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Research paper

Molecular epidemiology, evolution and phylogeny of foot-and-mouth disease virus

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ABSTRACT

Foot-and-mouth disease virus (FMDV) is responsible for one of the most economically important infectious diseases of livestock. The virus spreads very easily and continues to affect many countries (mainly in Africa and Asia). The risks associated with the introduction of FMDV result in major barriers to trade in animals and their products. Seven antigenically distinct forms of the virus are known, called serotypes, but serotype C has not been detected anywhere for many years and may now be extinct. The serotypes have been further divided into topotypes (except for serotype Asia-1 viruses, which comprise a single topotype), genotypes, lineages and sublineages, which are usually restricted to specific geographical regions. However, sometimes, trans-regional spread of some strains occurs. Due to the error-prone replication of the RNA genome, the virus continuously evolves and new strains frequently arise (e.g. with modified antigenicity). Using nucleotide sequencing technologies, this rapid evolution of the viral genome can be followed. This allows the tracing of virus transmission pathways within an outbreak of disease if (near) full-length genome sequences can be generated. Furthermore, the movement of distinct virus lineages, from one country to another can be analyzed. Some important examples of the spread of new strains of FMD virus are described.

1. Introduction

Foot-and-mouth disease (FMD) is an economically important disease of cloven-hoofed animals, affecting a variety of different species of domestic and wild livestock, including cattle, pigs, sheep, goats and buffalo. Although Europe and North America are usually free of the disease, it is still endemic in large parts of Asia and Africa (Jamal and Belsham, 2013). Sporadic cases of the disease occur in some South American countries. Outbreaks due to serotype O FMDV were reported in Colombia in June 2017 (OIE, 2017a) although no clinical disease had previously been detected anywhere in South America since 2013 when disease was reported in Venezuela. The causative agent of the disease, foot-and-mouth disease virus (FMDV), has a single stranded, positive sense, RNA genome (ca. 8400 nucleotides (nt) in length) and belongs to the *Aphthovirus* genus within the family *Picornaviridae*. Within the virus particles, the genome is packaged in a protein shell that is composed of 60 copies of 4 different proteins, termed VP1, VP2, VP3 and VP4 (Fig. 1). These individual virus proteins are produced by proteolytic processing of the polyprotein that is encoded by a single, large, open reading frame (ORF, ca. 7000 nt) within the viral RNA (Fig. 1). The polyprotein of FMDV includes two separate proteases (L^{pro} and $3C^{pro}$). The viral structural proteins are derived from the P1-2A capsid precursor (Fig. 1). The non-structural proteins (NSPs) are mainly involved in the replication of the virus and modifying the host cell responses to infection (reviewed in Martinez-Salas and Belsham, 2017). The virus exists in seven antigenically distinct forms, called serotypes, i.e. O, A, C, Asia-1, Southern African Territories (SAT) 1, 2 and 3. The serotype is determined by the surface exposed capsid proteins VP1, VP2 and VP3 (note that the VP4 is located internally within the virus particle, see Acharya et al., 1989). There is no cross-protection between these serotypes so animals vaccinated or infected with one serotype remain susceptible to infection by the other serotypes (Rodriguez and Grubman, 2009).

The serotypes O, A and C have had a worldwide distribution, with serotype O being responsible for the majority of the outbreaks globally. In contrast, serotype Asia-1 and the SAT viruses are normally restricted to Asia and sub-Saharan Africa, respectively (Rweyemamu et al., 2008). However, there has been an incursion of serotype Asia-1 viruses as far west as Greece in 2000 (Valarcher et al., 2008) and occasional incursions of SAT 1 and SAT 2 viruses from Africa into the Middle East (Rweyemamu et al., 2008; see below in Section 3.8). The last outbreaks of FMD due to serotype C viruses were recorded in 2004 in Brazil and

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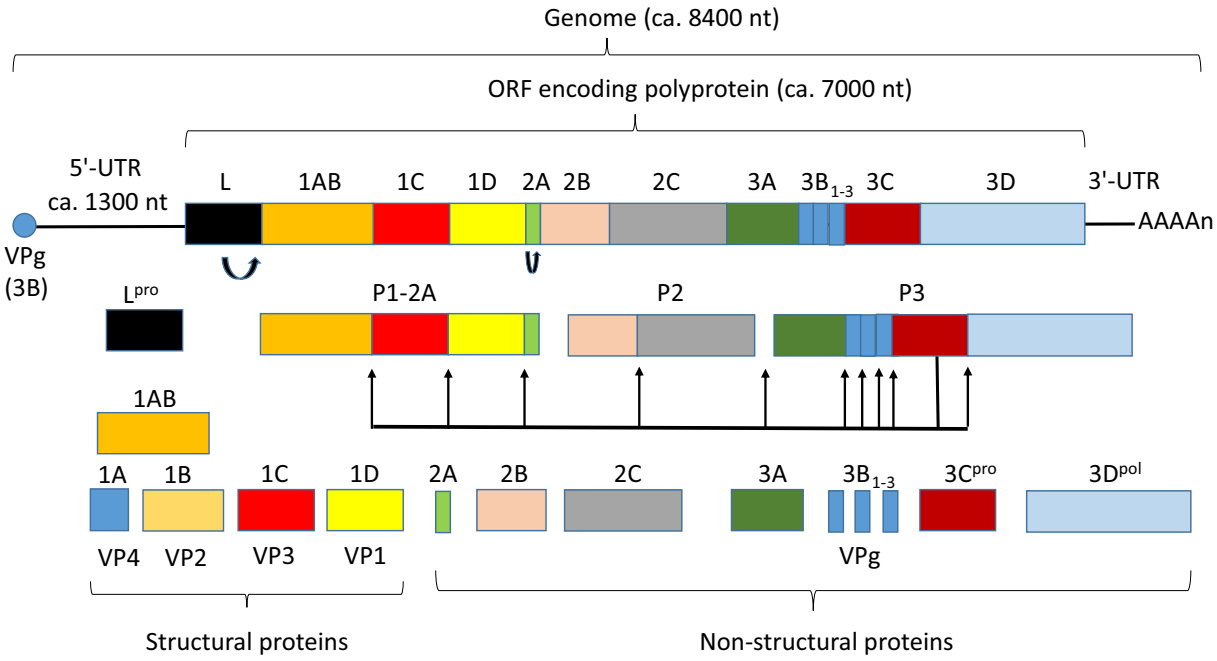

Fig. 1. Genome organization of FMDV. The RNA genome encodes a polyprotein that is processed, largely by virus-encoded proteases into 15 mature products. The virus capsid comprises 60 copies of VP1, VP2, VP3 and VP4 while the non-structural proteins are mainly involved in virus replication (e.g. $3D^{pol}$).

Kenya (Rweyemamu et al., 2008; Roeder and Knowles, 2009; Sangula et al., 2011) and this serotype is now apparently extinct. It is, however, worth mentioning that a SAT 3 serotype FMDV was identified from Uganda from a sample collected in 2013 after a period of more than 15 years without detection in that region. Indeed, the most recently reported isolation of SAT 3 FMDV, previous to this, was in South Africa in 2006. The recent Ugandan virus is of a different lineage than the South African viruses (Dhikusooka et al., 2015) and this latest isolate is most closely related to other, earlier, Ugandan strains of SAT 3 FMDV. The lack of detection of this virus in Uganda in the intervening years probably reflects the relatively infrequent sampling of the African buffalo populations in which these viruses are maintained.

Different subtypes exist within each serotype and new subtypes arise over time as the virus continually evolves (see below). Sometimes there is a lack of efficient cross-protection between different subtypes within a single serotype and thus characterization of subtypes becomes necessary for selection of appropriate vaccines to control an outbreak in countries where vaccination is employed. Different subtypes within each serotype are usually restricted to distinct geographical regions (Rweyemamu et al., 2008; Knowles et al., 2016a). Thus, FMDV subtypes within each serotype throughout the world have been sub-divided into seven regional pools (e.g., pool 2 comprises viruses from countries within the Indian sub-continent, except Pakistan, while pool 4 includes FMDVs from countries in North-eastern Africa). These pools are based on genetic and antigenic analyses (see Di Nardo et al., 2011; Jamal and Belsham, 2013; Sumption et al., 2012). However, occasional spread of subtypes beyond their usual regions occurs.

1.1. Error-prone replication of FMDV

As with other picornaviruses, FMDV RNA replicates within the cytoplasm of infected cells (see Belsham and Bostock, 1988; Paul and Wimmer, 2015). This process requires the virus encoded RNA-dependent RNA polymerase ($3D^{pol}$, see Fig. 1). This enzyme catalyses the synthesis of a negative strand copy of the positive strand viral genome and then the nascent negative strand is used as the template for the production of new positive strand RNA molecules (reviewed in Paul and Wimmer, 2015). Far more positive strand RNAs are made than negative strands. The positive strand RNAs can be packaged by the virus capsid proteins to make new virus particles. All the FMDV RNA is linked at its 5′-terminus to one of the different versions of the 3B peptide ($3B_{1–3}$, also known as VPg, (King et al., 1980), see also Fig. 1). The VPg acts as a protein primer for the synthesis of the viral RNA (Paul and Wimmer, 2015). The replication process is error prone, i.e. incorrect nt are incorporated into the RNA copies. Assessments of the error rate suggest that, on average, about 1 error is made for every 10,000 nt that are synthesized (Castro et al., 2005), i.e. nearly every genome has an error since almost 17,000 nt have to copied to make one new positive strand (by copying the negative strand). There is no known mechanism of proof reading activity in picornaviruses and thus the viral RNA represents a pool of closely related sequences; this pool is known as a quasi-species (see Domingo et al., 2012). It seems that there is a balance between the need to maintain a functional RNA sequence and the need to be able to adapt to new conditions. Modifications to the fidelity (that either increase or decrease the error rate) of the $3D^{pol}$ from picornaviruses reduce the “fitness” of the virus (see Korboukh et al., 2014). As a result of this continuous generation of errors, the virus population is always evolving. The differences in sequence can modify the biology of the virus, (e.g. in its antigenicity or speed of replication) but can also be useful for identifying the sources of viruses that cause disease outbreaks.

A more dramatic form of genome evolution, involving RNA recombination, can also occur between picornavirus genomes. During the process of RNA replication, it is possible for the RNA polymerase ($3D^{pol}$) to switch from copying one positive strand template to another. This process can result in the formation of “chimeric” genomes, e.g. with the capsid coding sequences derived from one parental virus and some of the non-structural protein coding sequences derived from a different strain of virus (e.g. see Jamal et al., 2011b; Jackson et al., 2007). Clearly, it requires that an animal is co-infected with distinctly different strains of the virus to enable this process to be detected.

1.2. Analysis of FMDV sequence evolution in the field

There have been significant advances in the understanding of FMD epidemiology during the last three decades largely due to the