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Comparative Evaluation of Three Commercial ELISA Kits for Detection of Antibodies to a Nonstructural Protein of Foot-and-Mouth Disease Virus

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ABSTRACT. In this study, we validated three commercial ELISA (NSP-ELISA) kits that detect antibodies to a nonstructural protein of foot-and-mouth disease virus (FMDV) in terms of their specificities and sensitivities. Although the specificities of the NSP-ELISA kits were as high as that of liquid-phase blocking ELISA (LPBE) in non-infected, non-vaccinated animals, the sensitivities of the NSP-ELISA kits were significantly lower than those of the present LPBE and did not agree with the findings of a previous report on infected animals in the field. Therefore, although countries can adopt both a "vaccination-to-kill" policy and a "vaccination-to-live" policy after emergency vaccination during an FMD epidemic, the NSP-ELISA kits do not seem to be suitable for the latter policy in Japan. These results should be useful for choosing appropriate control measures for potential future FMD epidemics in Japan and elsewhere.

KEY WORDS: antibody, ELISA, foot-and-mouth disease virus, nonstructural protein, vaccine.

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Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals that occurs endemically or sporadically in numerous countries around the world. It is caused by the FMD virus (FMDV), which belongs to the genus *Aphthovirus*, the family *Picornaviridae*. FMDV is divided into seven serotypes: O, A, C, Asia1, SAT1, SAT2 and SAT3. There is no evidence of cross-protection among the seven distinct serotypes [11].

When FMD occurs in FMD-free countries where vaccination is not practiced, in general, stamping out the affected and contact animals and control of livestock movement inside affected regions are applied as control measures. In addition, emergency vaccination can also be applied. Although countries can adopt both a "vaccination-to-kill" policy and a "vaccination-to-live" policy after emergency vaccination, the Terrestrial Animal Health Code drawn up by the World Organization for Animal Health (OIE) establishes that countries that adopt the former policy can regain their previous FMD-free status earlier than countries that adopt the latter policy [10]. Countries that adopt the latter policy must also discriminate serologically between FMDV-infected animals and the vaccinated animals to show there is no FMDV infection. This is because, irrespective of vaccination status, FMDV-infected ruminants may be long-term carrier animals of FMDV and discharge it only intermittently. Detection of carrier animals is important as a control measure of FMD, because carrier animals have the potential to serve as new infectious sources to other susceptible animals in future outbreaks.

In recent years, commercial FMD vaccines have been produced as follows: First, FMDV is grown in BHK cell suspension culture and is inactivated with ethylene imines [3]. The inactivated FMDV is concentrated and is purified by industrial ultrafiltration and chromatography in order to remove unwanted cellular protein contaminants and viral nonstructural proteins (NSPs). Therefore, we can differentiate between FMDV-infected animals and vaccinated animals by examining antibodies to NSPs, because non-infected, vaccinated animals theoretically have no antibodies to NSPs.

In 2010, FMD occurred in Japan for the first time since 2000 [5, 7]. Emergency vaccination was carried out as one of the control measures, and approximately eighty thousand vaccinated animals were destroyed. However, destroying such a large number of animals in the event of a future outbreak would pose serious problems in terms of environmental contamination, animal welfare, food security and conservation of scarce genetic resources. A policy of "vaccination-to-live" that relies upon effective detection systems would be far preferable.

At present, there are several commercial and "in-house" ELISA kits that detect antibodies to the NSPs (NSP-ELISA) [1, 2, 8, 9], but such ELISA kits, which could be used in support of the "vaccination-to-live" policy, must be validated for that purpose. Although the ELISA kits have been evaluated in several foreign countries [1], none has been sufficiently validated for use in support of a "vaccination-to-live" policy in Japan. In this study, we validated three commercial NSP-ELISA kits for the aforementioned purpose.

MATERIALS AND METHODS

Ethics: The Animal Care and Use Committee of the National Institute of Animal Health approved all animal procedures prior to initiation of this study.

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Table 1. Information on animals administered with foot-and-mouth disease vaccines, the vaccines used and the schedules of vaccinations

Animals	Breeds[a)]	Ages[b)]	Sexes[c)]	Serotypes[d)]	PD_{50}/dose[e)]	Administered times[f)]	Nos. of the sera[g)]
Bovine 1/vac/06	JB	6	F	O	6	1	31
Bovine 2/vac/06	JB	6	F	O	6	1	23
Bovine 3/vac/06	JB	6	F	Asia1	6	1	31
Bovine 4/vac/06	JB	6	F	Asia1	6	1	31
Bovine 5/vac/07	JB	6	F	O	6	1	31
Bovine 6/vac/07	JB	6	F	O	6	1	31
Bovine 7/vac/07	JB	6	F	Asia1	6	1	31
Bovine 8/vac/07	JB	6	F	Asia1	6	1	31
Bovine 57/vac/11	H	6	Fr	O	6	4 (at 2-week intervals)	14
Bovine 46/vac/11	H	6	Fr	A	6	4 (at 2-week intervals)	14
Swine 1/vac/06	LW	3	F	O	6	1	31
Swine 2/vac/06	LW	3	F	O	6	1	31
Swine 3/vac/06	LW	3	F	O	6	1	31
Swine 4/vac/06	LW	3	F	Asia1	6	1	26
Swine 5/vac/06	LW	3	F	Asia1	6	1	31
Swine 6/vac/06	LW	3	F	Asia1	6	1	31
Swine 7/vac/07	LW	3	F	O	6	1	31
Swine 8/vac/07	LW	3	F	O	6	1	31
Swine 9/vac/07	LW	3	F	O	6	1	31
Swine 10/vac/07	LW	3	F	Asia1	6	1	31
Swine 11/vac/07	LW	3	F	Asia1	6	1	31
Swine 12/vac/07	LW	3	F	Asia1	6	1	31
Swine 2/vac/11	LW	6	F	A	6	4 (at 2-week intervals)	14
Swine 8/vac/11	LW	6	F	Asia1	6	4 (at 2-week intervals)	14

a) JB, Japanese Black; H, Holstein; LW, Cross between Landrace and Large White. b) χ months. c) F, Female; Fr, Freemartin. d) Serotypes of strains of the vaccines used. e) 50% protection dose per 1 dose of the vaccines used. f) Administered times of the vaccines used to the animals. g) Number of sera collected from each animal.

Vaccines: The Aftpor vaccines (Merial, Lyon, France) used in this study are preserved for emergency use by the Ministry of Agriculture, Forestry and Fisheries in Japan. As shown in Table 1, each vaccine is formulated with serotypes O, A and Asia1 FMDVs, respectively, and contained six 50% protection dose (PD_{50}) per dose. In general, 2 m*l* of the vaccine is administered to cattle and pigs, and 1 m*l* of the vaccine is administered to goats and sheep.

Sera obtained from non-infected, non-vaccinated animals: A total of 203 serum specimens were obtained from cattle raised on 14 farms in Japan in 2010. The cattle had never been infected with FMDV nor administered any FMDV vaccines. A total of 225 serum specimens were collected from pigs raised on 18 farms in Japan in 2010. The pigs had never been infected with FMDV and had never been administered any FMDV vaccines.

Sera obtained from non-infected, vaccinated animals: Ten cattle and 14 pigs were administered the FMDV vaccine intramuscularly, and a total of 663 serum specimens were collected routinely from the animals (268 bovine sera and 395 swine sera). Table 1 displays information on the animals and the vaccine, and the schedules of vaccinations. The day when the animals were administered the vaccine was designated as 0 days postvaccination (DPV). In the case of animals administered a single dose of vaccine, the serum specimens were collected daily until 10 DPV, at 3–4 days intervals until 21 DPV and at approximately 1–2 weeks intervals after that, and the animals were raised for approximately 8 months. The exceptions were that Bovine 2/vac/06 and Swine 4/vac/06 were administered a single dose of vaccine and raised for approximately 4 and 5 months, respectively. In the case of animals administered four doses of vaccine, serum specimens were collected daily until 4 DPV, at 3–4 days intervals until 22 DPV and at approximately 1 week intervals after that, and were raised for approximately 2 months. In addition, 60 serum specimens were collected from 40 cattle and 20 pigs administered the vaccine as a control measure in the 2010 epidemic in Japan.

Sera obtained from infected, non-vaccinated animals: A total of 102 serum specimens were obtained from cattle raised on 66 farms where FMD occurred during the 2010 epidemic. A total of 50 serum specimens were collected from pigs raised on 38 farms where FMD occurred during the epidemic. All of these animals were assumed to have been exposed to FMDV and were expected to carry the NSP antibodies. In addition, two 2-month-old pigs (Swine 1/vir/10 and 2/vir/10), which were designated as pigs 1 and 2, respectively, in our previous report, were inoculated with the isolate O/JPN/2010-1/14C as described previously [4]. The day when the pigs were inoculated with the isolate was designated as 0 days postinoculation (DPI). Four 2-month-old pigs (Swine 3/vir/10, 4/vir/10, 5/vir/10 and 6/vir/10), which were designated as pigs 3–6, respectively, in the aforementioned report, were combined with the inoculated pigs at 1

DPI and housed in the same cubicle for 21 days. The day when the pigs were placed in contact with the inoculated pigs was designated as 0 days postcontact (DPC). A total of 116 serum specimens were collected from the inoculated pigs at 0–17, 19 and 21 DPI and from the pigs placed with the inoculated pigs at 0–16, 18 and 20 DPC.

NSP-ELISA kits: Three commercial NSP-ELISA kits were compared in this study in terms of their specificities and sensitivities: the PrioCHECK FMDV NS (Prionics AG, Schlieren-Zurich, Switzerland) [9], the SVANOVIR FMDV 3ABC-Ab (Svanova, Uppsala, Sweden) [8] and the IDEXX CHEKIT FMD-3ABC bo-ov (IDEXX Laboratories, Westbrook, ME, U.S.A.) [2]. These kits detect antibodies to the FMDV NSP 3ABC, which is expressed as a recombinant protein in different expression systems. In brief, the NSP 3ABC of the PrioCHECK kit was expressed by a baculovirus expression system. In contrast, those of the SVANOVIR kit and the IDEXX kit were expressed by *Escherichia coli* expression systems. Methodologically, the PrioCHECK kit is a blocking ELISA and can examine serum specimens obtained from cattle, pigs, goats and sheep. In contrast, the SVANOVIR and IDEXX kits are indirect ELISAs and use anti-species conjugates. The SVANOVIR kit can examine only bovine sera, and the IDEXX kit can examine only bovine and ovine sera. In the PrioCHECK kit, 3ABC antigens are trapped by an anti-3ABC monoclonal antibody. In the other kits, the antigens are directly coated on microplates. Antibody tests using these kits were carried out according to each manufacturer's instruction.

Liquid-phase blocking ELISA (LPBE): An LPBE (Institute for Animal Health, Surrey, U.K.) was carried out for the detection of antibodies to structural proteins of FMDV according to the manufacturer's instructions and the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2011 [11]. In principle, the LPBE is an assay to detect antibodies to structural proteins of FMDV. And as aforementioned, there are seven serotypes, which do not show serological cross-reactivity with each other, in FMDV [11]. Therefore, the LPBE is a serotype-dependent assay, and it is necessary to apply an appropriate FMDV strain as its antigen. The results of the LPBE in this study were obtained using the FMDV O Manisa strain as the antigen. In contrast, a serotype of an FMDV, which causes an FMD epidemic, is not identified in its early phase. Therefore, it is necessary to apply several FMDV strains with different serotypes as the antigens in the early phase. We applied serotypes O, A, C and Asia1 FMDV strains, which are serotypes that are endemic in East Asian countries, as the antigens of the LPBE during an early phase of the 2010 epidemic in Japan.

Diagnostic specificity and sensitivity: A total of 203 sera obtained from non-infected, non-vaccinated cattle, 308 sera obtained from non-infected, vaccinated cattle, 225 sera obtained from non-infected, non-vaccinated pigs and 415 sera obtained from non-infected, vaccinated pigs were used to calculate the specificities of the LPBE and the three NSP-ELISA kits. The specificity was calculated as the number of serum specimens judged as negative in the three NSP-ELISA kits divided by the number of serum specimens used, multiplied by 100. A total of 102 sera obtained from infected cattle in the field, 50 sera obtained from infected pigs in the field and 81 of 116 sera obtained from experimentally infected pigs were used to calculate the sensitivities of the LPBE and the three NSP-ELISA kits. The sensitivity was calculated as the number of serum specimens judged as positive in the three NSP-ELISA kits divided by the number of serum specimens used, multiplied by 100. The Pearson's chi-square test was used for analyzing the statistical significance of the differences in specificities and sensitivities among the LPBE and the three NSP-ELISA kits. In addition, the test was used for analyzing the statistical significance of the differences in sensitivities of the NSP-ELISA kits in this study and those of the same kits described in a previous report [1].

RESULTS

Comparative diagnostic specificities of the LPBE and the three NSP-ELISA kits in cattle: There were no statistically significant differences in specificity among the LPBE and the three NSP-ELISA kits in non-infected, non-vaccinated cattle, with the exception that the specificity for the SVANOVIR kit was statistically lower than those for the LPBE and the IDEXX kit ($P<0.05$, Table 2); the specificities of the LPBE and the three kits ranged from 96.1 to 99.5%. Similarly, the specificities of the NSP-ELISA kits in non-infected, vaccinated cattle were high; they ranged from 98.4 to 99.7%. In addition, no antibody was detected from the cattle administered the vaccine four times by the NSP-ELISA kits.

Comparative diagnostic specificities of the LPBE and the PrioCHECK kit in pigs: The specificities shown by the LPBE and the PrioCHECK kit in non-infected, non-vaccinated pigs were both 100% (Table 2). The specificity shown by the PrioCHECK kit in non-infected, vaccinated pigs was also very high at 99.8%. In addition, no antibody was detected from 27 of 28 serum specimens collected from the pigs administered the vaccine four times by the PrioCHECK kits.

Comparative diagnostic sensitivities of the LPBE and the three NSP-ELISA kits in cattle: The sensitivities shown by the LPBE and the three NSP-ELISA kits in infected, non-vaccinated cattle in the field showed statistically significant differences ($P<0.01$, Table 3); although the sensitivity shown by the LPBE was 100%, those shown by the NSP-ELISA kits ranged from 21.6 to 28.4%.

Comparative diagnostic sensitivities of the LPBE and the PrioCHECK kit in pigs: The sensitivities shown by the LPBE and the PrioCHECK kit in infected, non-vaccinated pigs in the field and experimentally infected, non-vaccinated pigs showed statistically significant differences ($P<0.01$, Table 3); although the sensitivities shown by the LPBE were both 100%, those shown by the PrioCHECK kit were 4.0 and 85.2%, respectively.

Comparison between antibody titers shown by the LPBE and the results of the NSP-ELISA kits in infected, non-vaccinated cattle and pigs: The antibody titers shown by the LPBE in infected, non-vaccinated cattle and pigs were compared with the results of the NSP-ELISA kits in the same animals, because the sensitivities of the NSP-ELISA kits

Table 2. Diagnostic specificity in non-infected, non-vaccinated animals and non-infected, vaccinated aminals

Animals		Numbers of specimens	ELISA kits			
			LPBE (%)	PrioCHECK (%)	SVANOVIR (%)	IDEXX (%)
Non-infected, non-vaccinated cattle		203	99.5	99.0	96.1	99.5
Non-infected, vaccinated cattle	Cattle administered vaccine once	240	NC[a)]	100	99.6	99.6
	Cattle administered vaccine four times	28	NC	100	100	100
	Cattle administered vaccine in the field	40	NC	95.0	90.0	100
	Total	308	NC	99.4	98.4	99.7
Non-infected, non-vaccinated pigs		225	100	100	ND[b)]	ND
Non-infected, vaccinated pigs	Pigs administered vaccine once	367	NC	100	ND	ND
	Pigs administered vaccine four times	28	NC	96.4	ND	ND
	Pigs administered vaccine in the field	20	NC	100	ND	ND
	Total	415	NC	99.8	ND	ND

a) Not calculated because specificity is a percentage of animals that are correctly confirmed as antibody-negative among animals that do not have antibodies to SPs or NSPs while the LPBE kit measures antibodies to SPs and vaccinated animals have antibodies to SPs. b) Not determined because the SVANOVIR and IDEXX kits cannot examine porcine sera.

Table 3. Diagnostic sensitivity in infected animals in the field and experimentally infected pigs

Animals	Numbers of specimens	ELISA kits			
		LPBE (%)	PrioCHECK (%)	SVANOVIR (%)	IDEXX (%)
Infected cattle in the field	102	100	28.4	28.4	21.6
Infected pigs in the field	50	100	4.0	ND[a)]	ND
Experimentally infected pigs	81[b)]	100	85.2	ND	ND

a) Not determined because the SVANOVIR and IDEXX kits cannot examine porcine sera. b) Specimens in which antibody titers were ≥45 were judged as positive in the LPBE and they were only used to calculate the sensitivity of the PrioCHECK kit.

were significantly lower than that of the LPBE, as aforementioned. Several specimens that had high LPBE titers were judged as positive in the NSP-ELISA kits (Table 4). However, many specimens were judged as negative in the kits, although the LPBE titers were high, especially in infected, non-vaccinated cattle and pigs in the field. Differences between the sensitivities shown by the NSP-ELISA kits were also observed in infected, non-vaccinated cattle in the field. In contrast, in the LPBE, antibodies were initially detected at 5 DPI in experimentally inoculated pigs (Swine 1/vir/10 and Swine 2/vir/10), and at 5–8 DPC in pigs placed in contact with them (Swine 3/vir/10–Swine 6/vir/10) (Table 5). In the PrioCHECK kit, antibodies were initially detected at 7–8 DPI in the experimentally inoculated pigs, and at 7–10 DPC in the pigs placed in contact with them.

DISCUSSION

The objective of this study was to evaluate three commercially available NSP-ELISA kits for their effectiveness in emergency FMD vaccination for the control of FMD outbreaks in Japan. The performance of these NSP-ELISA kits is critical to national veterinary authorities who are involved in the design of scientifically based serosurveillance sampling strategies.

Although the specificity of the SVANOVIR kit in non-infected, non-vaccinated cattle was statistically lower than those of the LPBE and the other NSP-ELISA kits ($P<0.05$), we confirmed that the specificities of the three NSP-ELISA kits were as high as that of the LPBE in non-infected, non-vaccinated animals (Table 2). Similarly, the specificities of the three NSP-ELISA kits were high in non-infected, singly vaccinated animals in this study. In addition, no antibody was detected from almost all animals administered the vaccine four times by the NSP-ELISA kits. Although multiple vaccinations in a short period will not be applied as a control measure in FMD-free countries where vaccination is not practiced, such as Japan, it was performed here to analyze the purity of the FMD vaccine preserved for emergency use in Japan. In the aforementioned OIE manual, the OIE recommends that vaccine manufacturers perform the following test to confirm the purity of their FMDV vaccines: (1) Administer a vaccine at least three times to several calves over a period of 3–6 months; (2) Test for presence of antibodies to NSPs at 30–60 days after the last vaccination; (3) Negative results in the test support claims that the vaccine has high purity and does not induce any antibodies to NSPs [11]. Although the period of our test was slightly shorter than that of the test recommended by the OIE, we could confirm in this study that the FMD vaccine preserved for emergency use in Japan likely has high purity and does not induce any antibodies to NSPs.

Brocchi *et al.* reported that the sensitivities of the NSP-ELISA kits that were used in this study were 100% in non-vaccinated, experimentally infected cattle at 7–27 DPI [1]. Additionally, they reported that the sensitivity of the Prio-

Table 4. Comparison between antibody titers shown by the LPBE and results of the NSP-ELISA kits in infected cattle and pigs

Animals	Antibody titers shown by LPBE	PrioCHECK			SVANOVIR			IDEXX		
		+[a]	–[b]	Sensitivity (%)	+	–	Sensitivity (%)	+	–	Sensitivity (%)
Infected cattle in the field	45	1	25	3.8	0	26	0	1	25	3.8
	64	0	7	0	2	5	28.6	0	7	0
	90	0	16	0	3	13	18.8	1	15	6.3
	128	0	1	0	0	1	0	0	1	0
	181	4	10	28.6	6	8	42.9	4	10	28.6
	256	2	2	50.0	0	4	0	1	3	25.0
	362	1	0	100	1	0	100	1	0	100
	≥362	21	12	63.6	17	16	51.5	14	19	42.4
	Total	29	73	28.4	29	73	28.4	22	80	21.6
Infected pigs in the field	45	0	16	0	ND[c]	ND	ND	ND	ND	ND
	64	0	5	0	ND	ND	ND	ND	ND	ND
	90	0	11	0	ND	ND	ND	ND	ND	ND
	128	0	1	0	ND	ND	ND	ND	ND	ND
	181	1	4	20.0	ND	ND	ND	ND	ND	ND
	256	0	2	0	ND	ND	ND	ND	ND	ND
	≥362	1	9	10.0	ND	ND	ND	ND	ND	ND
	Total	2	48	4.0	ND	ND	ND	ND	ND	ND
Experimentally infected pigs	45	0	4	0	ND	ND	ND	ND	ND	ND
	90	6	3	66.7	ND	ND	ND	ND	ND	ND
	128	3	1	75.0	ND	ND	ND	ND	ND	ND
	181	28	2	93.3	ND	ND	ND	ND	ND	ND
	256	1	0	100	ND	ND	ND	ND	ND	ND
	362	30	2	93.8	ND	ND	ND	ND	ND	ND
	724	1	0	100	ND	ND	ND	ND	ND	ND
	Total	69	12	85.2	ND	ND	ND	ND	ND	ND

a) The number of samples that were judged as positive by each NSP-ELISA kit. b) The number of samples that were judged as negative by each NSP-ELISA kit. c) Not determined because the SVANOVIR and IDEXX kits cannot examine porcine sera.

Table 5. Detection of antibodies in the FMDV-inoculated pigs and the pigs placed in contact with them by the LPBE and the PrioCHECK kit

Pigs	DPI and DPC																			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	21
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	18	20
Swine 1/vir/10	–/–[a]	–/–	–/–	–/–	–/–	+/–	+/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Swine 2/vir/10	–/–	–/–	–/–	–/–	–/–	+/–	+/–	+/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Swine 3/vir/10	NS[b]	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Swine 4/vir/10	NS	–/–	–/–	–/–	–/–	–/–	+/–	+/–	+/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Swine 5/vir/10	NS	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	+/–	+/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Swine 6/vir/10	NS	–/–	–/–	–/–	–/–	–/–	+/–	+/–	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+

a) Detection by the LPBE/detection by the PrioCHECK kit. b) Not sampled.

CHECK kit was 100% in non-vaccinated, experimentally infected pigs at >20 DPI. By contrast, the sensitivities of the NSP-ELISA kits in this study were significantly lower than those of their report in infected cattle and pigs in the field ($P<0.01$, 21.6–28.4% and 4.0%, respectively, Table 3). One possible explanation for the finding that the sensitivities of the NSP-ELISA kits were significantly lower in our report than in theirs may be that the serum specimens were collected from the infected cattle and pigs in the field before their bodies had had time to produce antibodies to the NSP. Although we cannot know the precise day when the infected cattle and pigs in the field were infected with FMDV, several infected cattle and pigs in the field that had high LPBE titers were judged as negative by the NSP-ELISA kits in this study (Table 4). Therefore, the sensitivities of the NSP-ELISA kits were likely to be lower than those reported previously. In addition, differences in the sensitivities of the three NSP-ELISA kits were observed in infected, non-vaccinated cattle in the field. Methodologically, the PrioCHECK kit is a blocking ELISA, and the SVANOVIR and IDEXX kits are indirect ELISAs. The NSPs 3ABC expressed by *Escherichia coli* expression systems are antigens in the latter two kits, but

the expression systems are different. In addition, different conjugates are applied in the kits. Therefore, differences in the sensitivities of the three kits in infected, non-vaccinated cattle in the field are likely attributable to the methods, antigens and conjugates of the kits.

In this study, all the serum specimens collected from experimentally inoculated pigs and the pigs placed in contact with them were judged as positive after 8 DPI and 10 DPC by both the LPBE and the PrioCHECK kit (Table 5). These results were inconsistent with the aforementioned results in infected cattle and pigs in the field. It is not clear why the results in experimentally infected pigs were different from those in infected animals in the field. At the very least, we can speculate that antibodies are detected later by the NSP-ELISA kits than by the LPBE. In addition, Brocchi *et al.* reported that the sensitivities of the NSP-ELISA kits were 38.3–74.5% in vaccinated cattle exposed to infection at between 7 and >100 DPI [1]. They also reported that the sensitivities of the PrioCHECK kit were 11.9 and 55.5% in vaccinated, experimentally infected pigs at between ≤14 and >20 DPI. These results suggest that the sensitivities of the NSP-ELISA kits in vaccinated, infected animals are considerably lower than those in non-vaccinated, infected animals. In contrast, when emergency vaccination is applied as a control measure in FMD epidemics, it is possible that vaccinated animals are infected with FMDV after the vaccination. In such a case, vaccinated, infected animals may be judged as negative by the NSP-ELISA kits. Because antibodies are detected later by the NSP-ELISA kits than by the LPBE and the sensitivities of the NSP-ELISA kits in vaccinated, infected animals are lower than those in non-vaccinated, infected animals, serological surveillance when a "vaccination-to-live" policy is adopted in FMD epidemics in FMD-free countries where vaccination is not practiced should be carried out as follows: (1) Confirm that vaccinated animals are negative for the antibody before vaccination using the LPBE; (2) Confirm that they are positive for the antibody at approximately 14–21 DPV using the LPBE and that they are negative for the antibody on the same day using the NSP-ELISA kits. However, because vaccinated animals should be examined twice at intervals of approximately 14–21 days by at least two tests, the "vaccination-to-live" policy is not likely to be convenient as a control measure for FMD epidemics except in rare cases, such as for animals raised in zoos.

As aforementioned, there are seven serotypes in FMDV, and serological cross-reactivity is not observed between distinct serotypes [11]. In addition, antigenic diversity is confirmed between the same serotype [6]. Therefore, it is important to confirm the serological relationship between a field strain and a vaccine strain when vaccination is practiced as one of the control measures in an FMDV outbreak. The r_1 value, which demonstrates the serological relationship between FMDV strains, is calculated as follows: a reciprocal arithmetic titer in a neutralization test of a reference vaccine serum against a field strain divided by a reciprocal arithmetic titer in the test of the reference vaccine serum against a vaccine strain [11]. An r_1 value greater than 0.3 indicates that a field strain is sufficiently similar to a vaccine strain and use of a vaccine based on the vaccine strain is likely to confer protection against a challenge with the field strain. The vaccine strain that was applied for emergency vaccination in the 2010 epidemic in Japan was the O Manisa strain. Our preliminary serological examination showed that the r_1 value between the O Manisa strain and the O/JPN/2010 strain isolated in the 2010 epidemic in Japan was greater than 0.3 (unpublished data). In fact, the number of FMD cases was gradually reduced in the epidemic, after emergency vaccination was practiced as one of the control measures [7].

In conclusion, we confirmed the following in this study: (1) the specificities of the NSP-ELISA kits are as high as that of the LPBE in non-infected, non-vaccinated animals; (2) the sensitivities of the NSP-ELISA kits in infected animals should be investigated in greater detail, because those of the NSP-ELISA kits were significantly lower than those of the LPBE and did not agree with the findings of a previous report on infected animals in the field. These results are useful for deciding on appropriate control measures for possible future FMD epidemics in Japan and elsewhere.

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