

Construction and characterization of a full-length infectious cDNA clone of foot-and-mouth disease virus strain O/JPN/2010 isolated in Japan in 2010

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### 1 Short communication

2	Construction and characterization of a full-length infectious cDNA clone of foot-and-mouth
3	disease virus strain O/JPN/2010 isolated in Japan in 2010
4	
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# 17 Abstract

18	A full-length infectious cDNA clone of the genome of a foot-and-mouth disease virus isolated
19	from the 2010 epidemic in Japan was constructed and designated pSVL-f02. Transfection of Cos-7
20	or IBRS-2 cells with this clone allowed the recovery of infectious virus. The recovered virus had the
21	same in vitro characterization as the parental virus with regard to antigenicity in neutralization and
22	indirect immunofluorescence tests, plaque size and one-step growth. Pigs were experimentally
23	infected with the parental virus or the recombinant virus recovered from pSVL-f02 transfected cells.
24	There were no significant differences in clinical signs or antibody responses between the two groups,
25	and virus isolation and viral RNA detection from clinical samples were similar. Virus recovered from
26	transfected cells therefore retained the in vitro characteristics and the in vivo pathogenicity of their
27	parental strain. This cDNA clone should be a valuable tool to analyze determinants of pathogenicity
28	and mechanisms of virus replication, and to develop genetically engineered vaccines against
29	foot-and-mouth disease virus.

30

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Keywords: Foot-and-mouth disease virus, full-length cDNA, Pathogenicity, O/JPN/2010 strain

32	Foot-and-mouth disease (FMD) is the most contagious disease of cloven-hoofed animals.
33	Outbreaks of FMD cause enormous economic damage to the livestock industry by reducing
34	livestock productivity and interrupting international trade of animals and their products. FMD virus
35	(FMDV) is classified into the genus Aphthovirus within the family Picornaviridae. The genome of
36	FMDV is composed of a single-stranded positive-sense RNA approximately 8.5 kb nucleotides in
37	length with a poly (C) sequence at the 5' terminus of this genome, and a poly (A) sequence at the 3'
38	terminus (Mason et al., 2003).
39	In Japan, FMD outbreaks occurred in 2000 and 2010; strains O/JPN/2000 and O/JPN/2010
40	were isolated from cattle during each outbreak, respectively (Sakamoto et al., 2002; Muroga et al.,
41	2012). In the 2000 epidemic, cases were limited to four cattle farms and eradicated by slaughter of
42	740 cattle (Sugiura et al., 2001). Typical clinical signs were confirmed only in pigs following
43	experimental infections with O/JPN/2000; inoculated Japanese Black cattle showed only mild
44	clinical signs, and Holstein cattle and goats did not demonstrate clinical signs of infection, nor viral
45	shedding (Yamakawa et al., 2002). In contrast, the FMD outbreak in 2010 spread to 292 farms and
46	inoculated pigs, Holstein cattle and goats showed clinical signs and significant viral shedding
47	following experimental infections with O/JPN/2010 (Fukai et al., 2011; Onozato et al., 2014). These

48	differences in infectivity and pathogenicity were suspected to contribute to the relative severity of
49	the 2010 outbreak compared to the one in 2000; however, the molecular mechanisms underlying the
50	pathogenicity of O/JPN/2000 and O/JPN/2010 are not well understood.
51	To date, infectious cDNA clones have been constructed for the purposes of understanding viral
52	replication and pathogenicity at the molecular level as well as for vaccine development (Zibert et al.,
53	1990; Rieder et al., 1993; Garcia-Arriaza et al., 2004; Liu et al., 2004; van Rensburg et al., 2004;
54	Hema et al., 2009; Xin et al., 2009; Rajasekhar et al., 2013) and recombinant and site-directed
55	mutants have been used for genomic studies (Falk et al., 1992; Piccone et al., 1995; Ellard et al.,
56	1999; Beard and Mason, 2000; Pacheco et al., 2003; Botner et al., 2011;Seago et al., 2013; Xin et al.,
57	2014). In the present study, a full-length infectious cDNA clone of O/JPN/2010 (pSVL-f02) was
58	constructed and the recovered virus was compared to its parental strain by in vitro characterization
59	and experimental infection of pigs.
60	The FMDV used in this experiment (O/JPN/2010 290-1E) was isolated from the epithelial tissue
61	of diseased cattle from a farm, which was confirmed as the 235th affected premises of 292 in total
62	during the 2010 epidemic in Japan. Viral RNA of O/JPN/2010 290-1E was extracted using a High
63	Pure Viral RNA Kit (Roche Diagnostics, Tokyo, Japan). First-strand cDNA synthesis was performed

64	using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostics) and random hexamer
65	primers. Polymerase chain reaction (PCR) was performed using Platinum Taq Polymerase High
66	Fidelity (Life Technologies, Tokyo, Japan), using primers which were designed based on the
67	sequence of the O/JPN/2010 290-1E isolate (GenBank accession number LC036265) or other
68	reports (Sup. Table 1). The PCR fragments were inserted into the pGEM-T Easy cloning vector
69	(Promega, Tokyo, Japan). The full-length cDNA was assembled with five cDNA clones covering the
70	complete genome of O/JPN/2010 290-1E using appropriate restriction enzymes and cloned
71	downstream of the SV40 late promoter in the pSVL vector (formerly Pharmacia Biotech, WI, USA)
72	(Fig. 1). The 5N-2 clone had a poly $(C)_{18}$ tract and the 3R-L4 had a poly $(A)_{27}$ tail confirmed by
73	sequencing.
74	African green monkey kidney cell line Cos-7 and porcine kidney cell line IBRS-2 were grown
75	in Minimum Essential Medium (MEM) (Nissui Pharmaceutical, Tokyo, Japan) in a 12 well culture
76	plate. At 70-90% confluency, the cells were transfected with 0.1-1000 ng of pSVL-f02 using
77	Lipofectamine 3000 (Life Technologies) and incubated at 37°C, in 5% CO <sub>2</sub> . In Cos-7 and IBRS-2
78	monolayers transfected with 1000 ng of pSVL-f02 DNA, distinct cytopathic effect (CPE) was
79	observed as soon as 1 day following transfection. At other doses, weak CPE were observed at 1 day

80	post-transfection in both cell types transfected with 100 ng of pSVL-f02, but not in cells transfected
81	with 0.1-10 ng construct up to 3 days post-transfection. The supernatants and cells were collected at
82	3 days post-transfection, and virus was titrated in porcine kidney cell line, LFBK-alphavbeta6
83	(LaRocco et al., 2013); at least 100 ng and 10 ng of the plasmid were necessary to produce
84	infectious virus in Cos-7 and IBRS-2 cells, respectively (Sup. Table 2A). After transfection using
85	1000 ng of pSVL-f02, supernatant or supernatant and cells were subjected to three freeze/thaw
86	cycles, clarified and collected at 1 to 3 days post-transfection. The viral titers of these samples were
87	determined as 50% tissue culture infectious dose (TCID <sub>50</sub> ) in LFBK- $\alpha_v\beta_6$ cells. The highest titers
88	were detected at 2 and 1 day(s) post-transfection in Cos-7 and IBRS-2 cells, respectively (Sup. Table
89	2B).
90	The infectious virus from Cos-7 cells was passaged three times on the fetal goat tongue cell
91	line, ZZR-127 (Brehm et al., 2009), then subsequently on baby hamster kidney cell line, BHK and
92	ZZR-127 cells to obtain a high titer viral sample ( $10^{7.3}$ TCID <sub>50</sub> /0.1mL) for subsequent studies. The
93	antigenicity of the recovered virus vSVL-f02 was examined by virus neutralization tests using sera
94	collected from pigs inoculated with FMDV O/JPN/2010 290-1E isolate (Nos. 1 and 3) or the
95	recovered virus vSVL-f02 (Nos. 5 and 6) were determined using each virus as an antigen (Sup. Table

96	3). There were no remarkable differences between the antibody titers. The antigenicity of the
97	vSVL-f02 was also examined by an indirect immunofluorescence test using FMDV-specific
98	monoclonal antibody (MAb) 1H5 which was raised against the O/JPN/2000 strain and reacts with all
99	seven serotypes of FMDV (Morioka et al., 2009) (Fig. 2). The MAb 1H5 reacted with the recovered
100	virus vSVL-f02. The plaques of the vSVL-f02 and the parental 290-1E isolate on LFBK and IBRS-2
101	cell monolayers were visualized by staining with crystal violet, and similar plaque growth was
102	confirmed (Fig. 3). In addition, vSVL-f02 and the parental 290-1E isolate were inoculated onto
103	LFBK cell monolayers cultured in 25-cm <sup>2</sup> flasks at a multiplicity of infection (MOI) of 2, then the
104	culture supernatant was harvested at subsequent time points and the amount of virus present was
105	determined (Fig. 4). Growth curves of both viruses reached a plateau at 12 hours post inoculation
106	and there were no significant differences in virus titers at each time points of the growth step
107	calculated by Student's t-test. Therefore, growth rates of these viruses were not significantly
108	different, and the in vitro characteristics of the recovered virus vSVL-f02 were demonstrated to be
109	identical to those of the parental strain 290-1E.

110

To test pathogenicity, the parental 290-1E isolate or recovered virus vSVL-f02 were inoculated

111 into pigs. Six 2-month-old pigs were intradermally inoculated with 0.1 ml of  $10^{5.0}$  TCID<sub>50</sub> of the

112	viruses (group 1: 290-1E, group 2: vSVL-f02) at the right and front heel bulbs. Pigs were observed
113	for the appearance of clinical signs daily until 14 days post inoculation (dpi). Sera, saliva, and nasal
114	discharge were collected as described in our previous report (Fukai et al., 2015). Animal
115	experiments were authorized by the Animal Care and Use Committee of the National Institute of
116	Animal Health (NIAH) (authorization number: 14-060) and were performed in a high-containment
117	facility at the NIAH. All pigs infected with either 290-1E isolate or vSVL-f02 developed vesicles by
118	1 or 2 dpi on their feet, lips, and tongues (Table 1). Viruses were isolated from the sera (1-3 dpi),
119	saliva (1-6 dpi), and nasal swabs (2-3 dpi) of the 290-1E inoculated pigs, and from the sera (1-3
120	dpi), saliva (2-5 dpi) and nasal swabs (1-5 dpi) of the vSVL-f02 inoculated pigs by using LFBK
121	cells. Viral genes were detected in the sera (1-3 dpi), saliva (1-14 dpi) and nasal swabs (2-10 dpi)
122	collected from 290-1E inoculated pigs, and in the sera (1-8 dpi), saliva (2-14 dpi) and nasal swabs
123	(1-9 dpi) collected from vSVL-f02 inoculated pigs by reverse transcription PCR (RT-PCR) using the
124	FM8-9 primer set previously described (Sakamoto et al., 2002). Moreover, virus titers were
125	determined by the microtitration method using LFBK cells and viral RNA loads were determined by
126	real-time RT-PCR using primers and a probe described in the Manual of Diagnostic Tests and
127	Vaccines for Terrestrial Animals 2013 of World Organization for Animal Health. Viral titers in the

128	sera, saliva, and nasal swabs collected from pigs in group 1 were $10^{2.1}$ - $10^{6.1}$ TCID <sub>50</sub> /ml, $10^{2.6}$ - $10^{6.3}$
129	TCID <sub>50</sub> /ml and $10^{2.6}$ – $10^{6.6}$ TCID <sub>50</sub> /ml, respectively, while the viral RNA loads were $10^{6.6}$ – $10^{9.8}$
130	copies/ml, 10 <sup>7.5</sup> –10 <sup>10.6</sup> copies/ml and 10 <sup>7.7</sup> –10 <sup>10.2</sup> copies/ml (Sup. Tables 4 and 5). In group 2, viral
131	titers were $10^{2.8}-10^{5.3}$ TCID <sub>50</sub> /ml, $10^{2.6}-10^{5.6}$ TCID <sub>50</sub> /ml and $10^{2.6}-10^{6.9}$ TCID <sub>50</sub> /ml, respectively,
132	while the viral RNA loads were $10^{6.9}-10^{9.5}$ copies/ml, $10^{7.7}-10^{10.1}$ copies/ml and $10^{7.4}-10^{10.3}$
133	copies/ml (Sup. Tables 4 and 5). Antibodies were detected by liquid-phase blocking enzyme-linked
134	immuno-sorbent assay (LPBE) (Biological Diagnostic Supplies Limited, Scotland, UK) from 5 or 6
135	dpi in both groups and the antibodies against non-structural protein (NSP) of FMDV were detected
136	using the PrioCHECK FMDV NS Antibody ELISA Kit (Life Technologies) from 7 or 8 dpi, and 6-8
137	dpi in groups 1 and 2, respectively. Therefore, no significant differences in clinical signs, virus
138	isolation and titers, viral RNA loads and antibody responses were observed between groups 1 and 2.
139	In the present study, plasmid pSVL-f02 containing a full-length cDNA of FMDV O/JPN/2010
140	290-1E isolate was constructed and the vSVL-f02 virus was recovered following transfection of
141	mammalian cells. The pSVL-f02, which is based on pSVL, harbors a full-length FMDV cDNA
142	within the SV40 VP1 translational unit and carries the SV40 origin of replication. Therefore, the
143	replication efficiency of this plasmid should theoretically be greatly increased in Cos-7 cells which

144	express the SV40 large T antigen and result in high levels of protein expression following DNA
145	transfection (Mellon et al., 1981). In this study, contrary to expectations, transfection efficiency did
146	not increase in Cos-7 cells compared to IBRS-2 cells. The reason for this is unknown; however, it
147	was probably due to the difference in susceptibility to infection with FMDV between Cos-7 and
148	IBRS-2 cells.
149	The in vitro characteristics of vSVL-f02, including its antigenicity in the neutralization test,
150	indirect immunofluorescence, and plaque size and one-step growth, were identical to those of the
151	parental 290-1E isolate (Fig 2-4, Sup. Table 3). In the animal experiments, pigs inoculated with
152	either the vSVL-f02 or O/JPN/2010 290-1E isolate developed vesicles on their feet, lips and tongues
153	beginning at 1 or 2 dpi. In addition, there were no significant differences between groups 1 and 2 in
154	the dynamics of viral loads in serum, saliva, and nasal swabs or in the development of anti-FMDV
155	and anti-NSP antibodies (Table 1, Sup. Tables 4 and 5). These data demonstrate that the in vitro and
156	in vivo characteristics of the virus recovered from pSVL-f02 transfected cells were consistent with
157	those of parental virus. Therefore, we concluded that a full-length cDNA of FMDV O/JPN/2010
158	290-1E was successfully cloned and constructed. This infectious cDNA is therefore a valuable tool
159	to analyze the molecular mechanisms of differences in pathogenicity of O/JPN/2000 and

160	O/JPN/2010 strains, as well as to conduct further studies to understand the mechanisms of virus
161	replication and determinants of pathogenicity of FMDV, and to develop genetically engineered
162	vaccines.
163	
164	Declaration of conflicting interests
165	The author(s) declare no potential conflicts of interest with respect to the research, authorship,
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#### 176 **References**

- Beard, C.W., Mason, P.W., 2000. Genetic determinants of altered virulence of Taiwanese
  foot-and-mouth disease virus. Journal of Virology 74, 987-991.
- 179 Botner, A., Kakker, N.K., Barbezange, C., Berryman, S., Jackson, T., Belsham, G.J., 2011. Capsid
- 180 proteins from field strains of foot-and-mouth disease virus confer a pathogenic phenotype in
- 181 cattle on an attenuated, cell-culture-adapted virus. Journal of General Virology 92, 1141-1151.
- 182 Brehm, K.E., Ferris, N.P., Lenk, M., 2009. Highly sensitive fetal goat tongue cell line for detection
- and isolation of foot-and-mouth disease virus. Journal of Clinical Microbiology 47,
- 184 3156–3160.
- 185 Ellard, F.M., Drew, J., Blakemore, W.E., Stuart, D.I., King, A.M., 1999. Evidence for the role of
- 186 His-142 of protein 1C in the acid-induced disassembly of foot-and-mouth disease virus
- 187 capsids. Journal of General Virology 80, 1911-1918.
- 188 Falk, M.M., Sobrino, F., Beck, E., 1992. VPg gene amplification correlates with infective particle
- 189 formation in foot-and-mouth disease virus. Journal of Virology 66, 2251-2260.
- 190 Fukai, K., Morioka, K., Yoshida, K., 2011. An experimental infection in pigs using a foot-and-mouth
- 191 disease virus isolated from the 2010 epidemic in Japan. Journal of Veterinary Medical Science

# 192 73, 1207-1210.

193	Fukai, K., Yamada, M., Morioka, K., Ohashi, S., Yoshida, K., Kitano, R., Yamazoe, R., Kanno, T.,
194	2015. Dose-dependent responses of pigs infected with foot-and-mouth disease virus
195	O/JPN/2010 by the intranasal and intraoral routes. Archives of Virology 160, 129-139.
196	Garcia-Arriaza, J., Manrubia, S.C., Toja, M., Domingo, E., Escarmis, C., 2004. Evolutionary
197	transition toward defective RNAs that are infectious by complementation. Journal of Virology
198	78, 11678-11685.
199	Hema, M., Chandran, D., Nagendrakumar, S.B., Madhanmohan, M., Srinivasan, V.A., 2009.
200	Construction of an infectious cDNA clone of foot-and-mouth disease virus type O 1 BFS
201	1860 and its use in the preparation of candidate vaccine. Journal of Biosciences 34, 45-58.
202	LaRocco, M., Krug, P.W., Kramer, E., Ahmed, Z., Pacheco, J.M., Duque, H., Baxt, B., Rodriguez,
203	L.L., 2013. A continuous bovine kidney cell line constitutively expressing bovine $\alpha\nu\beta6$
204	integrin has increased susceptibility to foot-and-mouth disease virus. Journal of Clinical
205	Microbiology 51, 1714-1720. Author Correction. 2015. Journal of Clinical Microbiology 53,
206	755

207 Liu, G., Liu, Z., Xie, Q., Chen, Y., Bao, H., Chang, H., Liu, X., 2004. Generation of an infectious

cDNA clone of an FMDV strain isolated from swine. Virus Research 104, 157-164.

- Mason, P.W., Grubman, M.J., Baxt, B., 2003. Molecular basis of pathogenesis of FMDV. Virus
  Research 91, 9-32.
- 211 Mellon, P., Parker, V., Gluzman, Y., Maniatis, T., 1981. Identification of DNA sequences required for
- 212 transcription of the human  $\alpha$ 1-globin gene in a new SV40 host-vector system. Cell 27,
- 213 279-288.
- 214 Morioka, K., Fukai, K., Yoshida, K., Yamazoe, R., Onozato, H., Ohashi, S., Tsuda, T., Sakamoto, K.,
- 215 2009. Foot-and-mouth disease virus antigen detection enzyme-linked immunosorbent assay
- using multiserotype-reactive monoclonal antibodies. Journal of Clinical Microbiology 47,
- 217 3663-3668.
- 218 Muroga, N., Hayama, Y., Yamamoto, T., Kurogi, A., Tsuda, T., Tsutsui, T., 2012. The 2010
- foot-and-mouth disease epidemic in Japan. Journal of Veterinary Medical Science 74,
  399-404.
- 221 Onozato, H., Fukai, K., Kitano, R., Yamazoe, R., Morioka, K., Yamada, M., Ohashi, S., Yoshida, K.,
- 222 Kanno, T., 2014. Experimental infection of cattle and goats with a foot-and-mouth disease
- virus isolate from the 2010 epidemic in Japan. Archives of Virology 159, 2901-2908.

224	Pacheco, J.M., Henry, T.M., O'Donnell, V.K., Gregory, J.B., Mason, P.W., 2003. Role of
225	nonstructural proteins 3A and 3B in host range and pathogenicity of foot-and-mouth disease
226	virus. Journal of Virology 77, 13017-13027.
227	Piccone, M.E., Rieder, E., Mason, P.W., Grubman, M.J., 1995. The foot-and-mouth disease virus
228	leader proteinase gene is not required for viral replication. Journal of Virology 69, 5376-5382.
229	Rajasekhar, R., Hosamani, M., Basagoudanavar, S.H., Sreenivasa, B.P., Tamil Selvan, R.P.,
230	Saravanan, P., Venkataramanan, R., 2013. Rescue of infective virus from a genome-length
231	cDNA clone of the FMDV serotype O (IND-R2/75) vaccine strain and its characterization.
232	Research in Veterinary Science 95, 291-297.
233	Reid, S.M., Ferris, N.P., Hutchings, G.H., Samuel, A.R., Knowles, N.J., 2000. Primary diagnosis of
234	foot-and-mouth disease by reverse transcription polymerase chain reaction. Journal of
235	Virological Methods 89, 167-176.
236	Rieder, E., Bunch, T., Brown, F., Mason, P.W., 1993. Genetically engineered foot-and-mouth disease
237	viruses with poly(C) tracts of two nucleotides are virulent in mice. Journal of Virology 67,
238	5139-5145.

239 Sakamoto, K., Kanno, T., Yamakawa, M., Yoshida, K., Yamazoe, R., Murakami, Y., 2002. Isolation

240	of foot-and-mouth disease virus from Japanese black cattle in Miyazaki Prefecture, Japan,
241	2000. Journal of Veterinary Medical Science 64, 91-94.
242	Seago, J., Juleff, N., Moffat, K., Berryman, S., Christie, J.M., Charleston, B., Jackson, T., 2013. An
243	infectious recombinant foot-and-mouth disease virus expressing a fluorescent marker protein.
244	Journal of General Virology 94, 1517-1527.
245	Sugiura, K., Ogura, H., Ito, K., Ishikawa, K., Hoshino, K., Sakamoto, K., 2001. Eradication of foot
246	and mouth disease in Japan. Scientific and Technical Review 20, 701-713
247	van Rensburg, H.G., Henry, T.M., Mason, P.W., 2004. Studies of genetically defined chimeras of a
248	European type A virus and a South African Territories type 2 virus reveal growth determinants
249	for foot-and-mouth disease virus. Journal of General Virology 85, 61-68.
250	World Organization for Animal Health (2013) Chapter 2.1.5. Foot and mouth disease. Manual of
251	Diagnostic Tests and Vaccines for Terrestrial Animals 2013. Available from
252	http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf
253	Xin, A., Li, H., Li, L., Liao, D., Yang, Y., Zhang, N., Chen, B., 2009. Genome analysis and
254	development of infectious cDNA clone of a virulence-attenuated strain of foot-and-mouth
255	disease virus type Asia 1 from China. Veterinary Microbiology 138, 273-280.

256	Xin, A., Zhu, M., Hu, Q., Miao, H., Peng, Z., He, Y., Gao, L., Li, H., 2014. Effect of amino acid
257	mutation at position 127 in 3A of a rabbit-attenuated foot-and-mouth disease virus serotype
258	Asia1 on viral replication and infection. Virologica Sinica 29, 291-298.
259	Yamakawa, M., Kanno, T., Kaku, Y., Morioka, K., Yoshida, K., Sakamoto, K., 2002. Pathogenicity
260	of a Japanese isolate of foot-and-mouth disease virus, strain O/JPN/2000, to susceptible
261	animals. Topics in Animal Health Research 2, 49-50 (in Japanese).
262	Zibert, A., Maass, G., Strebel, K., Falk, M.M., Beck, E., 1990. Infectious foot-and-mouth disease
263	virus derived from a cloned full-length cDNA. Journal of Virology 64, 2467-2473.
264	

### 266 Figure Legends

267 Fig. 1. Construction of full-length cDNA clone of the FMDV O/JPN/2010 290-1E isolate. Five

268 cDNA clones comprising the genome of the 290-1E isolate were ligated to each other using

- 269 appropriate restriction enzymes and inserted into the pSVL plasmid to create a full-length cDNA
- identified as pSVL-f02.
- 271 Fig. 2. Indirect immunofluorescence test of IBRS-2 cells infected with vSVL-f02. Alexa Fluor

272 488-conjugated goat anti-mouse IgG (H+L) antibody (Life Technologies) was used for detection.

- 273 Coverslips were mounted using ProLong Gold Antifade Reagent with DAPI (Life Technologies). (a)
- vSVL-f02, (b) No infection.
- **Fig. 3.** Comparison of plaque size of 290-1E isolate and vSVL-f02. (a) and (c) 290-1E isolate
- 276 inoculated onto IBRS-2 or LFBK monolayer cells, respectively, (b) and (d) vSVL-f02 inoculated
- 277 onto IBRS-2 or LFBK monolayer cells, respectively. The cultures were fixed 1 day after the
- 278 inoculation and stained with crystal violet.
- Fig. 4. One-step growth curves of 290-1E isolate and vSVL-f02. The LFBK cell monolayers were
- 280 inoculated with each virus at a MOI of 2 and incubated at 37°C. Samples of supernatant were
- 281 collected at the indicated times and viral infectivity was determined using a standard plaque assay.

Group	Pig	* 7*	Clinical Days post infection												
No.	No.	Virus	sample	0	1	2	3	4	5	6	7	8	9	10	13/14
1	1	290-1E	Serum	-/- <sup>a</sup>	+/+	+/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
			Saliva	-/-	+/+	+/+	+/+	+/+	-/+	-/+	-/+	-/+	-/+	-/+	_/_
			Nasal swab	-/-	-/-	+/+	+/+	-/+	-/+	-/+	-/-	_/_	_/_	-/+	-/-
			$LPBE^{b}$	<32	< 32	<32	< 32	<32	45	90	181	256	181	362	181
			NSP <sup>c</sup>	-	-	-	-	-	-	-	+	+	+	+	+
	2		Serum	-/-	+/+	+/+	+/+	-/-	-/-	-/-	-/-	_/_	_/_	_/_	_/_
			Saliva	-/-	-/-	+/+	+/+	+/+	-/+	+/+	-/+	-/+	-/+	_/_	-/+
			Nasal swab	-/-	-/-	+/+	+/+	-/+	-/+	-/+	-/+	-/+	_/_	_/_	_/_
			LPBE	<32	< 32	<32	< 32	<32	< 32	45	90	181	181	256	181
			NSP	-	-	-	-	-	-	-	+	+	+	+	+
	3		Serum	-/-	+/+	+/+	+/+	-/-	_/_	-/-	-/-	-/-	-/-	-/-	-/-
			Saliva	-/-	_/_	+/+	+/+	+/+	-/+	-/+	-/+	-/+	-/+	-/+	-/-
			Nasal swab	-/-	-/-	+/+	+/+	-/+	-/+	-/+	-/+	-/+	_/_	-/+	_/_
			LPBE	<32	< 32	<32	< 32	<32	<32	32	45	90	181	256	362
			NSP	-	-	-	-	-	-	-	-	+	+	+	+
2	4	vSVL-f02	Serum	-/-	+/+	+/+	-/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

Table 1. Isolation of viruses, detection of viral genes, and detection of antibodies in clinical samples obtained from infected pigs

	Saliva	-/-	-/-	+/+	+/+	-/+	-/+	-/+	-/-	-/+	-/+	_/_	-/-
	Nasal swab	_/_	-/-	+/+	+/+	-/+	-/+	-/+	-/+	-/+	-/+	-/-	-/-
	LPBE	< 32	< 32	< 32	< 32	< 32	< 32	45	90	90	90	90	181
	NSP	-	-	-	-	-	-	-	-	+	+	+	+
5	Serum	-/-	+/+	+/+	+/+	-/-	-/-	_/_	-/-	-/-	-/-	-/-	-/-
	Saliva	-/-	-/-	+/+	+/+	+/+	+/+	-/+	-/+	-/+	-/+	-/+	-/-
	Nasal swab	-/-	+/+	+/+	+/+	+/+	+/+	-/+	-/+	-/+	-/-	_/_	_/_
	LPBE	< 32	<32	<32	< 32	<32	32	45	90	181	181	181	256
	NSP	-	-	-	-	-	-	-	+	+	+	+	+
6	Serum	-/-	+/+	+/+	+/+	-/-	_/_	-/-	-/+	-/+	-/-	-/-	-/-
	Saliva	-/-	-/-	+/+	+/+	+/+	-/+	-/+	-/+	_/_	-/+	_/_	-/+
	Nasal swab	-/-	-/-	+/+	+/+	+/+	-/+	-/+	-/+	-/+	-/-	_/_	-/-
	LPBE	< 32	< 32	<32	< 32	<32	64	181	362	362	256	181	256
	NSP	-	-	-	-	-	-	+	+	+	+	+	+

Boxes in the table indicate the day at which obvious vesicles appeared in each pig.

<sup>a</sup> Isolation of virus/detection of virus gene.

<sup>b</sup>Antibodies against foot-and-mouth disease virus (FMDV) were detected and titrated by liquid-phase blocking enzyme-linked immuno-sorbent assay (LPBE).

<sup>c</sup> Antibodies against non-structural protein (NSP) of FMDV were detected using the PrioCHECK FMDV NS Antibody ELISA Kit.

Fig. 1



pSVL-f02









