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Site-specific substitution (Q172R) in the VP1 protein of FMDV isolates collected from asymptomatic carrier ruminants in Vietnam

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ABSTRACT

The epidemiological significance of asymptomatic persistent foot-and-mouth disease virus (FMDV) infection in carrier animals, specifically its ability to seed new clinical outbreaks, is undetermined, and consistent viral determinants of FMDV persistence have not been identified. We analyzed 114 FMDV O/ME-SA/PanAsia VP1 sequences from naturally infected animals in Vietnam, of which 31 were obtained from persistently infected carrier animals. A site-specific substitution was identified at VP1 residue 172 where arginine was present in all 31 of the carrier-associated viruses, whereas outbreak viruses typically contained glutamine. Additionally, we characterized multiple viruses from a single persistently infected animal that were collected over the course of eight months and at multiple distinct anatomic sites (larynx, dorsal soft palate and dorsal nasopharynx). This work sheds new light on naturally occurring viral mutations within the host and provides a basis for understanding the viral evolution and persistence mechanisms of FMDV.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals. FMD is endemic in much of Asia and Africa and is of significant importance to the transboundary trade in animals and products. The clinical presentation of FMD is characterized by fever, loss of appetite, lameness, and vesicular lesions in, on, or around the feet, mouth and teats (reviewed in Alexandersen and Mowat, 2005). FMD outbreaks can result in devastating economic losses owing to decreased productivity, culling and trade limitations (Knight-Jones and Rushton, 2013). There are seven distinct serotypes of FMD virus (FMDV) (family *Picornaviridae*, genus *Aphthovirus*) which may be further subdivided into topotypes, genotypes and lineages, each with varying levels of antigenic variation (reviewed in Jamal and Belsham, 2013; Brito et al., 2015). Phylogenetic analysis of one of the four

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structural proteins of FMDV (VP1) has been extensively used to characterize viral evolution, identify epidemiological trends and trace outbreaks (Knowles and Samuel, 2003; de Carvalho Ferreira et al., 2015).

In addition to the numerous other challenges associated with controlling outbreaks of FMDV, the virus can persist in the nasopharynx of infected ruminants even years after clinical signs have disappeared (reviewed in Arzt et al., 2011; Alexandersen et al., 2002), while suids do not become long-term carriers of infectious FMDV (Stenfeldt et al., 2016a). Persistently infected FMDV carriers are defined as asymptomatic animals from which FMDV can be recovered ≥28 days after infection (OIE, 2012). The length of the carrier state is believed to be influenced by an undetermined combination of host and viral factors (Moonen and Schrijver, 2000; Gebauer et al., 1988; Salt, 1993), however the identification of these influential factors remains elusive. Circumstantial data have linked carrier cattle to outbreaks (Salt, 2004), but recent work exploring their possible role in seeding new infections in naïve animals showed that this risk is very low under controlled conditions (Parthiban et al., 2015; Tenzin et al., 2008). Currently, the African buffalo (*Syncerus caffer*) is the only species that has been demonstrated to initiate new FMDV infections from a persistently infected carrier animal (Dawe et al., 1994; Vosloo et al., 1996; Hedger and Condy, 1985; Bengis et al., 1986; Sutmoller et al., 2000; Thomson et al., 2003). While the role of carriers in the evolution and epidemiology of FMDV is undetermined, they are still considered a possible source of infection and this forms the basis of current FMD control policies (Salt, 2004).

Several studies have examined possible viral determinants associated with persistent infection of FMDV under experimental conditions (Parthiban et al., 2015; Horsington and Zhang, 2007; O'Donnell et al., 2014; Barros et al., 2007). One study examined approximately 850 nucleotides of the genomic region that encodes the viral structural proteins and observed a tyrosine (Y) to histidine (H) substitution in the B-C loop of VP2 in four out of six carrier cattle 28 days after experimental infection with FMDV type O UKG/34/2001 (Horsington and Zhang, 2007). Another study examined the complete genomic sequences of viruses from carrier cattle experimentally infected with the same virus (UKG/34/2001) and found the same VP2 Y to H substitution in two of the four carrier cattle and in four of the seven viral sequences obtained from these four cattle (Parthiban et al., 2015). Among the six full-length sequences of viruses from three persistently infected cattle in the latter study, no single substitution relative to the inoculum was observed in all of the viruses, leading to the conclusion that there was no evidence of viral determinants influencing persistent infection (Parthiban et al., 2015). To our knowledge, no previous studies have reported specific substitutions within the FMDV genome associated with persistent infection under field conditions.

There are different selection pressures influencing viral genomic changes in persistent infections relative to viruses obtained from clinical samples. Reports have estimated the nucleotide substitution rate per site per year (s/s/y) to be approximately 0.9×10^{-2} to 7.4×10^{-2} in the VP1-coding region of FMD viruses from carriers (Gebauer et al., 1988; Barros et al., 2007), while the rate across the entire viral genome has been estimated to be approximately 1.9×10^{-2} to 9.2×10^{-2} s/s/y (Parthiban et al., 2015; Barros et al., 2007). While the viral serotype and lineage may influence the substitution rate, in general a lower rate of 6.3×10^{-3} to 7.8×10^{-3} s/s/y was estimated in the VP1-coding region of FMD viruses from outbreaks (Jamal et al., 2011; Subramaniam et al., 2015) while the rates across the entire viral genome were estimated to be approximately 8×10^{-3} to 9×10^{-3} s/s/y (Hanada et al., 2004; Valdazo-Gonzalez et al., 2012). Additionally, the nucleotide substitution rate can vary between persistently infected animals (de Carvalho Ferreira et al., 2015; Parthiban et al., 2015; Barros et al., 2007). The sum of these data demonstrates complex and varying influences on the viral genome that may contribute to establishing and maintaining persistent subclinical infections in susceptible animals.

The aim of the current study was to investigate viral determinants of FMDV persistence in naturally infected hosts and to explore the molecular dynamics of within-host evolution of persistent FMDV with regard to time and anatomic loci. This work further supports the hypotheses that specific viral determinants are associated with FMDV persistence and that persistently infected animals may serve as a source of new outbreaks.

2. Methods

A recent study from our group described the phylogenetic relationships of VP1 sequences of FMDVs collected from carrier animals and related outbreak viruses of the serotype O ME-SA/PanAsia lineage in Vietnam between 2010 and 2013 (de Carvalho Ferreira et al., 2015). The present study included the viruses in the previously described dataset and 27 novel viral sequences obtained from either carrier animals (4 sequences, accession numbers KX869894-KX869897) or outbreak (clinically diseased) animals (23 sequences, accession numbers KX944714-KX944736, N.J. Knowles and J. Wadsworth, unpublished data) naturally infected in Vietnam and collected between 2012 and 2014. Carriers were defined as animals from which FMDV was recovered from oropharyngeal fluid and which were situated at farms at which clinical cases of FMD had not been identified within the previous or subsequent 30 days relative to sample collection (de Carvalho Ferreira et al., 2015). The new sequences from carrier animals were obtained from tissues collected at necropsy and sequenced either directly from raw material or from first-passage supernatant as previously described (de Carvalho Ferreira et al., 2015; Pacheco et al., 2010). Additional outbreak sequences were obtained from previously unpublished samples submitted to the World Reference Laboratory for FMD (Pirbright, United Kingdom). The final dataset contained 114 VP1 sequences collected from either outbreaks ($n = 83$) or carriers ($n = 31$), collected in either Vietnam ($n = 112$), Kazakhstan ($n = 1$) or China ($n = 1$) between 2010 and 2014.

To identify specific genetic changes associated with FMDV persistence in Asian buffalo (*Bubalus bubalis*) and study the within-host genetic variability, viral sequences were obtained from two persistently infected animals (V-BU 1 and V-BU 10) sampled at different time points throughout infection and again at necropsy. From V-BU 10 (a bovine), a total of eight viral sequences were examined: five from oropharyngeal fluid samples collected by probang cup between April and November 2012 (the last collected on the day of necropsy) and three from tissue samples of the larynx, dorsal soft palate (DSP) and dorsal nasopharynx (DNP),