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Research article

Direct typing and molecular evolutionary analysis of field samples of foot-and-mouth disease virus collected in Viet Nam between 2006 and 2007

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ABSTRACT

In this study, we used universal or duplex serotype-specific (O and Asia 1) RT-PCR to analyze clinical field samples of foot-and-mouth disease virus (FMDV) or virus isolates collected in Viet Nam between 2006 and 2007. We found viral serotypes O and Asia 1 circulating concurrently during this period. Direct sequencing of type-specific RT-PCR products revealed the existence of three different topotypes of serotype O: Southeast Asia (SEA), Middle East-South Asia (ME-SA), and Cathay. Of these, SEA was most prevalent during the period. All samples of serotype Asia 1 belonged to genetic group V. Based on the rooted maximum likelihood phylogenetic trees inferred from the VP1 region, new lineages in topotype SEA were originating from Viet Nam, and group V strains of Asia 1 have undergone fewer passages from the common ancestor, compared with other genetic groups. The co-circulation of different types of FMDV may complicate the individual or population genomic structures of FMDV and make conventional multiplex diagnostic methods and phylogenetic analyses with relevant evolutionary models essential in Viet Nam.

1. Introduction

Foot-and-mouth disease virus (FMDV) of the family *Picornaviridae*, genus *Aphthovirus*, causes acute and systemic disease in domestic and wild cloven-hoofed animals. FMDV has a single-stranded, positive-sense RNA genome and comprises seven immunologically distinct serotypes: Euroasiatic serotypes A, O, C, and Asia 1 and South African Territories serotypes SAT1, SAT2, and SAT3.

In Southeast Asia, FMD is caused by multiple serotypes of FMDV, including O, A, and Asia 1 (Gleeson, 2002). It is endemic in several countries, and outbreaks of FMD have had significant impacts on their agricultural systems (Perry et al., 2002). In case of Lao PDR (bordering Viet Nam) between 1998 and 2006, type O was the dominant serotype, but serotype A viruses were detected only in 2003 and 2006 and serotype Asia 1 was identified only in 1996 and 1998 (Khounsy et al., 2009). In Viet Nam, three topotypes (Cathay, ME-SA, and SEA) of serotype O and genetic groups IV and V of serotype Asia 1 were discovered between 2005 and 2008, but sequence data were not published (Morrissy et al., 2008). For serotype Asia 1, genetic group V virus spread to China, Russia, and Mongolia in 2005, and to North Korea in 2007; evidence of inter-topotypic recombination of the 2C coding region in groups V and III has also been reported (Lee et al., 2009; Valarcher et al., 2009). Because Viet Nam is

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bordered by China to the north, the new genetic group V might have been introduced into Viet Nam, in contrast to genetic group IV, which was indigenous in Viet Nam and the rest of Southeast Asia until 2006 (Valarcher et al., 2009).

In the present study, we developed a duplex (types O and Asia 1) RT-PCR method for the molecular detection and characterization of FMD outbreaks in Viet Nam. We applied this method to clinical samples (or their isolates) that had been sent to the National Center for Veterinary Diagnosis (NCVD) from northern and central Viet Nam between March 2006 and December 2007. This method employed a single tube and buffer system for RT-PCR (Tosh et al., 1997) with serotype-specific primers (Giridharan et al., 2005; Rodriguez et al., 1992; Vangrysperre and De Clercq, 1996). All reagents were customized to be mixed and dried, to lessen the possibility of cross-contamination during diagnosis. The amplified PCR products were directly sequenced (Locher et al., 1995) for typing, after FMD detection. Although sets of type-specific primers for the detection of FMD serotypes O, A, C, and Asia 1 have been developed and validated for multiplex RT-PCR (Callens and De Clercq, 1997; Reid et al., 1999; Vangrysperre and De Clercq, 1996), we designed new sets of primers specific for types O and Asia 1, to account for newly reported sequences of diverse genotypes in the GenBank database.

Here, we detected FMDV infections in a type-specific manner and were able to determine their genotypes by direct sequencing of PCR products, and we confirmed the introduction of group V of type Asia 1 into Viet Nam in 2006 and its circulation in 2007. Moreover, we explained the uneven evolutionary patterns between different topotypes of type O or between different genetic groups of type Asia 1, uncovered in the large phylogenies using a maximum likelihood program (Guindon and Gascuel, 2003).

2. Materials and methods

2.1. Clinical samples and isolates

We tested 35 clinical field samples or cell culture isolates from clinical samples originating in 13 provinces of Viet Nam. The clinical samples were sent to the NCVD between March 2006 and December 2007. The sample number (assigned in order of collection date), date of collection in the field, regional origin, and host species for each sample are listed in Table 1. Viral RNAs were extracted using an RNeasy

Table 1
Description of clinical samples or cell culture isolates. Sample identification numbers were assigned in the order of the collection date in the field. The provincial origin, species, and typing results by duplex RT-PCR or direct sequencing are shown.

No.	Samples	Date of collection	Province	Host species	Serotyping result	Subtyping result	Sequence references[†]
1	Clinical samples	2006.03.31	Lao Cai	Buffalo	n.d.	n.a.	n.a
2	Cell supernatant	2006.06.21	Thanh Hoa	Cattle	Asia 1	V	Table 3
3	Clinical samples	2006.06.27	Vinh Phuc	Buffalo	O	SEA[a]	Fig. 1
4	Clinical samples	2006.07.10	Thai Nguyen	Cattle	O	SEA[a]	Fig. 1
5	Clinical samples	2006.08.04	Ha noi	Cattle	O	SEA[a]	Fig. 1
6	Clinical samples	2006.10.06	Thai Nguyen	Buffalo	O	SEA[a]	Fig. 1
7	Clinical samples	2006.10.10	Thai Nguyen	n.a	O	SEA[a]	Fig. 1
8	Clinical samples	2006.10.11	Ha noi	Pig	O	SEA[a]	GQ855801 (551 bp)
9	Clinical samples	2006.10.12	Lang Son	Cattle	O	SEA[a]	Fig. 1
10	Clinical samples	2006.10.17	Son La[1]	Pig	O	SEA[a]	Fig. 1
11	Clinical samples	2006.10.17	Son La[1]	Cattle	O	SEA[a]	GQ855802 (551 bp)
12	Clinical samples	2006.10.17	Son La[1]	Buffalo	O	SEA[a]	Fig. 1
13	Clinical samples	2006.10.20	Lang Son	Pig	O	SEA[a]	Fig. 1
14	Clinical samples	2006.10.20	Son La[2]	Cattle	O	SEA[a]	Fig. 1
15	Clinical samples	2006.10.20	Son La[2]	Cattle	O	SEA[a]	GQ855799 (551 bp)
16	Clinical samples	2006.10.22	Son La	Buffalo	O	SEA[a]	Fig. 1
17	Clinical samples	2006.10.25	Son La	Cattle	O	SEA[a]	Fig. 1
18	Clinical samples	2006.10.28	Son La[3]	Cattle	O	SEA[a]	Fig. 1
19	Clinical samples	2006.10.28	Son La[3]	Buffalo	O	SEA[a]	GQ855800 (551 bp)
20	Clinical samples	2006.10.28	Son La[3]	Cattle	O	SEA[a]	Fig. 1
21	Clinical samples	2006.10.28	Son La[3]	Cattle	O	SEA[a]	Fig. 1
22	Clinical samples	2006.10.28	Son La[3]	Cattle	O	SEA[a]	Fig. 1
23	Clinical samples	2006.10.29	Khanh Hoa	Unkno1wn	O	Pan Asia	GQ855798 (121 bp)
24	Clinical samples	2007.01.19	Lai Chau	Buffalo	n.d.	n.a.	n.a
25	Clinical samples	2007.02.13	Thai Nguyen	Cattle	O	SEA[a]	Fig. 1
26	Clinical samples	2007.04.21	Thai Nguyen	Cattle	O	SEA[a]	Fig. 1
27	Cell supernatant	2007.06.16	Quang Tri	Pig	Asia 1	V	Table 3
28	Clinical samples	2007.06.23	Thanh Hoa	Cattle	Asia 1	V	Table 3
29	Cell supernatant	2007.06.27	Quang Tri[4]	Cattle	Asia 1	V	GQ452295 (7020 bp)
30	Cell supernatant	2007.06.27	Quang Tri[4]	Cattle	Asia 1/O	V/SEA[b]	Fig. 1/Table 3
31	Clinical samples	2007.06.29	Quang Tri[5]	Cattle	Asia 1	V	Table 3
32	Cell supernatant	2007.06.29	Quang Tri[5]	Cattle	Asia 1	V	Table 3
33	Clinical samples	2007.07.15	Thua Thien Hue	Pig	O	Cathay	GQ855803 (551 bp)
34	Cell supernatant	2007.12.16	Nghe An	Cattle	O	SEA[b]	GQ855804 (551 bp)
35	Cell supernatant	2007.12.27	Ha Tinh	Cattle	O	SEA[b]	GQ855805 (551bp)

[1,2,3,4,5]The cases in which multiple individuals of the same or different species are sampled.
[a,b]For two sublineages, 1 and 2, respectively, found in O SEA topotype.
V: Genogroup V in serotype Asia 1; n.a.: not available; n.d.: not detected.
[†]All sequences determined are within VP1 protein coding region.