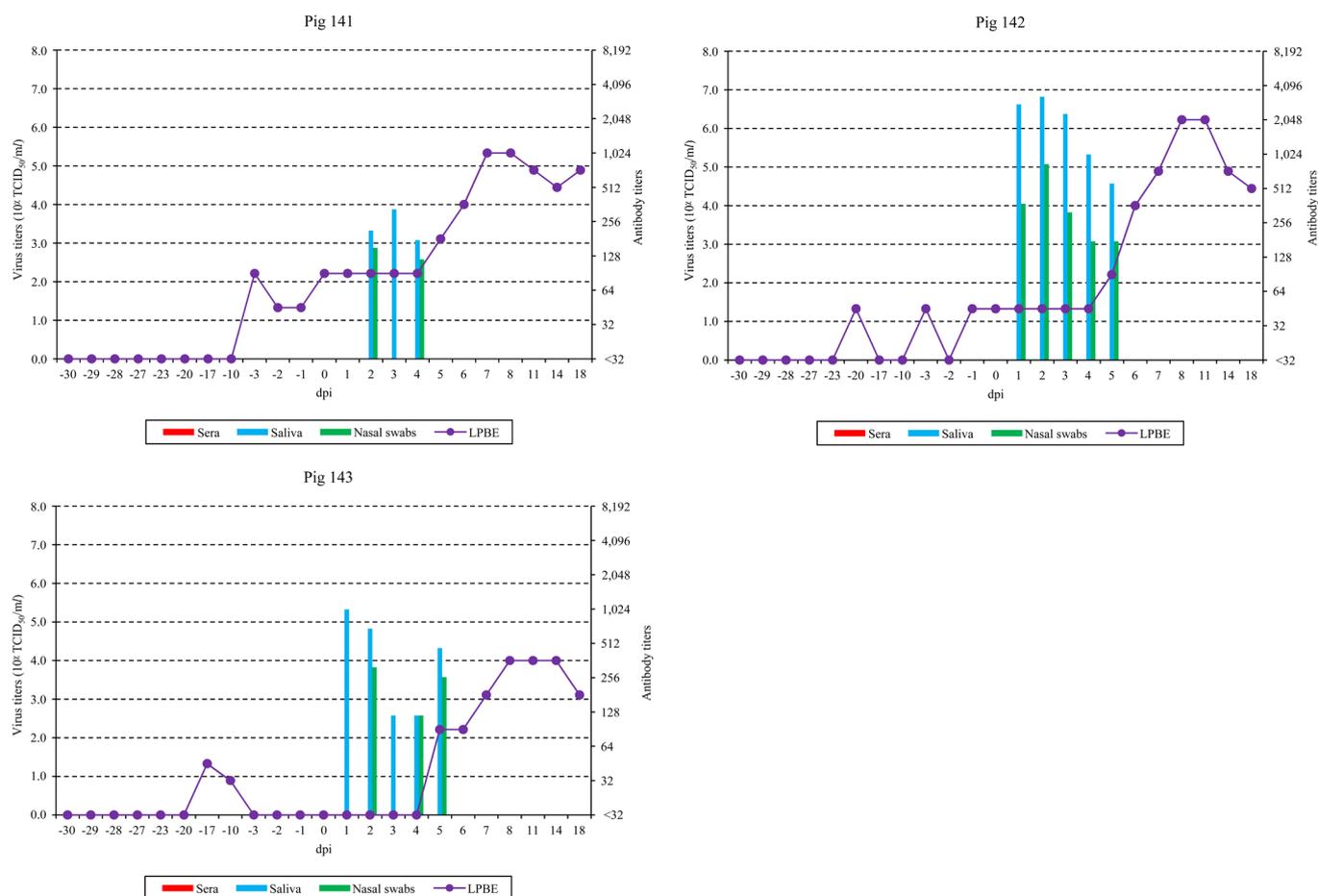


Fig. 4. Virus titers of the clinical samples collected from pigs vaccinated at 30 days before the virus infection.

18, 26]. However, the degree of the antibody responses that is necessary for such protection varies among different strains. One report argued that the use of an r_1 value to estimate the protection provided against a challenge by an FMDV is not logical because the difference in the antibody responses needed to achieve this protection is not taken into consideration [25]. Therefore, if the objective is to choose a vaccine that will best protect against infection with a field isolate, it would be better to look for a vaccine that can induce the highest antibody responses against the field virus strain instead of looking at the r_1 value of the field strain to the vaccine strain. In a recent study that analyzed the serological results of cross-protection tests, it was shown that the r_1 value of a serum sample from a vaccinated and heterologous-challenged cow did not predict protection against the challenge, but the serum titer against the challenge strain in the VNT was able to predict it [6]. The methods of choosing an appropriate vaccine for an FMDV need to be further explored as proposed in a previous report [6].

Clinical signs were completely eliminated as a result of vaccination at 30 dbv in both the cows and pigs in this study. Vesicular epithelia and fluid generally include large amounts of viruses [1]. Mitigating the clinical signs is therefore valuable in preventing the spread of viruses in the environment. In contrast, it should be noted that virus excretion to salivary and nasal discharge continued without any clinical signs in the animals that received the vaccination, especially the pigs. In addition, viruses and viral RNAs were obtained for a longer period in the pigs that received the vaccine at 3 dbv than in the infected pigs that did not receive the vaccination. Although horizontal transmission occurred from animals inoculated with 10^4 and 10^5 TCID₅₀ of an FMDV to susceptible contact animals, horizontal transmission did not occur from animals inoculated with the higher dose of 10^6 TCID₅₀ of the FMDV to susceptible contact animals [15]. When animals are inoculated with a high dose of an FMDV, immune responses and subsequent virus clearances may occur rapidly. Similarly, immune responses and virus clearance may have occurred more rapidly in the infected pigs that did not receive the vaccination than in the pigs administered the vaccine at 3 dbv in this study. Vaccinated and immediately infected pigs are likely to be more significant sources of infection for other animals than infected pigs that did not receive the vaccination. Pigs that are vaccinated as a control measure in an FMD outbreak therefore would need to be sacrificed immediately.

In conclusion, the following was confirmed in this study: (1) the clinical signs were completely eliminated in the animals vaccinated at 30 dbv; (2) the periods of virus excretion into the clinical samples were decreased in the cows by the vaccination; and (3) the periods of virus excretion into the clinical samples except for the sera were not decreased significantly in the pigs by the vaccination. The mitigation of clinical signs is important to prevent environmental contamination with viruses, because vesicular

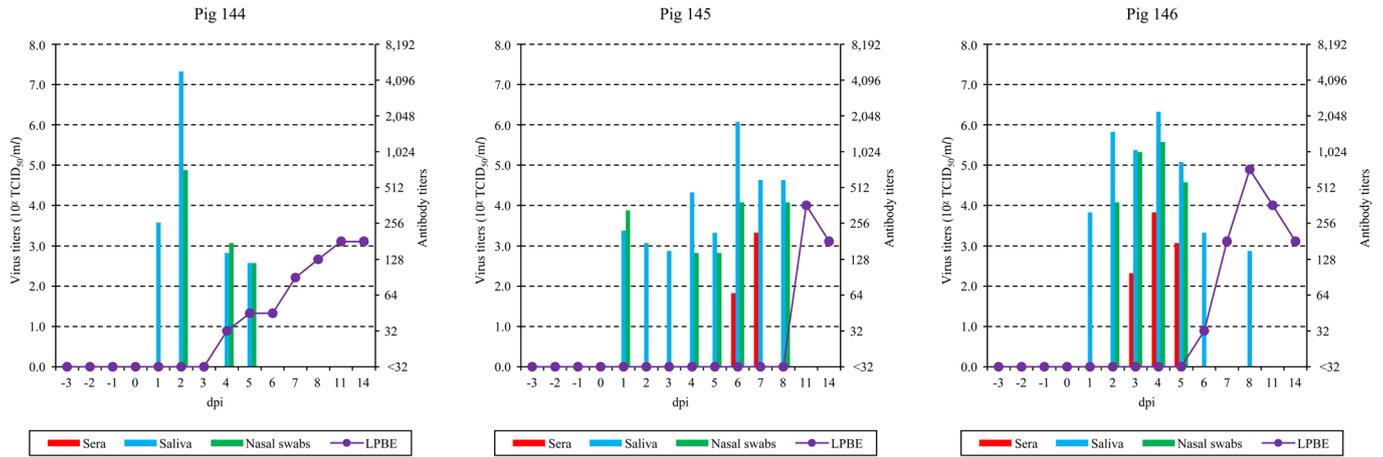


Fig. 5. Virus titers of the clinical samples collected from pigs vaccinated at 3 days before the virus infection.

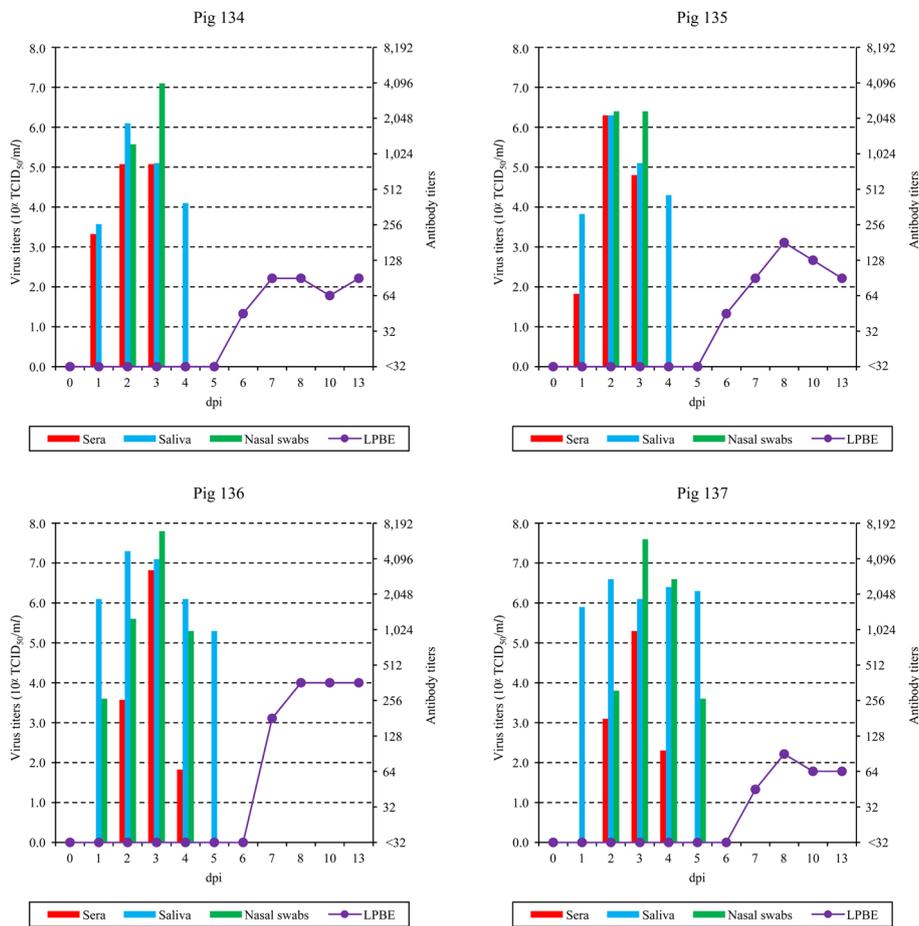


Fig. 6. Virus titers of the clinical samples collected from the infected pigs that did not receive the vaccination.

lesions generally have a large amount of viruses; however, it should be noted that vaccinated and subsequently infected animals, especially pigs, may excrete viruses into clinical samples without exhibiting any clinical signs.

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