Characteristics of Foot and Mouth Disease Virus in Taiwan

Chin-Cheng HUANG, Ming-Hwa JONG, and Shih-Yuh LIN

Department of Hog Cholera, National Institute for Animal Health, Council of Agriculture, Tamsui, Taipei, Taiwan 251, R. O. C.

ABSTRACT. Since March 1997 two strains of foot and mouth disease (FMD) virus have found their way into Taiwan, causing severe outbreaks in pigs and in Chinese yellow cattle. Outbreaks occurred in March 1997 were caused by a pig-adapted virus strain (O/Taiwan/97) which did not infect other species of cloven-hoofed animals by natural route. The epidemic spread over the whole region of Taiwan within two months and the aftermath was 6,147 pig farms infected and 3,850,746 pigs destroyed. In June 1999, the second strain of FMD virus (O/Taiwan/99) was isolated from the Chinese yellow cattle in the Kinmen Prefecture and in the western part of Taiwan. By the end of 1999, Chinese yellow cattle were the only species infected and those infected cattle did not develop pathological lesions. Seroconversions of serum neutralization antibody and on non-structural protein (NSP) antibodies were the best indicators for infection in non-vaccinated herds. The infected animals, however, excreted infectious levels of virus to infect new hosts. Based on the detection of the specific antibody to FMD virus, and virus isolation from oesophageal-pharyngeal (OP) fluid samples, ten herds of Chinese yellow cattle located in Kinmen and Taiwan were declared to have been infected. During the period of January to March 2000, however, five outbreaks caused by FMD virus similar to the O/Taiwan/99 virus occurred in four prefectures of Taiwan. The infected species included goats, Chinese yellow cattle and dairy cattle. Those outbreaks have caused high mortality in goat kids under two weeks old and also developed typical clinical signs of infection in dairy cattle.

KEY WORDS: Chinese yellow cattle, foot and mouth disease virus, non-structural protein, serotype O, VP1 gene.

Foot and mouth disease in Taiwan was recorded in 1913. Papers described by previous investigators indicated that outbreaks of FMD occurred in two periods, during 1913–1916 and 1924–1929, and those infected animals were destroyed by stamping out policy. No further outbreak of FMD was reported between 1929 and 1996.

In the past three years (1997 to 2000), however, the Council of Agriculture, Taipei, Taiwan, Republic of China has continually reported FMD outbreaks to the Office International des Epizooties in Paris [1–5, 10–12]. On March 20, 1997, three outbreaks of FMD, two in the Hsinchu Prefecture and one in the Taoyuan Prefecture, were first reported to the OIE [10]. A total of 1,570 pigs were infected and of those 615 had died. Samples from the infected animals were transported to the National Institute for Animal Health (NIAH), Taiwan, for examinations. Electron microsopio observation showed that the vesicular fluids from infected pigs contained Picorna-like virus particles. Samples from infected tissues and vesicles were inoculated onto BHK-21 cells inducing cytopathic effect (CPE) in 8 hrs. Vesicular fluids and their cultures were tested by ELISA antigen detection kit for FMDV (Pirbright product), indicating that the vesicular fluids contained the serotype O FMD virus (O/Taiwan/97) and the result was confirmed by the World Reference Laboratory (WRL) for FMD at Pirbright, UK.

The DNA fragments of the O/Taiwan/97 virus were amplified from vesicular fluids or from the cultures by reverse transcriptase-polymerase chain reaction (RT-PCR) method using a set of serotype O primers (P38: 5′-GCTGCCTACCTCCTTCAAA-3′; P33: 5′-AGCTTGTTACAGGGTTTGGGC-3′) [15] corresponding to the 1D and 2B genomic regions of the FMD virus strain O1K [8]. The DNA fragments were sequenced by the direct sequencing method, using BigDyeTM Terminator Cycle Sequencing kit and ABI 377 DNA sequencer (Applied Biosystems, Inc.). The study of the VP1 sequences in the international DNA data bank (NCBI) indicated that the O/Taiwan/97 virus was closely related to the viruses seen in Russia (O/1685/Moscow/Russia/95), Hong Kong and the Philippines [9; our unpublished data]. The O/Taiwan/97 virus is a pig-adapted strain of FMD virus which does not infect cattle by natural route [6].

The outbreaks caused by the O/Taiwan/97 virus spread from the northern to the southern and the eastern part of Taiwan. By March 30, 1997, a total of 251,706 pigs were slaughtered; by April 30, an accumulation of 2,971,524 pigs; by May 30, an accumulation of 3,847,444 pigs; by June 30, an accumulation of 3,850,536 pigs; by July 31, a total of 3,850,746 pigs were destroyed. This epidemic resulted in 6,147 (24.2%) pig farms infected and 3,850,746 pigs destroyed within five months.

A national vaccination of pigs was subsequently carried out in May, 1997. During March 26 to June 21, a total of 2,176 million doses of the FMD vaccines (costing 1.74 billion USD) were imported as an emergency measure. Following the vaccination policy, the epidemic was brought under control. Since January 1998 to April 1999, only twelve sporadic outbreaks were reported [2–5], of which three were in the Yunlin Prefecture, two in Tainan, two in Kaohsiung, one in Chanhwa, one in Penghu, one in Sinchu, and one in Pingtung. The last case caused by the O/Taiwan/97 virus was reported in April, 1999.

The direct economic consequences of this epidemic were a pause of an annual export trade to Japan of 6 million pig carcasses (approximately 1.57 billion USD) and an instant drop of hog price from 4,000 – 5,000 to 1,000 – 3,000 NTD/100 kg
continuously for at least three months. This epidemic also resulted in the close of pig farms, layoff of workers, regional bankruptcy, and consumer’s rejection to pork.

On 20 May 1999, the Food and Agriculture Organization (FAO) received an official report on type O FMD outbreaks from the Bureau of Animal Production and Health in Beijing, China [7]. Two outbreaks occurred in the province of Fujian, which is geographically close to the Kinmen Prefecture (only 4 kilometers in distance), a territory of Taiwan. To find out whether the virus from China has got into Kinmen, serum and OP fluid samples were collected from four herds of the Chinese yellow cattle in Kinmen and sent to NIAH on May 28, checking for FMDV-specific antibody. Serum neutralization antibody specific to FMD virus and the antibody to non-structural proteins of FMDV (ELISA kits based on a 3ABC protein or a 3B peptide) [13, 14] were measured, showing that the serum samples from two of four farms were FMD seropositive. By the time, the cattle in Kinmen had not received FMD vaccination. Therefore, the presence of FMD specific antibody indicated that the cattle might have been infected. However, there were no clinical signs of infection. By June 12, a Picorna-like virus was isolated from BHK-21 cells inoculated with the OP fluid samples. The virus was typed with ELISA antigen detection kit (Pirbright product) at NIAH and at Pirbright, indicating that the virus was a serotype O FMD virus (O/Taiwan/99). The DNA fragment of VP1 gene was subsequently amplified from the total RNA extracted from the infected cultures by the RT-PCR method using the p33 and p38 primer set [15]. The VP1 nucleotide sequences obtained in this study (AF 167307) [manuscript in preparation] was then compared to those available VP1 sequences from the international DNA data bank (NCBI), indicating that the virus (O/Taiwan/99) was a novel virus of Taiwan with the best similarity (96-97%) to the virus strains O/ONDRI/96 (X99069) and O/Saudi Arabia/94 (AJ004660), which were responsible for the outbreaks in the India and Saudi Arabia. We have studied the growth characteristics of the virus in cell cultures and in different species of cloven-hoofed animals. In a plaque assay system, the growth of the O/Taiwan/99 virus produced small plaques than the plaques induced by the O/Taiwan/97 virus. In addition, the O/Taiwan/99 virus in one-step growth assay produced low levels of infectious virus particles than the virus levels produced by the O/Taiwan/97 virus [manuscript in preparation].

Under the field conditions, we were incapable of detecting any lesions on the infected Chinese yellow cattle, however, the infected cattle could steadily transmit infectious levels of FMDV to susceptible animals. We have experimentally studied the susceptibilities of the Chinese yellow cattle, dairy cattle, goats and pigs to the O/Taiwan/99 virus. The Chinese yellow cattle (5–6 months old) receiving a titer of $10^{7.3}$ TCID$_{50}$ of the virus did not develop the clinical signs up to 14 days after exposure but the cattle were seroconverted to positive on the 6th days. In addition, virus could be harvested from the OP fluids during the 3rd and the 5th days postinfection. When two SPF pigs were put in contact with the infected Chinese yellow cattle, vesicular lesions appeared at the snout and at the coronary band of the feet on the 8th day after exposure. Dairy cattle (5–6 months old) received a titer of $10^{7.2}$ TCID$_{50}$ of the virus, and the formation of vesicles were observed on the nose and on the skin between and adjacent to the claws of the feet on the 2nd day postinfection. The viremia phenomenon and virus excretion into the OP fluids were also detectable on the dairy cattle and pigs. These laboratory experiments supported the field observations, showing that the O/Taiwan/99 virus would not produce pathological lesions on Chinese yellow cattle but were seroconverted to positive and excreted virus to infect new hosts.

Prior to June 1999, a number of the Chinese yellow cattle in Kinmen shipped to Taiwan for slaughter. FMD virus might have been carried by the asymptomatic cattle and brought into Taiwan. National surveillance of the cloven-hoofed animals was carried out from June to August. By August 31, a total of 14,000 serum samples collected from cattle, goats, buffaloes and deers were examined for the titers of neutralization antibody and for the NSP antibodies. At that time, all of the cattle in Kinmen and most of the cattle in Taiwan were not vaccinated against FMD. Therefore, positive serum antibodies specific to the FMD virus were the best indicators for infection. The OP fluid samples collected from the seroconverted herds were tested by RT-PCR to check for viral ribonucleic acid and to perform virus isolation in cell cultures. A total of ten herds, including 663 head of the Chinese yellow cattle, located in Kinmen and on the western part of Taiwan were declared to have had FMD virus infection. The outbreaks were quickly brought under control by stamping out policy, movement restrictions, and ring vaccination surrounding the infected herds.

Although all of the cattle herds in Taiwan had been screened for FMD antibodies, five outbreaks occurred in goats, dairy cattle and the Chinese yellow cattle during the period of January to March 2000 [11, 12]. The infected cows developed typical vesicular lesions on mouth and feet. The goat kids under two weeks old showed high mortality, but the adult goats did not develop pathological lesions in the field.

Marine transportation in Taiwan is very common. Therefore, it is possible to introduce novel viruses by illegal importation of livestock. During 1997 and 1999, the invasions by two O type FMD viruses have resulted in immune agricul-

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REFERENCES