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Short communication

Genome variability of foot-and-mouth disease virus during the short period of the 2010 epidemic in Japan

Tatsuya Nishi, Manabu Yamada, Katsuhiko Fukai, Nobuaki Shimada, Kazuki Morioka, Kazuo Yoshida, Kenichi Sakamoto, Toru Kanno, Makoto Yamakawa*

Exotic Disease Research Unit, Division of Transboundary Animal Diseases, National Institute of Animal Health, National Agriculture and Food Research Organization, 6-20-1, Josui-honcho, Kodaira, Tokyo, 187-0022, Japan

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ABSTRACT

Foot-and-mouth disease virus (FMDV) is highly contagious and has a high mutation rate, leading to extensive genetic variation. To investigate how FMDV genetically evolves over a short period of an epidemic after initial introduction into an FMD-free area, whole L-fragment sequences of 104 FMDVs isolated from the 2010 epidemic in Japan, which continued for less than three months were determined and phylogenetically and comparatively analyzed. Phylogenetic analysis of whole L-fragment sequences showed that these isolates were classified into a single group, indicating that FMDV was introduced into Japan in the epidemic via a single introduction. Nucleotide sequences of 104 virus isolates showed more than 99.56% pairwise identity rates without any genetic deletion or insertion, although no sequences were completely identical with each other. These results indicate that genetic substitutions of FMDV occurred gradually and constantly during the epidemic and generation of an extensive mutant virus could have been prevented by rapid eradication strategy. From comparative analysis of variability of each FMDV protein coding region, VP4 and 2C regions showed the highest average identity rates and invariant rates, and were confirmed as highly conserved. In contrast, the protein coding regions VP2 and VP1 were confirmed to be highly variable regions with the lowest average identity rates and invariant rates, respectively. Our data demonstrate the importance of rapid eradication strategy in an FMD epidemic and provide valuable information on the genome variability of FMDV during the short period of an epidemic.

Foot-and-mouth disease (FMD) is the most contagious disease of mammals, and causes severe economic damage to livestock industries. FMD virus (FMDV) is classified into the genus *Aphthovirus* of the family *Picornaviridae*. Its genome is composed of a single-stranded positive-sense RNA of approximately 8.4 kilobases (kb) in length which is divided into S-fragment and L-fragment by poly (C) sequence at the 5′ terminus of the genome. The open-reading frame of FMDV is composed of 12 proteins, L, VP1-4, 2A–2C, 3A–3D. The FMDV capsid surface is covered by VP1, 2 and 3, and held by VP4 which is buried within the virion (Mason et al., 2003). To date, a total of five primary antigenic sites have been identified on FMDV type O, three of which are located on the VP1 region (Kitson et al., 1990; Crowther et al., 1993). VP1 is especially considered to be a dominant protein which reveals characteristic of FMDV, and is usually sequenced for genetic analysis in diagnosis (World Organization for Animal Health, 2015).

In April 2010, an FMD outbreak occurred in Miyazaki Prefecture, in the south of Japan (Muroga et al., 2012). After confirmation of the first case on April 20th, the epidemic continued until July 4th and involved a total of 292 farms and over 200,000 cattle, swine, sheep and goats, and about 290,000 animals have been culled, including vaccinated animals. During the epidemic, the Japanese eradication strategy was implemented, which includes stamping out, movement restrictions and disinfection of contaminants. Finally, the epidemic was suppressed after a total of 76 days in one prefecture. A virus isolate from the first case, O/JPN/1/2010 (GenBank accession no. KF112885), was phylogenetically analyzed using sequences of the VP1 region, and classified into genotype Mya-98 of topotype Southeast Asia (Valdazo-González et al., 2013). In addition, from the results of experimental infections, this virus isolate was confirmed to be pathogenic in swine, cattle and goats, and to spread efficiently by direct contact (Fukai et al., 2011; Onozato et al., 2014).

* Corresponding author.
E-mail address: yamato@affrc.go.jp (M. Yamakawa).

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Table 1
Virus isolates obtained from the 2010 epidemic in Japan in the present study.

Isolate	Date of sampling	Species	Passage history[a]	GenBank accession no.
O/JPN/2010-1/14C	April, 09	cattle	BK1	LC149617
O/JPN/2010-2/5S	April, 20	cattle	BK1	LC149618
O/JPN/2010-3/2S	April, 20	cattle	BK1	LC149619
O/JPN/2010-5/6S	April, 22	cattle	BK1	LC149620
O/JPN/2010-6/1S	March, 31	buffalo	BK1LF1	LC149621
O/JPN/2010-8/1S	April, 24	cattle	BK1	LC149622
O/JPN/2010-9/1S	April, 27	cattle	BK1	LC149623
O/JPN/2010-10/396V	April, 27	swine	BK1LF1	LC149624
O/JPN/2010-11/4S	April, 27	cattle	BK1	LC149625
O/JPN/2010-15/1S	April, 28	cattle	BK1	LC149626
O/JPN/2010-17/593F	April, 29	swine	BK1	LC149627
O/JPN/2010-18/3S	April, 30	swine	BK1	LC149628
O/JPN/2010-21/3S	April, 30	cattle	BK1LF1	LC149629
O/JPN/2010-22/3S	May, 01	swine	BK1	LC149630
O/JPN/2010-23/2S	May, 02	cattle	BK1	LC149631
O/JPN/2010-27/3S	May, 03	swine	BK1LF1	LC149632
O/JPN/2010-28/2S	May, 04	swine	BK1	LC149633
O/JPN/2010-29/4S	May, 04	swine	BK1	LC149634
O/JPN/2010-39/2S	May, 05	swine	BK1LF1	LC149635
O/JPN/2010-46/3S	May, 06	cattle	BK1LF1	LC149636
O/JPN/2010-47/3S	May, 06	swine	BK1LF1	LC149637
O/JPN/2010-50/1S	May, 06	cattle	BK1LF1	LC149638
O/JPN/2010-55/1S	May, 07	swine	BK1LF1	LC149639
O/JPN/2010-61/1S	May, 07	cattle	BK1LF1	LC149640
O/JPN/2010-65/3S	May, 08	swine	BK1LF1	LC149641
O/JPN/2010-68/3S	May, 08	cattle	BK1LF1	LC149642
O/JPN/2010-72/3S	May, 09	swine	IB1LF1	LC149643
O/JPN/2010-82/1S	May, 09	cattle	IB1LF1	LC149644
O/JPN/2010-83/2S	May, 09	cattle	IB1LF1	LC149645
O/JPN/2010-84/1S	May, 09	swine	IB1LF1	LC149646
O/JPN/2010-85/1S	May, 10	cattle	IB1LF1	LC149647
O/JPN/2010-86/1S	May, 10	cattle	BK1LF1	LC149648
O/JPN/2010-87/1S	May, 10	swine	BK1LF1	LC149649
O/JPN/2010-88/5S	May, 10	cattle	BK1LF1	LC149650
O/JPN/2010-90/5S	May, 11	cattle	BK1LF1	LC149651
O/JPN/2010-92/3S	May, 11	swine	BK1LF1	LC149652
O/JPN/2010-93/2S	May, 11	swine	BK1LF1	LC149653
O/JPN/2010-94/5S	May, 11	cattle	BK1LF1	LC149654
O/JPN/2010-98/1S	May, 12	cattle	BK1LF1	LC149655
O/JPN/2010-100/5S	May, 12	cattle	BK1LF1	LC149656
O/JPN/2010-102/2S	May, 12	swine	BK1LF1	LC149657
O/JPN/2010-103/1S	May, 12	swine	IB1LF1	LC149658
O/JPN/2010-104/2S	May, 12	cattle	BK1LF1	LC149659
O/JPN/2010-105/2S	May, 12	cattle	BK1LF1	LC149660
O/JPN/2010-106/4S	May, 12	cattle	BK1LF1	LC149661
O/JPN/2010-107/5S	May, 13	cattle	BK1LF1	LC149662
O/JPN/2010-108/5S	May, 13	cattle	BK1LF1	LC149663
O/JPN/2010-110/5S	May, 13	cattle	BK1LF1	LC149664
O/JPN/2010-111/2S	May, 13	cattle	BK1LF1	LC149665
O/JPN/2010-113/2S	May, 13	cattle	BK1LF1	LC149666
O/JPN/2010-115/2S	May, 13	cattle	BK1LF1	LC149667
O/JPN/2010-118/1S	May, 14	cattle	BK1LF1	LC149668
O/JPN/2010-121/3S	May, 14	cattle	BK1LF1	LC149669
O/JPN/2010-122/4S	May, 14	cattle	BK1LF1	LC149670
O/JPN/2010-123/3S	May, 14	cattle	BK1LF1	LC149671
O/JPN/2010-124/5S	May, 14	cattle	BK1LF1	LC149672
O/JPN/2010-125/1S	May, 14	cattle	BK1LF1	LC149673
O/JPN/2010-126/3S	May, 14	cattle	BK1LF1	LC149674
O/JPN/2010-127/1S	May, 15	cattle	BK1LF1	LC149675
O/JPN/2010-128/3S	May, 15	swine	BK1LF1	LC149676
O/JPN/2010-129/2S	May, 15	cattle	BK1LF1	LC149677
O/JPN/2010-133/5S	May, 15	cattle	BK1LF1	LC149678
O/JPN/2010-134/2S	May, 15	swine	BK1LF1	LC149679
O/JPN/2010-135/1S	May, 15	cattle	BK1LF1	LC149680
O/JPN/2010-137/3S	May, 15	cattle	BK1LF1	LC149681
O/JPN/2010-138/3S	May, 15	cattle	BK1LF1	LC149682
O/JPN/2010-140/1S	May, 15	cattle	BK1LF1	LC149683
O/JPN/2010-141/3S	May, 15	swine	BK1LF1	LC149684
O/JPN/2010-142/1S	May, 15	cattle	BK1LF1	LC149685
O/JPN/2010-144/2S	May, 16	cattle	IB1LF1	LC149686
O/JPN/2010-145/5S	May, 16	cattle	BK1LF1	LC149687
O/JPN/2010-146/3S	May, 16	swine	IB1LF1	LC149688
O/JPN/2010-148/3S	May, 16	cattle	BK1LF1	LC149689
O/JPN/2010-151/1S	May, 16	swine	BK1LF1	LC149690
O/JPN/2010-152/2S	May, 16	swine	BK1LF1	LC149691

Table 1 (*Continued*)

Isolate	Date of sampling	Species	Passage history[a]	GenBank accession no.
O/JPN/2010-157/3S	May, 16	swine	BK1LF1	LC149692
O/JPN/2010-159/3S	May, 17	cattle	BK1LF1	LC149693
O/JPN/2010-163/3S	May, 17	cattle	BK1LF1	LC149694
O/JPN/2010-165/2S	May, 17	cattle	BK1LF1	LC149695
O/JPN/2010-168/3S	May, 17	cattle	BK1LF1	LC149696
O/JPN/2010-169/1S	May, 17	swine	BK1LF1	LC149697
O/JPN/2010-171/2S	May, 17	cattle	BK1LF1	LC149698
O/JPN/2010-172/1S	May, 17	cattle	BK1LF1	LC149699
O/JPN/2010-173/3S	May, 18	cattle	BK1LF1	LC149700
O/JPN/2010-174/1S	May, 18	swine	BK1LF1	LC149701
O/JPN/2010-180/3S	May, 18	swine	BK1LF1	LC149702
O/JPN/2010-188/2S	May, 19	cattle	BK1LF1	LC149703
O/JPN/2010-194/3S	May, 20	cattle	BK1LF1	LC149704
O/JPN/2010-195/3S	May, 20	cattle	BK1LF1	LC149705
O/JPN/2010-196/3S	May, 20	cattle	BK1LF1	LC149706
O/JPN/2010-198/2S	May, 20	cattle	BK1LF1	LC149707
O/JPN/2010-199/3S	May, 20	cattle	BK1LF1	LC149708
O/JPN/2010-200/3S	May, 20	cattle	BK1LF1	LC149709
O/JPN/2010-201/1S	May, 20	cattle	BK1LF1	LC149710
O/JPN/2010-202/3S	May, 20	cattle	BK1LF1	LC149711
O/JPN/2010-216/3S	May, 21	cattle	BK1LF1	LC149712
O/JPN/2010-244/3S	May, 24	cattle	BK1LF1	LC149713
O/JPN/2010-247/3S	May, 24	cattle	IB1LF1	LC149714
O/JPN/2010-253/2S	May, 24	cattle	IB1LF1	LC149715
O/JPN/2010-290/1S	May, 29	cattle	BK1	LC149716
O/JPN/2010-324/3	June, 01	cattle	BK1ZR1	LC149717
O/JPN/2010-351/1S	June, 09	cattle	BK1LF1	LC149718
O/JPN/2010-354/3	June, 10	cattle	BK1LF1	LC149719
O/JPN/2010-362/3	June, 11	swine	BK1LF1	LC149720

[a] BK, primary bovine kidney cell; LF, LFBK-αvβ6 cell; IB, IBRS-2 cell; ZR, ZZR-127 cell.

As with other RNA viruses, the FMDV genome has the high mutation rate of 10^{-3} to 10^{-5} per genomic replication, leading to enormous genetic variation due to poor fidelity of RNA replication (Drake and Holland, 1999). Several genetic analyses of FMDV have appeared, including sequence variability of FMDVs isolated from several areas and years (Abdul-Hamid et al., 2011; Subramaniam et al., 2015; Upadhyaya et al., 2014), comparative analysis of FMDVs representing seven different serotypes (Carrillo et al., 2005), and molecular epidemiological analysis of sporadic outbreaks (Cottam et al., 2006, 2008). To date, however, few studies have described the genome dynamics of the whole L-fragment gene of FMDV over a short period and limited scale of an epidemic in an FMD-free area, such as Japan. Here, we analyzed how FMDVs genetically evolved during the short period of the 2010 epidemic in Japan. Virus isolates obtained from each case were subjected to phylogenetic analysis, and their whole L-fragment genes and amino acid sequences were comparatively analyzed.

Clinical samples were submitted by the Miyazaki Prefectural Government for diagnosis of FMD occurring in 2010 in Japan. These samples were collected by veterinarians in accordance with the guidelines of the Act on Domestic Animal Infectious Diseases Control, in which the veterinarians collect samples, such as epitheliums or swabs from a lesion and soaks them in 2 ml of PBS. Virus isolation from clinical samples of cattle, swine and buffalo of the 2010 epidemic was conducted using the primary bovine kidney (BK), IBRS-2, LFBK-$\alpha_v\beta_6$ cells (LaRocco et al., 2013, 2015) and ZZR-127 cells (Brehm et al., 2009) according to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2015 (World Organization for Animal Health, 2015). One hundred and four virus isolates were obtained from samples collected between March 31st and Jun 11th (Table 1).

Viral RNA was extracted from the supernatant of infected cells using the High Pure Viral RNA Kit (Roche Diagnostics, Tokyo, Japan). First-strand cDNA synthesis was performed using the SuperScript III Reverse Transcriptase (Life Technologies, Tokyo, Japan) and FMDV-specific 2B331R primer (5′-GGCACGTGAAAGAGACTGGAGAG-3′) and 3′-2010R primer (5′-TGGATAAAGGAAACGGGAAAAGC-3′). Full length of the L-fragment gene of approximately 7.7 kb was amplified by polymerase chain reaction with PrimeSTAR Max DNA Polymerase (TaKaRa, Shiga, Japan) and two primer sets: set 1 consisted of 5′-2010F primer (5′-CGTTAAAGGGAGGTAACCACAAG-3′) and 2B331R primer and set 2 consisted of 2B217F primer (5′-ATGGCCGCTGTAGCAGCACGGTC-3′) and 3′-2010R primer. Their nucleotide sequences were analyzed using the Ion PGM system (Life Technologies). These sequences have been deposited to the GenBank (accession no. LC149617-LC149720). The following sequence ambiguity code was used: K (T/G), M (A/C), R (A/G), S (C/G), W (A/T), Y (C/T), B (C/T/G), D (A/T/G), H (A/C/T), V (A/C/G), and N (A/C/G/T).

L-fragment nucleotides of 104 virus isolates were phylogenetically analyzed with 14 closely related strains from other countries registered at GenBank by the maximum-likelihood (ML) method using the MEGA 6.0 software (http://www.megasoftware.net/) and Bayesian Markov Chain Monte Carlo (MCMC) method using the BEAST program (Drummond et al., 2012) based on the Tamura-Nei model (Fig. 1). Both results showed that the virus isolates of the 2010 epidemic in Japan were classified into a single group. It indicated that there was a single introduction of FMDV from other countries into Japan through the epidemic. In addition, in the ML phylogenetic tree, the virus isolate of O/JPN/2010-6/1S was the most closely related to a foreign virus (O/BY/CHA/2010), with a nucleotide sequence identity rate of 99.41%, and was thus speculated to be the initial isolate of these 104 virus isolates. This speculation is consistent with an epidemiological survey of this epidemic (Ministry of Agriculture, Forestry and Fisheries, 2010).

To analyze how FMDVs genetically evolved through the epidemic, L-fragment sequences of 104 virus isolates were compared each other and pairwise identity rates among them were calculated using the MEGA 6.0 and GENETYX Ver. 12 (GENETYX). Among these 104 virus isolates, no sequences were completely identical with each other, and no genetic deletion or insertion was seen. Pairwise identity rates of these 104 virus

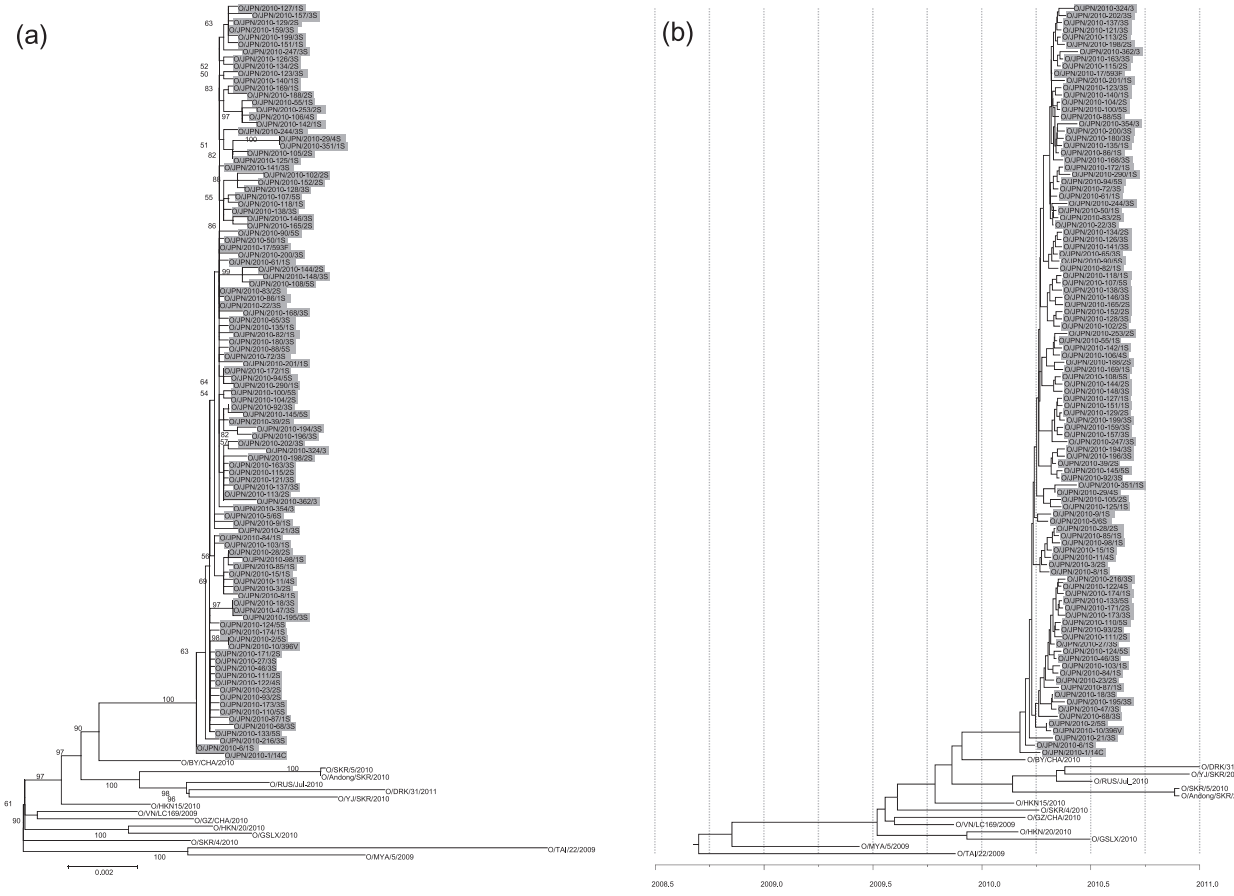

Fig. 1. Phylogenetic analysis of FMDV isolates using L-fragment gene. (a) 7668 nucleotide bases of the whole L-fragment of FMDVs were used for ML phylogenetic analysis based on the Tamura-Nei model. Horizontal distances are proportional to the minimum number of nucleotide differences required to join nodes and sequences. Numbers at each node indicate the confidence level in bootstrap analysis with 1000 replications. (b) 7668 nucleotide bases of the whole L-fragment of FMDVs were used for MCMC method using the BEAST program based on the Tamura-Nei model. Isolation dates were used to calibrate the molecular clock. MCMC chains were run for 10 million states, sampled every 1000 states. One-hundred and four virus isolates obtained in the present study were colored gray.

isolates ranged from 99.56% to 99.98% (average 99.83%) (Table 2). The mean rate of substitution of them was estimated to be 2.88×10^{-5} per site per day using the BEAST program. These results indicated that genetic substitutions of the virus isolates had occurred not drastically during the epidemic but gradually and constantly over this period. This genetic variability was presumably induced by the high mutation rate of FMDV during replication and transmission in the course of the epidemic.

Nucleotide and amino acid sequences of each of the 12 protein coding regions of the 104 virus isolates were compared and pairwise identity rates and invariant rates of them were calculated (Table 2). For these 12 proteins, average identities of amino acid sequences of VP4 and 2C were the highest, 99.93%, and invariant rates of them in both nucleotide and amino acid sequences were more than 96.0%. Therefore, among these 12 proteins, VP4 and 2C were confirmed to be the most highly conserved regions in the 2010 epidemic (Table 2). VP4 is one of the structural proteins holding viral structural conformation within the capsid, and is cleaved from VP0 together with VP2 in the final step of capsid assembly (Mason et al., 2003). The 2C region plays an important role in membrane binding during viral replication (Echeverri et al., 1998), and has ATPase and GTPase activities (Rodríguez and Carrasco, 1993). These protein coding regions have been previously reported to be highly conserved (Mason et al., 2003), consistent with our present results, and speculated to need to be conserved for efficient transmission among the epidemic.

Average identity rate of VP2 and invariant rate of VP1 in amino acid sequences were the lowest, 99.46% and 89.20%, respectively (Table 2). It indicates that these protein coding region were highly variable region even in the limited scale of epidemic. To analyze variable amino acid positions of FMDV among the epidemic, full amino acid sequences of 104 virus isolates were compared with consensus sequence of them (Fig. 2). As a result, six amino acid positions were confirmed as comparatively variable sites with more than 7.7% (8 of 104 virus isolates) proportion of samples with substitutions of amino acids, No.17 of L, No.78-79, 132 and 182 of VP2, No.63 of 3C and No.158 of 3D (Table 3). No.78-79 and 132 of VP2 are expected to be on the antigenic site 2 and reported as highly variable region (Kitson et al., 1990). In addition, No.79 of VP2 is reported to be associated with persistent infection in cattle (Horsington and Zhang, 2007), although persistent infection was not reported in this 2010 epidemic in Japan. High variety of amino acid substitution at No.17 of L and high fixed substitution at No.63 of 3C were confirmed (Table 3); however, relation between these variability and virus characterization were not analyzed. By using infectious cDNA of the O/JPN/2010, influences of these substitutions to biological feature of FMDV would be elucidated in the future study.

In the analysis of variable amino acid positions, any amino acid positions of VP1 which includes three of five antigenic sites and major receptor-binding sites and, thus, is expected to be variable region was not confirmed as highly variable. Similarly, other reports have indicated that substitutions in the VP1 region were no more frequent than in other parts of the genome through in vivo passages and epidemics (Carrillo et al., 2007; Cottam et al., 2006). These data suggest that genetic substitutions in the VP1 region

Table 2
Pairwise identity rates and invariant rates of each protein coding region of virus isolates obtained from the 2010 epidemic in Japan.

Genome region[a]		No. of Positions aligned	Average identity rate (%)	Invariant rate (%)
L-fragment	nt	7668	99.83 (99.56–99.98)	95.25
L	nt	603	99.81 (99.00–100)	93.70
	aa	201	99.67 (97.51–100)	89.55
VP4	nt	255	99.92 (99.21–100)	96.08
	aa	85	99.93 (97.64–100)	96.47
VP2	nt	654	99.69 (98.77–100)	93.27
	aa	218	99.46 (98.16–100)	93.12
VP3	nt	660	99.86 (98.93–100)	95.00
	aa	220	99.82 (97.72–100)	92.73
VP1	nt	639	99.86 (99.06–100)	94.05
	aa	213	99.76 (97.65–100)	89.20
2A	nt	48	99.76 (95.83–100)	89.58
	aa	16	99.88 (93.75–100)	93.75
2B	nt	462	99.77 (98.05–100)	94.59
	aa	154	99.75 (95.45–100)	91.56
2C	nt	954	99.90 (99.47–100)	96.96
	aa	318	99.93 (99.05–100)	96.54
3A	nt	459	99.84 (98.91–100)	94.12
	aa	153	99.76 (98.03–100)	92.81
3B	nt	213	99.54 (98.12–100)	93.90
	aa	71	99.60 (97.18–100)	91.55
3C	nt	639	99.90 (99.21–100)	97.34
	aa	213	99.74 (98.23–100)	95.77
3D	nt	1413	99.87 (99.36–100)	96.46
	aa	471	99.87 (98.93–100)	95.97

[a] nt, nucleotide; aa, amino acid.

were not fixed on defined position but occurred scatteredly through some selective environments of passages in animals.

In the present study, virus was isolated from clinical samples and replicated using BK, IBRS-2, LFBK-$\alpha_v\beta_6$ and ZZR-127 cells (Table 1), from which viral RNAs were extracted for sequencing. To verify the effect of passage through cell culture, whole L-fragment sequences of a virus derived directly from an epithelium of a pig inoculated with O/JPN/2010-1/14C and viruses passaged through cell cultures by all patterns used in the present study were determined and compared. Animal experiments were authorized by the Animal Care and Use Committee of the National Institute of Animal Health (NIAH) (authorization number: 13-084) and were performed in a high-containment facility at the NIAH. Through the passage, no significant substitution through passage were observed, except for three selections from ambiguity code R (A/G) at nucleotide No. 313 of VP3, M (A/C) at nucleotide No. 399 of VP1, and Y (C/T) at nucleotide No. 26 of 3D to a single nucleotide of A, C, and C, respectively. This result suggests that genetic substitutions in this study did not occur by passage through cell culture, but rather through animals during the epidemic.

To analyze the effect of host species tropism, amino acid sequences of 74 viruses isolated from cattle were compared with consensus sequence of 29 viruses isolated from swine, and vice versa, and identity rates of each amino acid positions were calculated. There was no amino acid position which showed less than 50% of identity to the consensus sequence of viruses isolated from another species, therefore, specific substitution related to host species was not confirmed. In addition, in this study, genetic mutants as reported deletion in the 3A region which significantly contribute to virulence in swine and cattle was not confirmed (Beard and Mason, 2000). Up until the 2010 epidemic, Japan had remained FMD-free since 2000, and in the 2010 epidemic, all infected, suspected and vaccinated animals were culled as soon as FMD was confirmed. Therefore, the selection pressure of transmission across hosts and immunity induced by infection or vaccination were presumably limited in this epidemic. The rapid eradication strategy mentioned above was assumed to have

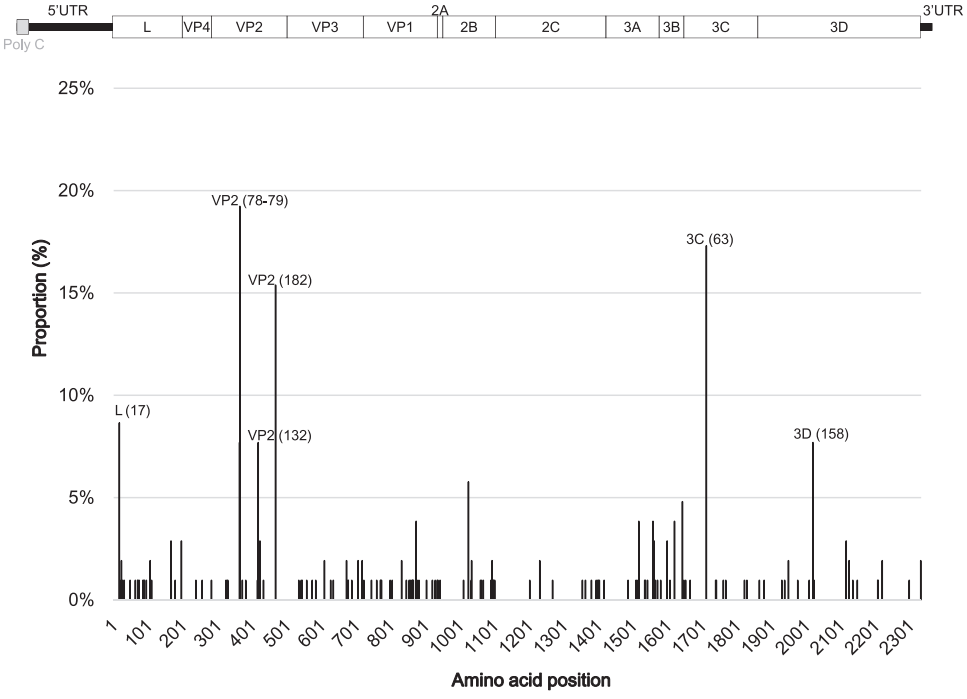

Fig. 2. Variable amino acid positions of FMDV isolates obtained from the 2010 epidemic in Japan. Proportion of samples with substitution of amino acids on each amino acid positions of 104 virus isolates compared with the consensus sequence of them were calculated. Six positions at which proportion of samples with substitution were more than 7.7% (8 of 104 virus isolates) were indicated with protein coding regions belonging and amino acid number.

Table 3
Variable amino acid positions of FMDVs obtained from the 2010 epidemic in Japan.

Genome region	aa no.[a]	aa	Proportion (%)	No. of viruses
L	17	Ile	91.4	95
		Thr	6.7	7
		Lys	1.0	1
		Met	1.0	1
VP2	78	Cys	92.3	96
		Tyr	4.8	5
		Tyr/Cys	2.9	3
	79	Tyr	80.8	84
		His	9.6	10
		Cys	5.8	6
		Tyr/His	3.8	4
VP2	132	Ile	92.3	96
		Thr	2.9	3
		Ile/Thr	4.8	5
VP2	182	Met	84.6	88
		Thr	6.7	7
		Val	4.8	5
		Met/Thr	3.8	4
3C	63	Ile	82.7	86
		Thr	17.3	18
3D	158	Ala	92.3	96
		Val	7.7	8

[a] aa, amino acid.

contributed to suppress the scale of the epidemic and, as a result, prevented generation of an extensive mutant virus. By comparing our data with those of epidemics in endemic areas with high selective pressure, it would be elucidated how selective pressure influences the genetic variability of FMDV.

In the present study, genome variability of FMDV during the short period of the 2010 epidemic in Japan was analyzed by phylogenetic and comparative analyses using whole L-fragment genes of virus isolates obtained from each case. More precise transmission pathways based on nucleotide sequences, reports of clinical signs and antibody titers of infected animals, and epidemiological information, such as movements of concerned people and vehicles, would be conducted as a further research. Few studies have examined the genome variability of the whole genome of FMDV within the short period and limited scale of a sporadic epidemic in an FMD-free area. Our data should therefore provide valuable information about the genome dynamics of the highly contagious RNA virus like FMDV.

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