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ORIGINAL ARTICLE

Incursion of Foot-and-Mouth Disease (FMD) Serotype O East Africa Topotype -3 (O/EA-3) in Nigeria

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SUMMARY

Foot-and-mouth disease (FMD) is an endemic transboundary animal disease that affects livestock health across most of sub-Saharan Africa. Since the first official report of FMD in Nigeria in 1924, serotypes O, A, SAT 1 and SAT 2 have been documented within the country. Molecular epidemiology has been used to trace the origin of FMD outbreaks in the case of animal movement, inter-species transmissions and trans-continental introductions. Phylogenetic analyses of VP1 nucleotide sequences of the twelve isolates (n=12) provide evidence for the presence of type O/EAST AFRICA 3 (EA-3) in Nigeria. The epidemiological situation of FMD in Nigeria and other West African countries is further complicated by the emergence of the O/EA-3 lineage that is causing new outbreaks in the region in addition to the West Africa (WA) topotype that has been known to be in circulation in Nigeria and other parts of West African and Central African regions. These recent development in west and central Africa, indicates the dynamic and complex nature of FMD epidemiology in the region and this is not un-connected to the nomadism in the region. In addition the un-restricted animal movement across the porous border in the region in search of pasture and water for their animal has contributed to the spread of diseases across the region. From the results the phylogenetic analysis of the O/EA-3 has close identity and was closely related to O/EA-3 from the 2009 outbreak in Sudan. Therefore, based on these findings a sustained surveillance is required to yet understand the epidemiology of FMD in West and Central Africa that will inform the type of vaccine and target areas in other to control the disease. The need to restrict animal movement across the border should be put in place and where necessary all animals that must move from one point to another must have proper evidence of vaccine certification before it should be allow access into the area. Finally for effective FMD control, regional vaccination and surveillance should be advocated and it should be backed by law. The need to have FMD vaccination at national as well as regional FMD control policy is strongly advocate for effective FMD control in Nigeria and across the regions.

Key words: Topotype, West African; FMD and Nigeria

INTRODUCTION

Foot-and-mouth disease (FMD) is one of the most economically vital trans boundary animal disease that affects livestock health across most of sub-Saharan Africa. FMD is caused by foot-and-mouth disease virus (FMDV; family *Picornaviridae*, genus *Aphthovirus*) that exists as seven distinct serotypes [A, O, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3] (OIE, 2008), with multiple variants occurring within each serotype (Knowles and Samuel, 2003). The disease affects cloven-hoofed domestic animals, including cattle, swine, sheep and goats, as well as more than 70 species of wild animals (Ferris and Donaldson, 1992). Clinical signs in infected animals include fever, lameness, and vesicular lesions on the tongue and oral cavity, feet, snout and teats of lactating cows (Grubman and Baxt, 2004). In sheep and goats, the disease is generally mild and can be difficult to distinguish from other common vesicular diseases (Blanco et al., 2002).

FMDV is single-stranded, positive-sense RNA genome of approximately 8,500 nucleotides surrounded by an icosahedral capsid that comprises four structural proteins (Rueckert, 1990). Antigenic sites have been identified for all of the serotypes, except SAT 1 and SAT 3. At least four antigenic sites have been identified, each involving one or more of the capsid proteins (VP1, VP2, and VP3); however, each serotype may not contain all the four sites (Grubman and Baxt, 2004). Molecular epidemiology of FMDV usually focuses on the region of the viral genome (VP1) that encodes important antigenic sites on the surface of the virion (Knowles and Samuel, 2003). FMDV antigenic diversity increases with time, likely due to immunological pressures placed on the virus (Haydon et al., 2001; Domingo et al., 2003).

The disease was first reported in Nigeria in 1924, serotypes O, A, SAT 1 and SAT 2 have been documented within the country (Fasina *et al.*, 2013; Lazarus *et al.*, 2012; FAO, 2010a; FAO, 2010b, FAO, 2014a, FAO, 2014b, FAO, 2014c). Among these reported cases, SAT 2 was the most prevalent FMDV serotype between 1974-1991 in West Africa (Sangare et al., 2004). It has been speculated by farmers that many of these FMD outbreaks were associated with the trade of cattle entering Nigeria from the neighbouring countries (Fasina et al., 2013; Lazarus et al., 2012). Also, the pastoral farming system in the Sudan/Sahel region, which is characterized by long-distance movement of livestock due to either transhumance or trade, has been suggested to contribute to FMD outbreaks in Nigeria (Lazarus et al., 2012).

Molecular epidemiology has been used to trace the origin of FMD outbreaks in the case of illegal animal movement, inter-species transmissions and trans-continental introductions (Samuel et al., 1999; Sangaré et al., 2004).Therefore, the aim of this study was to characterize the recent Incursion of FMD Serotype O East Africa Topotype -3 (O/EA-3) viruses circulating in Nigeria for better understanding of the epidemiology of the disease in Nigeria.

MATERIALSAND METHODS

Clinical specimens

Epithelial samples were collected from suspected cases of FMD in cattle in Benue, Kaduna and Plateau State, Nigeria between 2007-2014 (i.e. animals displaying clinical signs suggestive of FMD). These epithelial samples were collected from unruptured and freshly ruptured vesicles, and stored in vials containing in-house prepared transport media [glycerol and isotonic Penicillin/Streptomycin/Gentamycin/Amphoteric in B (PSGA)] solution (1:1 media:glycerol with antibiotics). Specimens were transported on ice to the FMD National Reference Laboratory [National Veterinary Research Institute (NVRI), Vom, Nigeria] and stored at -80°C until processed.

Virus isolation and serotype identification at NVRI

Epithelial tissues (n =12) were washed in PBS (pH 7.2-7.4) before removing 1.0 g for sample processing. The tissues were homogenized using a sterile pestle and mortar, sand and tissue culture medium containing penicillin, streptomycin, gentamycin and amphotericin-B (5 X-PSGA). The tissue homogenates were added to 10 ml of medium and centrifuged at 1125 x g for 15 min. 500 µl of the filtered supernatant (0.22 µm pore size, Millipore) was inoculated onto foetal goat cells (ZZ-R 127) monolayer in a 25 cm^3 tissue culture flask containing 5 ml of in-house prepared Eagle's minimum essential (EMEM) media. Cells were incubated at 37°C for 48 hours and checked for cytopathic effect (CPE). Samples were considered negative if no CPE was observed after 72 hours on the second passage. For the samples where CPE was observed, the supernatant was clarified (1125 x g for 5 min) and then tested with an antigen detection ELISA kit for FMDV serotypes O, A, SAT1 and SAT2 (IZSLER Biotech Laboratory, Brescia, Italy).

Testing of specimens at WRLFMD

For each of the suspect FMDV samples (n =12) a portion of the original sample (tissue) were submitted to the World Reference Laboratory for FMD (WRLFMD; Pirbright, UK) for serotyping and molecular characterization. Samples were processed and subsequently tested using virus isolation (primary bovine thyroid cells (Snowdon 1966) and IB-RS-2 cell cultures (De Castro, 1964), antigen detection ELISA (Ferris & Dawson, 1988) and real-time RT-PCRs targeting the 5′ UTR and 3D regions of the FMDV genome (Callahan et al., 2002; Shaw et al., 2007). Only samples with detectable FMDV genome and a FMDV serotype were included in the molecular characterization.

VP1 sequencing

Total RNA was extracted from the twelve (n =12) cell culture isolates using RNeasy® kit (Qiagen Ltd, Crawley, West Sussex, UK). The VP1 region of the FMDV genome was amplified using a one-step RT-PCR kit (Qiagen Ltd, Crawley, West Sussex, UK), as described previously (Knowles et al., 2009). The oligonucleotide primers used were as follows:O-1C244F or O-1C272F (forward primers) and EUR-2B52R (reverse primer) for serotype O; A-1C562F o. Amplification conditions for serotype A viruses (Knowles et al., 2009), and for SAT 2 and O viruses (Habiela et al., 2010) were as previously described. The amplicons were sequenced using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) using the following oligonucleotides: NK72 for all serotypes; O-1D296F and O-1D628R f. DNA sequencing was performed on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences covering the complete VP1-coding region were assembled using SeqMan Pro™ (Lasergene 8.0 software, DNAStar Inc., Madison, WI, USA).

Phylogenetic analysis

Complete VP1 nucleotide sequences were aligned using BioEdit 7.0.5.3 (Hall, 1999) and Clustal W 1.83 (Thompson et al., 1994). These alignments were used to construct distance matrices using the Kimura-2-parameter nucleotide substitution model (Kimura, 1980), as implemented in the program MEGA 6.06 (Tamura et al., 2013). Midpoint-rooted Neighbor-joining (NJ) trees were then constructed using MEGA 6.06. The robustness of each tree topology was assessed with 1000 bootstrap replicates. VP1 sequences obtained from FMDV samples collected in Nigeria between 2007-2009 were also included in the midpoint-rooted NJ trees, as well as other serotype-specific sequences from GenBank (Ehizibolo et al., 2014). The FMD viruses were classified into geographically restricted clusters, also known as topotypes, as previously described (Vosloo et al., 2002; Knowles and Samuel, 2003).

RESULTS

Of the twelve (n=12) suspected FMDV samples analysed, FMDV were isolated from all the samples at NVRI, Nigeria. The samples showed CPE within 24 hrs of inoculation on a monolayer cell line of ZZR-127 foetal goat cell line. All tissue culture positive sample were serotyped using antigen ELISA. At the WRLFMD Pirbright UK, FMDV were also isolated and serotyped as FMDV serotpes O

Phylogenetic analysis of serotype O viruses

The relationship of all the Nigerian isolates with GenBank sequences is indicated in table I and with the percentage nucleotide identity ranging from 92-100% for the EA-3

Phylogenetic analysis of the complete VP1 sequences showed that the first incursion serotype O isolate (O/NIG/1/2007) from Bauchi clustered with isolates from Sudan (O/SUD/3/2005) within the EAST AFRICA 3 (EA-3) topotype (figure I). Subsequent isolates (O/NIG/15/2009, O/NIG/1/2011, O/NIG/8/2011 and O/NIG/10/2011), each collected from a different Nigerian state of Benue, Kaduna and Plateau, also clustered within the EAST AFRICA 3 (EA-3) topotype (figure 1). These four viruses were closely related to a FMD virus from Sudan reported by Habiela et al., 2011 (e.g. O/SUD/1/2009, 92.64-94.05 % nt id) table 1. Additionally, seven of the isolates (O/NIG/3/2011. O/NIG/4/2012, O/NIG/5/2012, O/NIG/1/2014, O/NIG/2/2014, O/NIG/3/2014, O/NIG/4/2014) clustered within the WEST AFRICA topotype (figure 2) reported by Ularamu et al., 2016. The virus isolate from 2011 was most closely related to viruses collected from Benin, Ghana and Togo, however, the remaining six were not closely related to any of the other viruses in this topotype.

DISCUSSION

This study describes the molecular analysis of serotypes O viruses isolated from Nigeria, 2007-2014. Epithelial tissue samples, collected from Bauchi, Benue, Kaduna and Plateau States, were

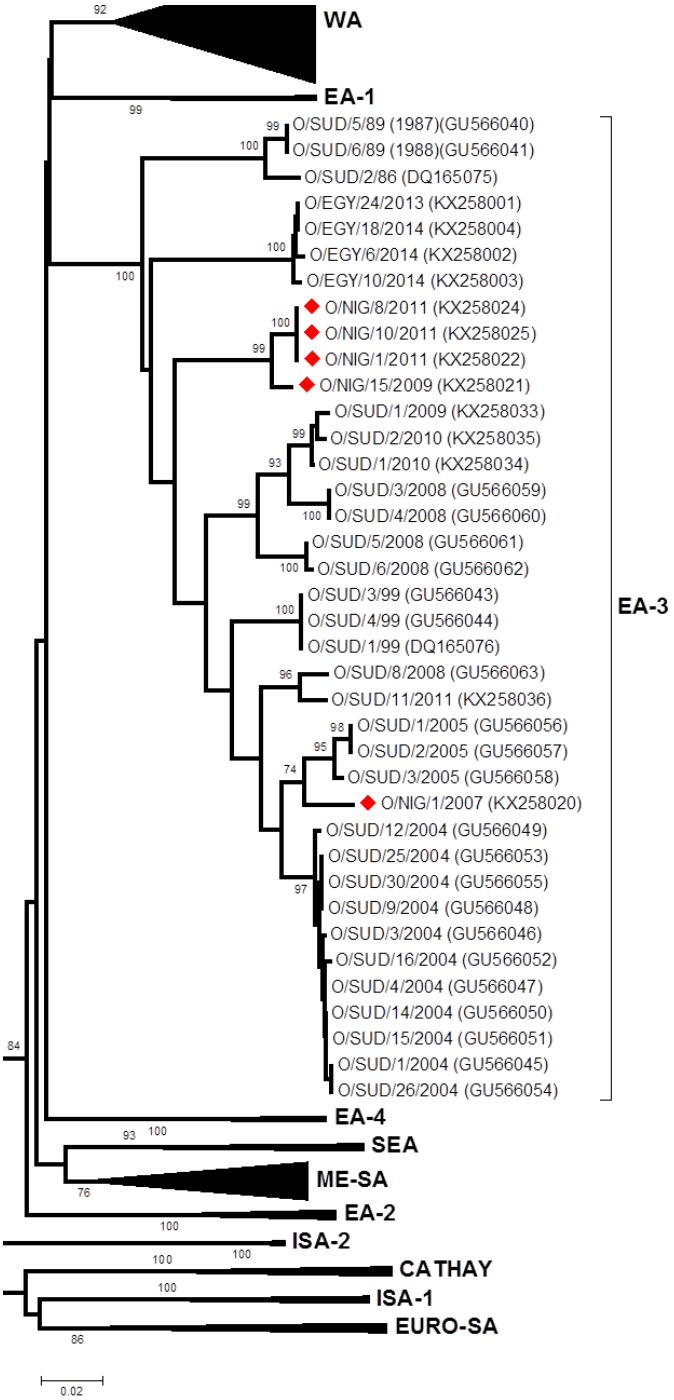

FIGURE. 1: Midpoint-rooted Neighbor-joining tree showing the relationships between the serotype O viruses collected from Nigeria (highlighted with red diamonds). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Reference numbers were assigned by the WRLFMD.

TABLE I: Nigeria serotype O isolates (EA-3 topotype) closely related with other type O GenBank sequences

Most Closely Related Viruses									
Pos.	Virus name	Filename	No. comp.	ntNo. match.	ntNo. ambig.	of% Id.	% Diff.	Topotype	Strain
1	O/NIG/10/2011	NIG11-10	639	639	0	100.00	0.00	EA-3	unnamed
2	O/NIG/8/2011	NIG11-08	639	639	0	100.00	0.00	EA-3	unnamed
3	O/NIG/15/2009	NIG09-15	639	630	0	98.59	1.41	EA-3	unnamed
4	O/SUD/3/2005 (GU566058)	SUD05-03	639	602	0	94.21	5.79	EA-3	unnamed
5	O/SUD/1/2005 (GU566056)	SUD05-01	639	601	0	94.05	5.95	EA-3	unnamed
6	O/SUD/2/2005 (GU566057)	SUD05-02	639	601	0	94.05	5.95	EA-3	unnamed
7	O/NIG/1/2007	NIG07-01	639	594	0	92.96	7.04	EA-3	unnamed
8	O/SUD/3/2004 (GU566046)	SUD04-03	639	593	0	92.80	7.20	EA-3	unnamed
9	O/SUD/12/2004 (GU566049)	SUD04-12	639	592	0	92.64	7.36	EA-3	unnamed
10	O/SUD/14/2004 (GU566050)	SUD04-14	639	592	0	92.64	7.36	EA-3	unnamed
Most Closely Related Reference Viruses (see http://www.wrlfmd.org/fmd_genotyping/prototypes.htm)									
Pos.	Virus name	Filename	No. comp.	ntNo. match.	ntNo. ambig.	of% Id.	% Diff.	Topotype	Strain
1	O/SUD/2/86 (DQ165075)	SUD86-02	639	572	0	89.51	10.49	EA-3	unnamed
2	O/ETH/1/2007 (FJ798137)	ETH07-01	639	557	0	87.17	12.83	EA-3	unnamed
3	O/IND/53/79 (AF292107)	IND79A53	639	548	0	85.76	14.24	ME-SA	unnamed
4	O/GHA/5/93 (AJ303488)	GHA93-05	639	547	0	85.60	14.40	WA	unnamed
5	O/UGA/17/98 (HM211075)	UGA98-17	639	546	0	85.45	14.55	EA-4	unnamed
6	O/ETH/2/2006 (FJ798127)	ETH06-02	639	545	0	85.29	14.71	EA-3	unnamed
7	O/ETH/3/2004 (FJ798109)	ETH04-03	639	545	0	85.29	14.71	EA-3	unnamed
8	O/UKG/35/2001 (AJ539141)	UKG01-35	639	542	0	84.82	15.18	ME-SA	PanAsia
9	O/CIV/8/99 (AJ303485)	CIV99-08	639	541	0	84.66	15.34	WA	unnamed
10	O/K83/79* (AJ303511)	KEN79B83	638	540	1	84.64	15.36	EA-1	unnamed

Most Closely Related Viruses									
Pos.	Virus name	Filename	No. nt comp.	No. nt match.	No. of ambig.	% Id.	% Diff.	Topotype	Strain
1	O/NIG/5/2012	NIG12-05	639	639	0	100.00	0.00	WA	unnamed
2	O/NIG/3/2014	NIG14-03	639	628	0	98.28	1.72	WA	unnamed
3	O/NIG/4/2014	NIG14-04	639	628	0	98.28	1.72	WA	unnamed
4	O/NIG/1/2014	NIG14-01	639	627	0	98.12	1.88	WA	unnamed
5	O/NIG/2/2014	NIG14-02	639	627	0	98.12	1.88	WA	unnamed
6	O/CAR/16/2000 (HM211080)	CAR00-16	639	562	0	87.95	12.05	WA	unnamed
7	O/CAR/17/2000 (HM211081)	CAR00-17	639	562	0	87.95	12.05	WA	unnamed
8	O/SUD/1/76	SUD76-01	639	560	0	87.64	12.36	WA	unnamed
9	O/MAI/1/2005	MAI05-01	639	559	0	87.48	12.52	WA	unnamed
10	O/MAI/2/2005	MAI05-02	639	559	0	87.48	12.52	WA	unnamed
Most Closely Related Reference Viruses (see http://www.wrlfmd.org/fmd_genotyping/prototypes.htm)									
Pos.	Virus name	Filename	No. nt comp.	No. nt match.	No. of ambig.	% Id.	% Diff.	Topotype	Strain
1	O/CIV/8/99 (AJ303485)	CIV99-08	639	554	0	86.70	13.30	WA	unnamed
2	O/GHA/5/93 (AJ303488)	GHA93-05	639	553	0	86.54	13.46	WA	unnamed
3	O/PAK/16/2003 (DQ165068)	PAK03-16	639	546	0	85.45	14.55	ME-SA	Pak-98
4	O/SUD/2/86 (DQ165075)	SUD86-02	639	545	0	85.29	14.71	EA-3	unnamed
5	O/IND/53/79 (AF292107)	IND79A53	639	540	0	84.51	15.49	ME-SA	unnamed
6	O/KUW/3/97 (DQ164904)	KUW97-03	639	540	0	84.51	15.49	ME-SA	Ind-2001a
7	O/ETH/1/2007 (FJ798137)	ETH07-01	639	539	0	84.35	15.65	EA-3	unnamed
8	O1/Manisa/TUR/69 (AY593823)	TUR69--G	639	539	0	84.35	15.65	ME-SA	unnamed
9	O/K83/79* (AJ303511)	KEN79B83	638	537	1	84.17	15.83	EA-1	unnamed
10	O/UAE/4/2008	UAE08-04	636	535	0	84.12	15.88	ME-SA	Ind-2001c

Table 2: Nigeria serotype O isolates (WA topotype) closely related with other type O GenBank sequences

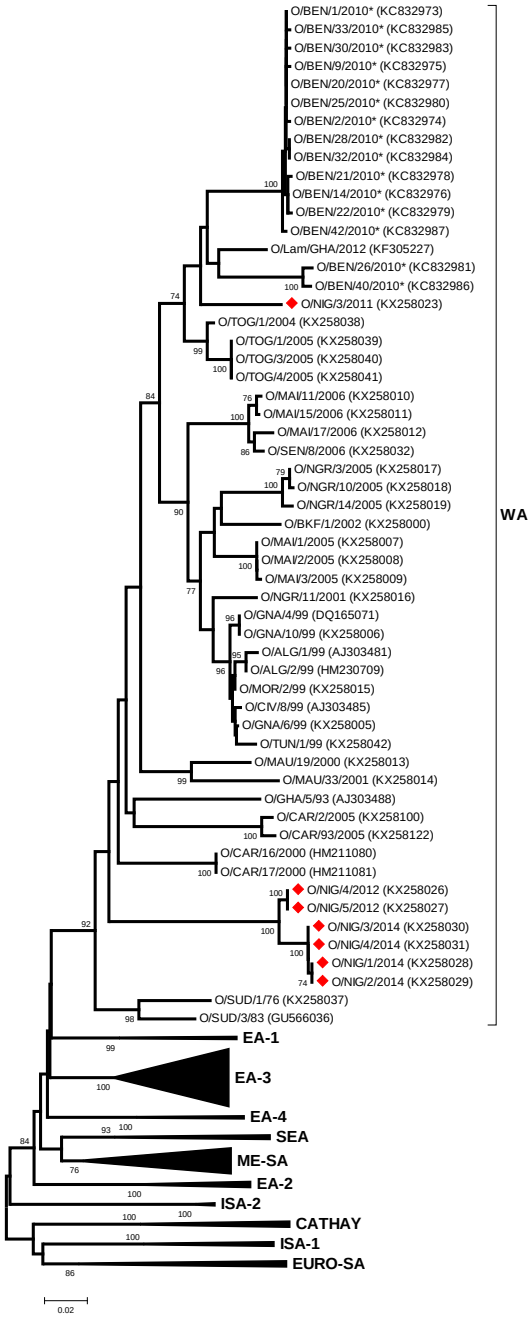

FIGURE.2: Midpoint-rooted Neighbor-joining trees showing the relationships between the serotype O viruses collected from Nigeria (highlighted with red diamonds) WEST AFRICA topotypes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Reference numbers were assigned by the WRLFMD.

analysed for the detection and typing of FMDV. Viruses were isolated and serotyped from samples at NVRI, Vom; same samples were analysed at WRLFMD (Pirbright, UK), viruses were isolated and serotyped from the same samples. Phylogenetic analyses were used to establish the relationships between Nigerian FMD viruses and those from neighbouring countries. From the analysis, the phylogenetic tree for Nigerian serotype O/EA-3 isolate (O/NIG/1/2007) from Bauchi been the first incursion shows a close relationship with FMD viruses from East Africa. The isolate of 2007 (O/NIG/1/2007) is closely related to FMD viruses from Sudan (O/SUD/3/2005) (Ehizibolo et al., 2014). In addition, a Nigerian isolate from 2009 (O/NIG/15/2009) is closely related to these 2011 isolates and this is indicating two separate virus introductions and the possibility of persistence from 2009 to 2011 within the country .To our knowledge, the 2007 isolate is the first report of O/EA-3 in both Nigeria and West Africa. Other recent serotype O isolates within **Table 2**: Nigeria serotype O isolates (WA topotype) closely related with other type O GenBank sequences the WA topotype grouped with FMD viruses collected from countries in West Africa. Together, these data for O/EA-3 and O/WA indicate that long-distance west-to-east as well as east-to-west animal movements through trade and pastoralism mediate the spread of FMD across sub-Saharan Africa.

CONCLUSION

Given that transhumance and pastoralism are traditional practices in many parts of Africa, Nigeria inclusive, and that borders among many sub-Saharan countries are uncontrolled, there are no restrictions on human and animal movements. Due to the lack of animal movement records, the vast number of cattle and the large expanse of the country, it is difficult to determine the source of

outbreaks or to trace the transmission of the disease over time. Further collection and analysis of samples, together with improved local epidemiological investigation into FMD outbreaks in sub-Saharan Africa, are required to improve our understanding of the complex epidemiology of FMD in the region.

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