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Short Communication

First isolation of border disease virus in Japan is from a pig farm with no ruminants☆

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ABSTRACT

The first isolation of border disease virus (BDV) in Japan was from a pig farm of the farrow-to-finishing type that kept no small ruminants or cattle. The infection was detected in the course of sero-surveillance for classical swine fever virus (CSFV) in Japan. The infected pigs had no clinical symptoms of CSFV or other disease; nevertheless, a high sero-positive rate of 58.5% was identified. A persistently infected pig with the BDV was found and suspected to be the cause of sero-prevalence in the farm. The isolated BDV was genetically close to BDV strains from New Zealand, but there was no epidemiological evidence concerning the route(s) of the invasion into the farm.

Pestivirus is a genus within the family *Flaviviridae*, comprising four main species of viruses causing disease in livestock: the classical swine fever (CSF) virus of pigs, bovine viral diarrhea (BVD) virus 1 and 2 of cattle, and border disease (BD) virus of sheep and goats (Simmonds et al., 2011). Other *Pestivirus* species are recognized in wild ruminants such as reindeer, giraffe and pronghorn antelope. Another pestivirus was found in South America in fetal bovine sera used for a cell culture supplement (Schirrmeier et al., 2004). These pestiviruses are antigenically close to each other, and cross-reactions are often found by serological tests for antibodies (Wensvoort et al., 1989; Becher et al., 2003).

It was believed that the natural hosts of pestiviruses—with the exception of CSF virus (CSFV)—were ruminants. However, the host of a new pestivirus, Bungowannah virus, found recently in Australia (which is free from CSFV) was identified as pigs, which were showing clinical signs of myocarditis (Kirkland et al., 2007).

In Japan, the eradication of CSF was achieved by 2007 through the nationwide ban of vaccinations for CSF and intensive sero-surveillance (Atagi, 2007). Monitoring of antibodies to CSFV is ongoing in order to confirm the CSF-free status using an enzyme-linked immunosorbent assay (ELISA) and neutralizing test (NT) for CSFV. On rare occasions, these tests found a few sero-positive reactions

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Table 1
Results of the clinical inspection, ELISA sero-survey and RT-PCR in 41 pigs selected at random from the Kanto-region farm.

Pig house	Numbers of pigs tested	First clinical test			Pig ID[b]	ELISA[c]		Sero-conversion (pre/post)
		Body temperature (°C) [min–max (Av.)]	WBC ($\times 10^2/\mu L$) [min–max (Av.)]	Sero-positive rate[a] (ratio)		Pre[d]	Post[d]	
Weaned	5	37.0–39.0 (37.8)	175–374 (294.6)	4/5 (80%)	W71	–	–	No
Growing	5	39.4–40.2 (39.9)	222–351 (293.0)	3/5 (60%)	W72	–	–	No
					W73	–	–	No
Finishing	20	36.0–40.2 (38.5)	163–409 (244.3)	15/20 (75%)	W68	–	–	No
					W69	–	–	No
					W70	–	–	No
					W74	–	–	No
					W75	–	–	No
Sow	11	38.0–39.0 (37.8)	150–200 (175.4)	4/5 (80%)	No.60	–	–	No
		ND	ND	3/6 (50%)	No.16	–	+	Yes
					No.19	–	–	No
					No.73	–	+	Yes

[a] The numerators indicate the numbers of pigs evaluated as sero-positive involving a doubt-positive by the ELISA of presera.
[b] This is an indication of sero-negative pigs by the ELISA of presera in all pigs tested.
[c] The S/P ratio as a sample-to-positive ratio was evaluated as "≥0.1" is positive (+), "<0.05" is negative (−), and "≥0.05 and <0.1" is doubt-positive (±).
[d] "Pre" and "post" indicate the first and second blood samples at an interval of 5 days, respectively.

in healthy pigs, resulting from a cross-reaction against BVD virus (BVDV), as confirmed by an antibody-differentiable NT.

In February 2012, pigs that were sero-positive to the E2 antigen of CSFV were detected by an ELISA (JNC Corp., Tokyo) at a pig farm of the farrow-to-finishing type, where a total of approx. 1100 pigs were kept, including 100 sows and 7 boars. The farm is located in the Kanto region of Japan, on the main island of Honshu. The sero-prevalence on the farm was 58.5% (24/41), and it reached 70.7% (29/41) when doubt-positive sera were added, which immediately converted to actual sero-positive (Table 1). Two sero-negative sows out of 11 at the initial tests turned sero-positive at the second test 5 days later, suggesting the circulation of the virus on the farm. Despite this evidence, the pigs showed no clinical symptoms suggesting CSF or other diseases.

Table 2
Differentiation of ELISA-positive sera by NT with CSFV, BVDV1 and BVDV2.

Titer of neutralizing antibodies against			Numbers of sera[a] (doubt positive)
CSFV GPE⁻	BVDV1 Nose	BVDV2 KZ-91	
<2	<2	<2	6 (5)
	2	<2	3 (0)
	4	<2	4 (1)
	8	<2	7 (1)
	16	<2	5 (0)
	32	<2	3 (1)
2	2	<2	1 (0)
	4	<2	2 (0)
	8	<2	6 (0)
	16	<2	2 (0)
	32	<2	4 (0)
	64	<2	2 (0)
4	16	<2	3 (0)
	32	<2	2 (0)
			50 (8)

[a] This is an indication of the numbers of pig sera evaluated as a sero-positive involving a doubt-positive by the ELISA

Then, three individual NTs for the GPE⁻ strain of CSFV (Shimizu et al., 1970), the Nose strain of BVDV 1 (Kodama et al., 1974) and the KZ-91 strain of BVDV 2 (Nagai et al., 1998) were conducted using 50 sero-positive sera including 8 doubt-positive samples (Table 2). The NT antibodies to the CSFV and the BVDV 1 were found in 22 and 44 sera, respectively, whereas no neutralizing antibody (1:<2) against the BVDV 2 was detected in any of the sera tested. We calculated the geometric mean (GM) titers of those positive sera to compare between the CSFV and the BVDV 1 antibody titers. The GM titers of the CSFV and the BVDV 1 antibodies were 1:2.34 (1:2–4) and 1:10.96 (1:2–64), respectively. Since the BVDV1 antibodies were markedly higher than the CSFV antibodies in both of the numbers and the GM titers of positive sera, infection with BVDV 1 (and neither CSFV nor BVDV 2) was suspected.

We conducted a reverse transcriptase-polymerase chain reaction (RT-PCR) to identify the transmissible source in the farm, which suggested the existence of such persistently infected (PI) animals due to the high sero-prevalence without clinical signs. The RT-PCR was performed with a set of primers, 324 (5′-ATGCCCWTAGTAGGACTAGCA-3′) and 326 (5′-GTACATGGCACATGGAGTTGA-3′) detectable for the 5′-nontranslating region (5′-NTR) of most pestiviruses (Vilcek et al., 1997). Approximately 200 blood samples involving all sera from 91 pigs used for serological tests and 100 nasal swabs collected mainly from pigs that sero-converted and pigs kept together with sero-converted and underdeveloped piglets were used for the RT-PCR. Consequently, the pestiviral gene was detected by the RT-PCR in four pigs (Table 3). One of these pigs was a healthy, fattening 120–130-day-old kept at the finishing piggery, identified as pig ID W68 in Table 1; it had no detectable antibody by the ELISA.

We suspected that the fattening W68 of the RT-PCR-positive pigs was a PI animal which had continuously