An Experimental Infection in Pigs Using a Foot-and-Mouth Disease Virus Isolated from the 2010 Epidemic in Japan

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ABSTRACT. In this study, we carried out an experimental infection in pigs using a foot-and-mouth disease virus isolated from the 2010 epidemic in Japan to analyze the clinical manifestation, antibody response and virus shedding patterns in pigs. We found that the virus was virulent in pigs, producing a synchronous disease in the inoculated pigs and efficient spread to direct contact pigs. These results are useful for epidemiologically investigating the 2010 epidemic in Japan and improving the measures for controlling possible future FMD outbreaks in Japan or elsewhere.

KEY WORDS: experimental infection, foot-and-mouth disease virus, Japan, swine.

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. The causative agent, FMD virus (FMDV), is divided into 7 serotypes, O, A, C, Asia1 and SAT1–3. Serotype O, which is the most predominant, has been divided into several topotypes by molecular analysis of a structural protein VP1-coding sequence [5]. Recent FMD outbreaks caused by the Southeast Asia (SEA) topotype of the serotype O FMDV have been confirmed in many Asian countries [8]. An outbreak also occurred in Japan between April and July 2010 and was the first outbreak since 2000. Two hundred and ninety-two cases were confirmed in Japan during this period. Approximately 0.3 million heads of affected, contact and vaccinated animals were destroyed as a control measure.

In general, FMDV-infected pigs exhale more virus particles than infected cattle [1]. Therefore, an FMD outbreak may spread rapidly if it occurs at a pig farm in an FMD-free country where vaccination is not practiced [3, 4]. In the FMD outbreak in Japan, the confirmed cases dramatically increased after an FMD outbreak occurred at a pig farm. In this study, we carried out an experimental infection in pigs using an FMDV isolated from the 2010 epidemic in Japan to analyze the clinical manifestation, antibody response and virus shedding patterns in pigs. The results obtained provide useful information for epidemiologically investigating the 2010 epidemic in Japan and for improving measures to control a possible future FMD outbreak in Japan or elsewhere.

The FMDV used in the experimental infection was isolated from a crust specimen obtained from cow No.14 raised on a farm where the first FMD case was confirmed in 2010 (the isolate O/JPN/2010-1/14C). It was the first case confirmed by the authorities concerned, but was assumed to actually be the second case by a later epidemiological surveillance [6]. It was initially isolated by primary bovine kidney cells and confirmed as serotype O using an antigen detection ELISA kit (Institute of Animal Health, Surrey, UK). It was also confirmed to belong to the SEA topotype by a molecular analysis of the VP1-coding sequence. It was subsequently passed twice by BHK-21 cells and used in the experimental infection.

Two 2-month-old pigs (pigs 1 and 2) were inoculated intradermally with 1 ml of 10^5.3 TCID50 of the isolate at the right and front heel bulbs. The titer of the isolate was identified by the microtitration method using IB-RS-2 cells according to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2010 [10]. The day when the pigs were inoculated with the isolate was designated as 0 days postinoculation (dpi). To demonstrate the ability of direct transmission, four 2-month-old pigs (pigs 3–6) were combined with the inoculated pigs at 1 dpi and housed in the same cubicle for 11 days. The day when the pigs were placed in contact with the inoculated pigs was designated as 0 days postcontact (dpc). The clinical signs were observed daily. Sera and saliva were collected from the pigs daily. All pigs were maintained in a biosafety level 3e-approved biocontainment facility at our institute. The Animal Care and Use Committee of the National Institute of Animal Health approved all animal procedures prior to initiation of this study.

Viral RNA was extracted from the clinical specimens using a High Pure Viral RNA Kit (Roche Diagnostics, Basel, Switzerland). The FMDV-specific gene was detected from the extracted RNAs by a RT-PCR according to Sakamoto et al. [9] and a real-time RT-PCR according to the above-mentioned OIE Manual. RNAs extracted from tenfold dilution series of the previously titrated 1/14C were tested simultaneously with those of the clinical specimens in order to estimate the virus loads. Virus isolation from the clinical specimens was carried out using IB-RS-2 cells according to the above-mentioned OIE Manual. Isolated virus was confirmed as serotype O using the antigen detec-
tion ELISA kit. Antibody titer was measured by a liquid-phase blocking ELISA kit (LPBE; Institute of Animal Health) and virus neutralization (VN) test according to the above-mentioned OIE Manual. Another FMDV isolate, which was isolated from a nasal swab specimen obtained from cow No.13 raised on the above-mentioned farm (the isolate O/JPN/2010-1/13), was used as a challenge virus in the VN test because it showed a cytopathic effect (CPE) more clearly in IB-RS-2 cells than the 1/14C. It was initially isolated by IB-RS-2 cells and confirmed as serotype O using the antigen detection ELISA kit. It was subsequently passed twice by IB-RS-2 cells and used in the VN test. The 1/13 was confirmed to have similar antigenicity to the 1/14C by a VN test using sera obtained from the cattle the two isolates were obtained from and the pigs used in this study. The tested sera were inactivated at 56°C for 30 min before the VN test. Fifty \( \mu l \) of them were diluted twofold to between 1/4 and 1/8192 using 2 rows of a 96-well plate per serum. The same volume of 100 TCID50 of the 1/13 was added into the wells, and the plate was incubated at 37°C for 1 hr. One hundred \( \mu l \) of IB-RS-2 cells (approximately 10^5/ml) were added into the wells, and the plate was incubated in an atmosphere of 5% CO2 at 37°C for 3 days. The appearance of CPE was observed by microscope and titers were expressed as the final dilution of serum in the serum/virus mixture where 50% of the wells were neutralized.

Vesicular development was initially observed on the snouts, lips and feet of pigs 1 and 2 between 1 and 3 dpi. They also showed depression, reduced appetite and lameness between 1 and 5 dpi. Vesicular development was initially observed on the snouts, tongue, lips and feet of pigs 3–6 between 2 and 5 dpc. They also showed depression, reduced appetite and lameness between 2 and 10 dpc.

In pigs 1 and 2, FMDV-specific genes were detected from serum specimens between 1 and 3 dpi (Table 1). Viruses were isolated from sera between 1 and 2 dpi (Table 1). The virus loads in the sera were between 10^{0.1} and 10^{4.1} TCID50/ml (Fig. 1A). The genes were detected from saliva specimens between 1 and 10 dpi (Table 2). Viruses were isolated from saliva between 1 and 3 dpi (Table 2). The loads in the saliva were between 10^{0.1} and 10^{4.4} TCID50/ml (Fig. 1A). In pigs 3–6, FMDV-specific genes were detected from serum specimens between 1 and 7 dpc (Table 1). Viruses were isolated from sera between 2 and 6 dpc (Table 1). The virus loads in the sera were between 10^{–1.2} and 10^{5.0} TCID50/ml (Fig. 1A). The genes were detected from saliva specimens between 1 and 10 dpc (Table 2). Viruses were isolated from saliva between 1 and 5 dpc (Table 2). The loads in the saliva were between 10^{0.2} and 10^{4.3} TCID50/ml (Fig. 1B).

In pigs 1 and 2, antibodies were first observed at 4 dpi in LPBE (Fig. 2A). They achieved a peak at 6 dpi (pig 1) or 10 dpi (pig 2). The maximum titer was 1/362. Antibodies were first observed at 3 dpi in VN (Fig. 2B). They rose continually during the experimental period, and the maximum titer was 1/5792. In pigs 3–6, antibodies were first observed between 5 and 8 dpc in LPBE (Fig. 2A). They achieved a

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peak between 7 and 10 dpc. The maximum titer was 1/724. Antibodies were first observed at 3 dpc in VN (Fig. 2B). They rose continually during the experimental period, and the maximum titer was 1/5792.

Pacheco and Mason have evaluated the clinical manifestation, antibody response and virus shedding patterns in pigs by direct inoculations of six FMDV strains to pigs and direct contact of naïve pigs with the inoculated pigs for a limited period [7]. In their study, pigs inoculated directly with four (O/TAW/97, O/HKN/21/70, O/SKR/00 and O/UKG/35/01 strains) of the six FMDV strains showed vesicular development, viremia and antibody patterns similar to those observed in the present study. In contrast, several pigs inoculated directly with other strains (O/TAW/2/99 and O/SAR/19/00 strains) showed delayed appearance of vesicular development, viremia and antibody than the pigs inoculated directly with the four FMDV strains and the O/JPN/2010 strain. Therefore, the clinical manifestation, antibody response and virus shedding patterns of the O/JPN/2010 strain are probably similar to those of the four FMDV strains in a direct inoculation.

In general, pigs have low susceptibility to FMDV infection by an aerosol route [1]. The four FMDV strains (O/SKR/00, O/UKG/35/01, O/TAW/2/99 and O/SAR/19/00 strains) were poorly transmitted by direct contact of naïve pigs with the inoculated pigs. The pigs placed in contact with the O/SAR/19/00-inoculated pigs did not become infected with the virus during the experimental period [7]. In this study, we were able to confirm that the O/JPN/2010 strain was transmitted from the inoculated pigs to the contact pigs. Because we wanted to carry out an experimental infection in pigs that replicated the situation similar to that in the field, naïve pigs were placed in contact with inoculated pigs and housed in the same cubicle during the experimental period. Pacheco and Mason evaluated the transmissibility of the FMDV strains by direct contact of.
naïve pigs with inoculated pigs for a limited period. Further investigations are needed to analyze the transmissibility of the O/JPN/2010 strain for a shorter period.

Although the period that viruses were detected from the sera by RT-PCR was 1–2 days longer than the period that viruses were isolated from the sera using IB-RS-2 cells, the periods were almost identical (Table 1). In contrast, the period that viruses were detected from the saliva by RT-PCR was 5–8 days longer than the period that viruses were isolated from the saliva using IB-RS-2 cells (Table 2). In our preliminary study, the RT-PCR performed in this study could detect approximately 10^2–10^3 TCID_{50}/ml of viruses, and its sensitivity was higher than that of virus isolation using IB-RS-2 cells (unpublished data). Although viruses in the sera disappeared for a short period along with the appearance of antibody, small volumes of viruses were excreted into the saliva for a relatively long period. Alexandersen et al. reported that FMDV-infected pigs excreted viruses into their saliva for a maximum of 7 days [2]. Taken together, these results suggest that an FMDV-infected pig would become a source of infection for susceptible animals and a source of contamination in the environment for a relatively long period.

In conclusion, the results of this experimental infection showed that the SEA topotype FMDV isolated from the 2010 epidemic in Japan was virulent in pigs, producing a synchronous disease in the inoculated pigs and efficient spread to direct contact pigs. We believe that these results are useful for epidemiologically investigating the 2010 epidemic in Japan and improving the measures for controlling a possible future FMD outbreak in Japan or elsewhere. In the 2010 epidemic in Japan, FMD occurred not only at pig farms but also at cattle and goat farms. In the future, we intend to carry out experimental infections in ruminants using the O/JPN/2010 strain to analyze the clinical manifestation, antibody response and virus shedding patterns in ruminants.

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