

Pathogenicity of border disease virus FNK2012-1 strain isolated from a pig in the natural host, sheep

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ABSTRACT. A first isolation of border disease virus (BDV) in Japan was from a pig on a farm without keeping any ruminants. Our previous study showed that this BDV, termed the FNK2012-1 strain, replicated inefficiently in swine-derived cells compared with those of ruminant origin. Pigs inoculated with this virus showed neither clinical symptoms nor viremia. In this study, we evaluated the pathogenicity of the FNK2012-1 strain in sheep, its natural host. The inoculated sheep showed clinical symptoms and transient viremia. Seroconversion was observed in the inoculated sheep. These results suggest that the FNK2012-1 strain was introduced from sheep and has not yet adapted to swine. Therefore, surveillance of border disease in Japan is necessary among both the swine and ruminant populations.

KEY WORDS: border disease virus, host, pathogenicity, sheep

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The genus *Pestivirus* within the family *Flaviviridae* mainly comprises bovine viral diarrhoea virus (BVDV) of cattle, classical swine fever virus (CSFV) of pigs and border disease virus (BDV) of sheep [12]. Diseases caused by these viruses have a deleterious impact on livestock economy. These pestiviruses are genetically and antigenically related to each other. Thus, polyclonal antisera against pestiviruses generally fail to distinguish among the species [6]. Classification of pestiviruses previously referred to the host species from which they were isolated as described above. So far, numerous studies have shown that pestiviruses are not highly host-specific. BVDV and BDV can cause disease in domestic and wildlife ruminants, and swine. CSFV infection is restricted to swine in nature, but CSFV can experimentally infect cattle and goats [8, 13].

The first isolation of BDV in Japan was from the swine population [4]. The ailing pigs were kept in a sow-farrow-to-finish farm with no ruminants. We recently reported the biological, genetic and antigenic characterization of one strain of BDV, termed FNK2012-1, isolated from a persistently infected pig [7]. Experimental infection of weaned piglets with the FNK2012-1 strain revealed that this isolate could not replicate efficiently. Therefore, the FNK2012-1 strain is considered avirulent in pigs.

In the present study, we assessed the pathogenicity of

the BDV FNK2012-1 strain in sheep, its natural host. To this end, four 10- to 16-week-old Suffolk crossbred lambs (JAPAN LAMB, Hiroshima, Japan) were intranasally inoculated with 10^{6.0} 50% tissue culture infectious dose (TCID₅₀) of the FNK2012-1 strain per head from the virus seed stock [7]. All lambs were deemed free of the main ovine diseases by a regular passive survey and tested negative for BDV, BVDV, CSFV and their antibodies by a neutralization test. The rectal temperatures and clinical symptoms of this flock were monitored daily over the period of this experiment. Two inoculated lambs (#1 and #2) were euthanized on day 5 post-inoculation (pi), and the tissue samples were aseptically collected from their brains, tonsils, tracheas, lungs, spleens, adrenal glands, kidneys, mesenteric lymph nodes and colons for virus isolation. Nasal swabs and blood were collected on days 0, 1, 3 and 5 pi also for virus isolation. The remaining two infected lambs (#3 and #4) were kept for 33 days and then euthanized. All collected tissue samples were homogenized in Eagle's minimum essential medium (MEM) to obtain a 10% suspension. Swabs were collected from lambs #3 and #4 on days 0, 1, 3, 5, 7, 10, 15, 22, 25, 28 and 33 pi for virus isolation. Blood was collected on days 0, 1, 5, 7, 10, 15, 22, 25, 28 and 33 pi to test for FNK2012-1 neutralization activity and virus isolation. Total leucocytes were counted using a pochH-100iV Diff apparatus (Sysmex, Kobe, Japan) on days 0, 1, 3, 5, 7, 10, 22, 25, 28 and 33 pi. Virus isolation was performed by inoculation of samples from tissues, swabs and blood into monolayer of the swine kidney cell SK-L [10] on 6-well plates. Virus titration was conducted for virus antigen-positive samples. Their titers are expressed as TCID₅₀ per ml (blood and swab) or gram (tissue) following the formula of Reed and Muench [9]. Real-time PCR was conducted for the virus antigen-positive samples followed by the protocol of La Rocca and Sandvik [5]. Cells were

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Table 1. Virus recovery from tissues of lambs inoculated intranasally with FNK2012-1 strain on day 5 post-inoculation

Ovine ID	Virus recovery from tissue (\log_{10} TCID ₅₀ /g)									
	Brain	Tonsil	Trachea	Lung	Spleen	Kidney	Adrenal gland	Mesenteric lymph node	Colon	
1	-	5.8	-	+	+	-	-	3.0	-	-
2	-	2.8	-	-	-	-	-	-	-	-

-: Not isolated, +: Isolated in a 6-well plate and was lower detection limit of TCID₅₀ ($10^{1.8}$ TCID₅₀/ml) in a 96-well plate.

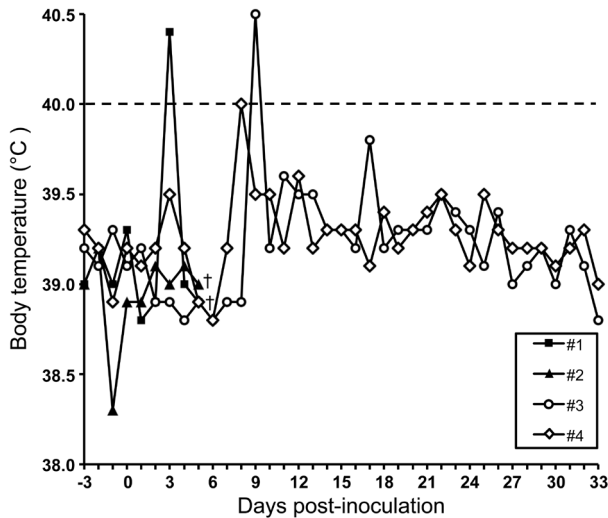


Fig. 1. Body temperature of lambs before and after inoculation with border disease virus FNK2012-1 strain. Four lambs were intranasally inoculated with the FNK2012-1 strain. Rectal temperature was monitored daily beginning 3 days before inoculation. A body temperature above 40.0°C was considered fever. "+" indicates the lambs were euthanized at that time.

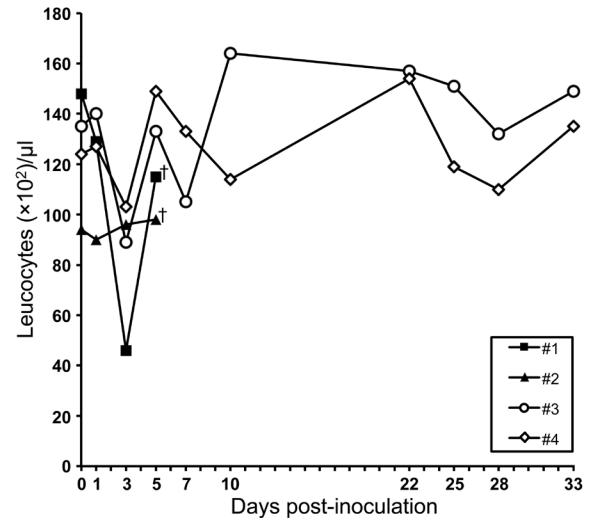


Fig. 2. Leucocyte counts in lambs inoculated with the FNK2012-1 strain. Blood was collected on days 0, 1, 3, 5, 7, 10, 22, 25, 28 and 33 post-inoculation (pi), and the leucocyte counts were determined for each time point. "+" indicates the lambs were euthanized at that time.

stained using the immunoperoxidase technique with anti-viral protein NS3 monoclonal antibody 46/1, as previously described [3, 11]. This animal experiment was conducted in a biosafety level-2 facility at the Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan. The institutional animal care and use committee of the Graduate School of Veterinary Medicine authorized this animal experiment (approval number: 14-0069). The experiment was performed according to the guidelines of this committee.

Infection of lambs with the FNK2012-1 strain resulted in mild clinical symptoms in 3 out of the 4 lambs: nasal discharge (#2 and #3) and depression (#3 and #4). Fever was observed in 3 of the 4 lambs within 10 days pi (Fig. 1). Transient leucopenia was also observed in the 3 lambs (Fig. 2). Hemorrhage was observed in the mesenteric lymph nodes of 1 lamb (#2) on day 5 pi. Virus was isolated from the tonsils of lamb #2 and from the tonsils, lungs, spleen and mesenteric lymph nodes of lamb #1 on day 5 pi (Table 1). The tonsil samples from lamb #1 and #2 were positive for virus genome by real-time PCR (data not shown). Transient viremia was observed in 2 of the 4 lambs within 5 days pi (Table 2). In contrast, virus was not recovered from any nasal swabs

obtained from the inoculated lambs (data not shown). These results indicate that the FNK2012-1 strain is pathogenic for sheep.

A neutralization test used to detect anti-BDV, BVDV and CSFV antibodies was performed as previously described [7]. Neutralizing antibody titer is expressed as the reciprocal of the highest serum dilution that showed complete viral neutralization. Seroconversion against the FNK2012-1 strain was detected in the remaining 2 lambs (#3 and #4) after day 22 pi (Fig. 3), even though virus was not isolated from lamb #4.

In a previous study, a BDV 87/6 strain isolated from diseased pigs caused mild clinical symptoms and viremia in weaned piglets, whereas infection was subclinical for sheep. In contrast, the ovine isolate 137/4 caused disorders in sheep, however, its pathogenicity in pigs was lower than that of the 87/6 strain [1]. The FNK2012-1 strain replicated inefficiently in swine cells, compared with the ruminant cells. The weaned piglets inoculated with the FNK2012-1 strain showed neither clinical symptoms nor viremia. A small amount of virus was recovered from only mesenteric lymph nodes of an infected pig [7]. The present study showed that infection of lambs with the FNK2012-1 strain resulted in fever and transit viremia. A large amount of virus was recov-

Table 2. Virus recovery from blood of lambs inoculated intranasally with FNK2012-1 strain

Ovine ID	Virus recovery from blood on days pi (\log_{10} TCID ₅₀ /ml)										
	0	1	3	5	7	10	15	22	25	28	33
1	-	-	+	≤1.3	NT	NT	NT	NT	NT	NT	NT
2	-	-	-	-	NT	NT	NT	NT	NT	NT	NT
3	-	-	+	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-

–: Not isolated, +: Isolated in a 6-well plate and was lower detection limit of TCID₅₀ ($10^{0.8}$ TCID₅₀/ml) in a 96-well plate, NT: Not tested.

ered from tonsils, and virus was also recovered from other tissues. All these data strongly suggest that the FNK2012-1 strain has kept the pathogenicity in sheep and has not yet adapted to pigs and was recently introduced to swine from the ovine population. However, there is limited information available how this BDV strain was introduced into the swine population. Only one epidemiological survey of BD among only the ovine population in Japan has been reported so far [2]. Surveillance of classical swine fever (CSF) and bovine viral diarrhoea (BVD) is routinely conducted by veterinary service in Japan. It is, therefore, important to perform surveillance of both virus isolation and antibody detection of BDV among ruminant and swine populations for further analysis, leading to control of not only BD but also CSF and BVD in Japan.

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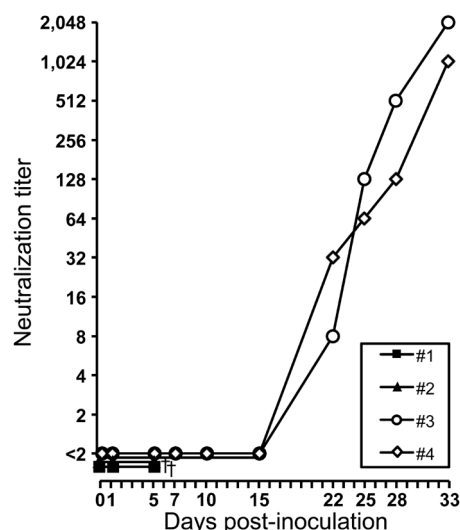


Fig. 3. Virus neutralization titers against the FNK2012-1 strain from inoculated lambs. Sera collected on days 0, 1, 5, 7, 10, 15, 22, 25, 28 and 33 pi were used to determine the virus neutralization titer. “+” indicates the lambs were euthanized at that time.

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