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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Phylogenetic analysis of foot-and-mouth-disease virus type a circulating in Tanzania during 2008-2013

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Abstract

Foot-and-mouth disease virus (FMDV) serotype A is endemic in Tanzania and is one of the serotype that is a major hindrance to the development of the national livestock industry. The serotype is widely spread in the whole country, causing a significant number of sporadic outbreaks throughout the year. The main objective of this study was to use genetic data to determine the distribution of FMDV type Atopotype and the possible sources of sporadic incursions. Complete VP1 sequences, obtained from 20 samples were collected between 2008 and 2013 from different Foot-and-Mouth disease (FMD) outbreaks areas. The sequences generated were compared with previous ones obtained from the public gene bank. Phylogenetic analysis of VP1 nucleotide sequences demonstrated a close relationship to recent FMDV isolates from African genotype I and II that circulate within the East Africa. However, the data showed that the viruses from Tanzania are genetically heterogeneous and both genotypes variants are endogenous to Tanzania. Furthermore, the FMDV in Tanzania were closely related to those from neighbouring countries indicating that there is a strong epidemiological link between neighbouring countries in the region.

Keywords: Serotype A; Phylogenetic; Tanzania; VP1 sequencing; Foot-and-Mouth Virus

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Introduction

FMDV is the causative agent of FMD, the virus is from the *Aphthovirus* genus, *Picornaviridae* family, within the *Picornavirales* order. FMDV genomic RNA (approx. 8400 bp) encodes 4 structural (VP1-4) proteins (Malirat et al., 2007), of which VP1 is a surface-exposed protein comprising 211 amino-acid residues and is the most antigenic and important protein for vaccine design. VP1 based phylogenetic analyses have been widely used to deduce evolutionary dynamics and the epidemiological relationship among the genetic lineages, and in tracing the authentic origin and movement of outbreak strains (Samuel & Knowles, 2001). The virus phylodynamics is marked by high rates of mutation and the same bias can be extended to 1D (10^{-2}-10^{-3} substitutions per site per year) (Sangula et al., 2010).

FMD is caused by seven immunologically distinct serotypes, designated O, A, C, Asia-1, and Southern African Territories 1, 2 and 3 (SAT 1, SAT 2, and SAT 3). FMD one of the most contagious and economically relevant animal disease and has a negative impact on socio-agricultural in countries where it is endemic. (Malirat et al., 2011). The disease also poses an alarming risk of being introduced into free areas, and is considered one of the most important barriers to world trade of livestock and animal products (Perry et al., 1999; Huang et al., 2000; Correa et al., 2002). In Tanzania, FMD is the most important viral

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transboundary animal disease (Swai et al., 2009). Since its first documentation in 1927 and first isolation of the virus in 1954, many FMD outbreaks have occurred across different areas of Tanzania (Kasanga et al., 2012). Unrestricted animal movements are an important mechanism by which FMD is spread within and across international borders (Kivaria, 2003; Paton et al., 2010; Di Nardo et al., 2011). The importance of the movement of infected animals as a mechanism responsible for the spread of FMD among countries in the Great Lakes region (Tanzania, Uganda, Kenya, Rwanda, Burundi and Democratic Republic of Congo) has been highlighted previously by Rweyemamu et al. (2008). In a FMD-endemic country like Tanzania, where 4 serotypes are prevalent (A, O, SAT1 & SAT2), the implementation of an effective vaccination program requires intensive molecular epidemiology surveillance of currently circulating field isolates and continuous monitoring to ensure that the vaccine strains are protective against field viruses.

Serotype A is considered to be one of the most diverse serotypes both antigenically and genetically, complicating control by vaccination (Kitching, 2005). The first serotype A outbreak in Tanzania was reported in 1958 while the last case before the 2008 occurred in 1980 (Mumford, 2007). Regular outbreaks of serotypes O, SAT1 and SAT2 have been reported from different parts of Tanzania since 1954 (Rweyemamu and Loretu, 1972; Mlangwa, 1983). The prevalence of these serotypes is due to unrestricted movement of susceptible animals (Kivaria 2003; Di Nardo et al., 2011), irregular vaccination, the existence of wild-life such as the African buffalo (*Synceruscaffer*), that can maintain the virus for long time (Vosloo et al., 1996), short duration of immunity and sharing of international porous boundaries with some of the endemic countries pose challenge in the control of the disease.

Therefore, understanding FMDV evolutionary dynamics through time and space would be of interesting to guide more efficient control measures and to evaluate the mechanisms of emergence of new strains (Konig et al., 2007; Landro, 2007). This study describes the genetic relationships between FMD serotype A viruses responsible for outbreaks of disease in Tanzania from 2008 to 2013. Phylogenetic analysis was done to reveal relationships that can be used to indicate whether FMD serotype A viruses are introduced on a regular basis from neighbour borders or are maintained for long periods of time.

Materials and Methods

Viruses and the study area

A total of 20 FMDV isolates typing as serotype A were collected from 2008 to 2013 from bovine. The isolates obtained from different areas of Tanzania used in this analysis as in Table 1 and Fig. 1. The collection procedures and preparation of samples was done according to the OIE procedures (OIE, 2012).

Fig. 1: Map of Tanzania showing the geographical locations of serotype A FMD outbreaks analysed in this study between 2008 and 2013. (The stars indicate the areas of outbreaks).

RNA extraction and DNA sequencing

Viral RNA was extracted from epithelial samples collected from cattle using the QIAamp® Viral RNA kit (Qiagen, Hilden, Germany) following the instructions of manufacturer. Samples were screened for FMDV presence using real time RT-PCR as described by Shaw et al. (2007). For sequencing, one-step RT-PCR was carried out as described previously (Knowles et al., 2009). The 639 bp VP1 coding region were PCR amplified using the forward primers A-1C562F (5' TAG CGC CGC CAA AGA CTT TCA 3') and reverse primer EUR-2B52R (5' GAC ATG TCC TGC ATC TGG TTG AT 3') and the Qiagen enzyme. The RT-PCR conditions were as described by Knowles et al. (2005, 2009). The PCR products of the correct size were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacture's recommendation. The purified PCR fragments were sequenced in both directions using the same primers as for PCR and the Big DYE® Terminator v3.1 Cycle Sequencing Kit (ABI, Warrington, UK).

Phylogenetic analysis

The consensus nucleotide sequences were exported to BioEdit (Hall, 1999) computer program and manually aligned using the same program. The identification of serotype using the nucleotide

sequences was done by computer-assisted comparisons of the nucleotide sequences. The comparisons of the similarities of nucleotides sequences were done by referring to the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) using BLASTN search program. Additional sequences comparison was done using FASTA search from European Bioinformatics Institute data base (EBI: http://www.ebi.ac.uk/service/) maintained by European Molecular Biology Laboratories (EMBL). Nucleotides sequences that encoded the VP1 region were translated to the (deduced) amino acid sequences by the EditSeq (DNAstar, Madison, USA). To compare within group sequences, additional sequences from the GenBank were used. Alignments of VP1 sequences were done using Crustal W algorithm method (MegAlign; DNAstar, Madison, USA) and Bio-Edit 7.0. These alignments were used to construct distance matrices by using the Kimura 2-parameter nucleotide substitution model in the program MEGA 5.1. Midpoint-rooted neighbour-joining trees were then constructed with MEGA 5.1 software. The robustness of the tree topology was assessed with 1,000 bootstrap replicates by using the model in MEGA 5.1 version (NJ/p-distance) (Tamura et al., 2011).

Results

The main focus of this study was to determine the emergence, escalating and sporadic spread of serotype A viruses in Tanzania in the past five. The complete nucleotide sequence of the VP1-coding region was determined for 20 serotype A viruses obtained from epithelial samples in 13 of the affected geographical endemic regions of Tanzania from 2008 to 2013. The serotype A FMDV population was classified into two major genotypes (I and II; Fig. 2a), and those from amino acids of VP1 coding region (639) were subjected to phylogenetic analysis to determine their genotypes and genetic relationship with others from African strains. These sequences were aligned and compared with those from African representative serotype A viruses from the recent outbreaks and the old isolates. The phylogenetic analysis showed that all emerging serotype A viruses in Tanzania circulating between the years 2008 to 2013 clustered as part of a clade, supported by 73 to 99% bootstrap values. The clustering pattern showed considerable genetic relationships of viruses with common boundaries, for examples isolates from Kenya (A/KEN/28/2009 and A/KEN/28/2008) clustered together with Tanzanian isolates (A/TAN/-CVL-2009-42 and A/TAN/-CVL-2009-52b) (Fig. 2a and 2b). At bootstrap 96% the two major clusters are separated within small groups showing genetically separation with four isolates (A/TAN/9/2009-Njombe, A/TAN/11/2008-Coast,

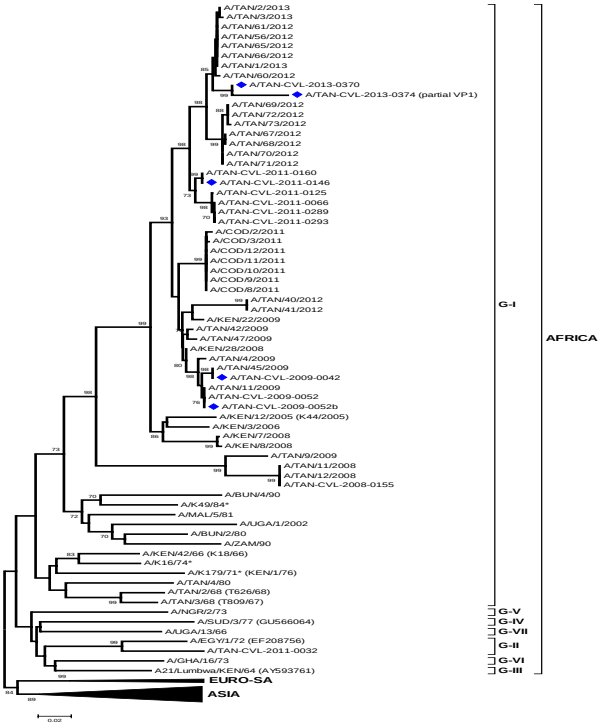

Fig. 2a: Midpoint-rooted neighbour-joining tree showing the relationships between the FMD serotype A virus isolates with other contemporary and reference viruses. Bootstrap support values above 99% are shown near the major nodes

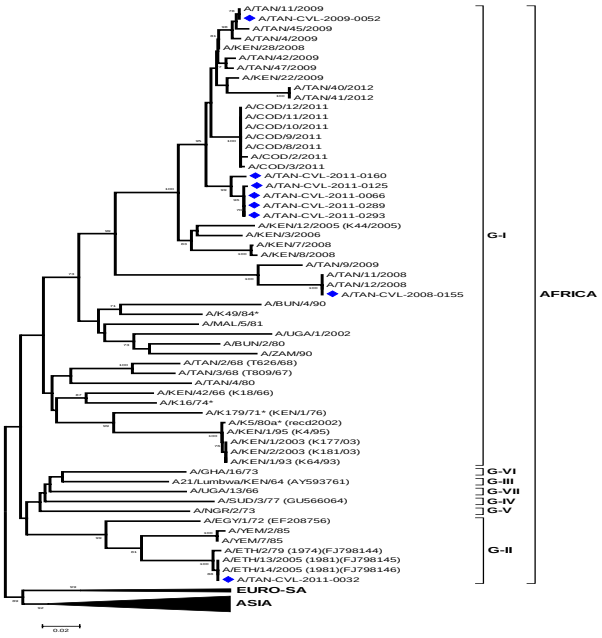

Fig. 2b: Midpoint-rooted neighbour-joining tree showing the relationships between the FMD serotype A virus isolates with other contemporary and reference viruses. Bootstrap support values above 73% are shown near the major nodes

Table 1: The number, date and origin of FMDV serotype a field isolates obtained from cattle

S No.	Isolate number	Date of Collection	Place of Origin	Reference
1.	A/TAN/2/1968	01/01/1968	Tanzania	Kasanga et al. (2014)
2.	A/TAN/3/1968	01/01/1968	Tanzania	Kasanga et al. (2014)
3.	A/TAN/2/1970	10/07/1970	Tanzania	1970 survey
4.	A/TAN/5/1971	30/11/1971	Tanzania	1971 survey
5.	A/TAN/6/1971	30/11/1971	Tanzania	1971 survey
6.	A/TAN/4/1980	17/10/1980	Arusha, Tanzania	Kasanga et al. (2014)
7.	A/TAN/6/1980	17/10/1980	Arusha, Tanzania	This study
8.	A/TAN/11/2008	01/08/2008	Iringa, Tanzania	Kasanga et al. (2014)
9.	A/TAN/12/2008	01/08/2008	Iringa, Tanzania	Kasanga et al. (2014)
10.	A/TAN-CVL-2008-0155	02/07/2008	Rukwa, Tanzania	This study
11.	A/TAN-CVL-2009-0042	01/05/2009	Dar Es Salaam, Tanzania	This study
12.	A/TAN-CVL-2009-0052	09/07/2009	Dar Es Salaam, Tanzania	This study
13.	A/TAN-CVL-2009-052b	09/07/2009	Dar Es Salaam, Tanzania	This study
14.	A/TAN/4/2009	01/05/2009	Morogoro, Tanzania	Kasanga et al. (2014)
15.	A/TAN/9/2009	13/06/2009	Njombe, Tanzania	Kasanga et al. (2014)
16.	A/TAN/11/2009	21/06/2009	Coast, Tanzania	Kasanga et al. (2014)
17.	A/TAN/42/2009	07/09/2009	Dodoma, Tanzania	Kasanga et al. (2014)
18.	A/TAN/45/2009	06/11/2009	Iringa, Tanzania	Kasanga et al. (2014)
19.	A/TAN/47/2009	21/11/2011	Coast, Tanzania	Kasanga et al. (2014)
20.	A/TAN-CVL-2011-0032	21/11/2011	Kigoma, Tanzania	This study
21.	A/TAN-CVL-2011-0066	21/11/2011	Tabora, Tanzania	This study
22.	A/TAN-CVL-2011-0125	21/11/2011	Mtwara, Tanzania	This study
23.	A/TAN-CVL-2011-0146	19/02/2011	Dar Es Salaam, Tanzania	This study
24.	A/TAN-CVL-2011-0160	19/02/2011	Dar Es Salaam, Tanzania	This study
25.	A/TAN-CVL-2011-0289	13/07/2011	Kigoma, Tanzania	This study
26.	A/TAN-CVL-2011-0293	13/07/2011	Kigoma, Tanzania	This study
27.	A/TAN-CVL-2013-0370	04/04/2013	Kagera, Tanzania	This study
28.	A/TAN-CVL-2013-0374	04/04/2013	Kagera, Tanzania	This study
29.	A/TAN/2/2013	18/01/2013	Serengeti, Tanzania	BBSRC Project*
30.	A/TAN/3/2013	11/02/2013	Serengeti, Tanzania	BBSRC Project*
31.	A/TAN/61/2012	15/08/2012	Serengeti, Tanzania	BBSRC Project*
32.	A/TAN/65/2012	18/08/2012	Serengeti, Tanzania	BBSRC Project*
33.	A/TAN/66/2012	18/08/2012	Serengeti, Tanzania	BBSRC Project*
34.	A/TAN/01/2013	18/01/2013	Serengeti, Tanzania	BBSRC Project*
35.	A/TAN/60/2012	14/08/2012	Serengeti, Tanzania	BBSRC Project*
36.	A/TAN/70/2012	03/10/2013	Serengeti, Tanzania	BBSRC Project*
37.	A/TAN/71/2012	05/10/2012	Serengeti, Tanzania	BBSRC Project*
38.	A/TAN/67/2012	15/09/2012	Serengeti, Tanzania	BBSRC Project*
39.	A/TAN/68/2012	15/09/2012	Serengeti, Tanzania	BBSRC Project*
40.	A/TAN/73/2012	16/10/2012	Serengeti, Tanzania	BBSRC Project*
41.	A/TAN/69/2012	03/10/2012	Serengeti, Tanzania	BBSRC Project*
42.	A/TAN/72/201	05/10/2012	Serengeti, Tanzania	BBSRC Project*

*Field collection and analysis done under the BBSRC Project

A/TAN/12/2008-Iringa and A/TAN/-CVL-2008-0155-Rukwa) with bootstrap of 99 to 100%. These isolates, except for isolate A/TAN/11/2008, was isolated from the Coastal region at the animal holding ground ready to be marketed. These mentioned isolates are most likely originated from Southern Highlands where other isolates were obtained during this period of five years. This can be evidenced by the outbreak which was caused in 2009 by the same virus from Tanzanian highlands. The isolate A/TAN/45/2009 was found in southern highlands of Tanzania Iringa while in the eastern part of the country, Dar Es Salaam an isolate A/TAN/-CVL-2009-042 both caused the outbreaks the same in month and year (Table 1, Fig. 2a &2b).

Discussion

The VP1 region of FMDV is widely used for genetic characterization because of its significance for virus attachment and entry, protective immunity and serotype specificity (Jackson et al., 2003; Burman et al., 2006). Like other RNA viruses, the FMDV continually evolves and mutates and the analysis of its genome has become a primary means of classification (Simmonds, 2006; Carrillo et al., 2007). When compared with the available sequences from viruses circulating in Tanzania for the past five years, a closer relationship with topotypes from neighbouring countries of serotype A viruses with related value of 87 to 86% was found.

As the nucleotide (nt) and amino acid (aa) comparisons showed the present circulating viruses shared about 92 to 100% nt and aa. This finding may indicate the dissemination of the same virus lineage of GI-Africa serotype A in Tanzania. In terms of phylogenetic interpretations, the FMDV that differs by 2 to 5% from each other are generally believed to have the same epizootic origin (Samwel et al., 1997). In this study, the most closely related viruses differed in the range of 1.25 to 5.92% showing little divergence. The strains A/TAN-CVL-2013-0370 and A/TAN-CVL-2013-374 came from recent outbreak of year 2013 and came from western part of Tanzania (Kagera) that are closely related by 99% Fig. 2a. These isolates are most closely related (96%) to viruses of years 2012/13 outbreaks from North-West of Tanzania, which shows that the same virus is circulating Fig. 2a. Isolate A/TAN-CVL-2011-0032 are closely related to the old Egyptian isolate (A/EGY/1/72 or EF 208756) are in genotype II, topotype Africa. From these observations, more analyses on antigenic characterization and genetic analyses should be undertaken to confirm whether these Tanzanian serotype A isolates are undergoing antigenic diversification or is vaccine strain. Therefore, we can predict that, differences in the genetic sequences of viruses from the same serotype do not necessarily reflect differences in antigenicity Esterhuyesen, 1994. But, it has also been shown that very limited genetic variations in the immunodominant region can alter the antigenic specificity of FMDV isolates (Mateu et al., 1990, 1996).

The improvement of diagnostic methods to detect and differentiate viruses of diverse serotype immediately would be commendable to use in order to implement effective disease control measures of the circulating strains. In epidemiological surveys of FMD, molecular characterization of FMDV genetic diversity can be used to provide insight into the relationships between different fields isolates (Le et al., 2010). The field collections, analysis of samples together with regular epidemiological investigation of FMD outbreaks in the country and the neighbouring borders is very important in disease eradication programs. Therefore, the findings which came from this study may prove beneficial in formulating control strategies and measures against FMD by the Tanzanian government and its neighbour countries.

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