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Molecular epidemiology and vaccine matching study on foot-and-mouth disease virus circulating in Ethiopia

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The study was conducted in three regional states of Ethiopia: Amhara, Oromia, and Addis Ababa with the aims of identifying the molecular epidemiology of Foot and Mouth Disease (FMD) virus in Ethiopia and to determine the appropriate vaccine strains of FMD virus. From the total of 33 bovine epithelial tissue cultured samples, 19 (57.57%) samples were showed cytopatic effect for FMD virus. Of these, three samples were found serotype A and Africa topotype and 16 samples were found serotype O and East Africa-3 topotype. Certain FMD isolates were characterized by two dimensional virus neutralization test (2dmVNT) and liquid phase block ELISA (LPBE) in order to choose an appropriate vaccine strain found at World Reference Laboratory for FMD (WRLFMD). The result indicates that most vaccine strains found at WRLFMD can protect against serotype O of Ethiopian isolates, while serotype A has highest antigenic variation and only few vaccine strains found at WRLFMD can provide protection. Various strains of foot and mouth disease virus (FMDV) were isolated in Ethiopia and therefore, continuous monitoring of newly emerging strains is necessary to perform vaccine matching studies to support the efficacy of actual vaccine formulations.

Key words: Ethiopia, foot and mouth disease virus (FMD), phylogeny, vaccine match, serotype, topotype.

INTRODUCTION

Foot and Mouth Disease (FMD) is a highly contagious viral disease of cloven-hoof animals and is one of the most important economic diseases of livestock (Bronsvoort et al., 2004) and the disease is characterized by fever, and vesicular eruptions in the mouth, feet and teats. It is caused by a virus of the genus *Aphthovirus*, in the family *Picornaviridae*, of which there are seven immunologically district serotypes; A, O, C, South African Territories (SAT) 1, SAT2, SAT3 and Asia1 (OIE, 2004). FMD serotypes O and C were first recorded in Ethiopia in 1957 (Martel, 1974). Serotypes A and SAT 2 were not identified until 1969 and 1989, respectively (Roeder et al., 1994). From 1988 to 1991, serotype O and serotype SAT- 2 were identified from outbreaks in Ethiopia (Roeder et al., 1994). The existence of SAT-1 in Ethiopia has also been reported in the first time in 2007 (Yoseph et al., 2013). The occurrence of FMD in Ethiopia has apparently increased since 1990; outbreaks throughout the country are reported frequently (Asfaw and Sintaro, 2000). FMD remains largely uncontrolled in the country because vaccination for prophylactic purpose is not being practiced except for a few dairy herds containing exotic animals (Sahle, 2004). With no control and preventive measures in place, FMD causes substantial economic loss to farmers and to the nation from embargoes of livestock and livestock product trade (Sahle et al., 2004; Megersa et al., 2009). Therefore, livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries.

The official data may not exhibit the reality of the

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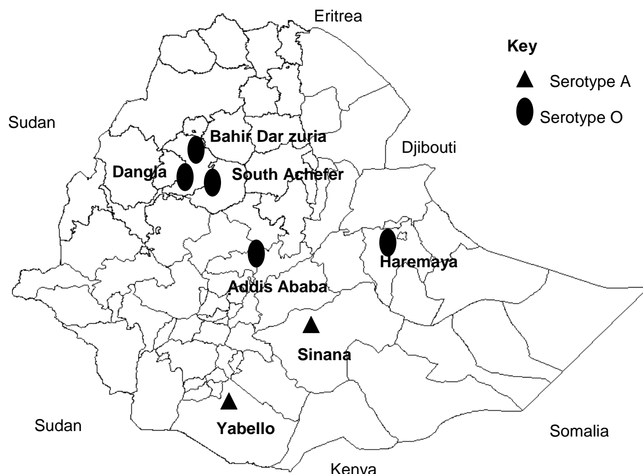

Figure 1. Map of Ethiopia showing the distribution of FMD virus serotype O and A isolated in Ethiopia during study period.

disease, due to the insidious nature of the disease, the unreported cases by farmers, as well as the few samples submitted to WRLFMD, Pirbright, for identification. Therefore, the present study was designed with the aims of identifying the molecular epidemiology of FMDV circulating in the three regions of Ethiopia and to determine the appropriate vaccine strains for the country.

MATERIALS AND METHODS

Study areas, study design and sampling methods

The study was conducted from August 2008 to April 2009 in three national regional states of Ethiopia: Amhara (Bahirdar zuria, Yilmana Densa, South Achefer, Dangela), Oromia (Haremaya, Sinana, Yabello) and Addis Ababa city council (Akaki sub city) as shown in Figure 1. The study design was purposive sampling. Animals were clinically examined for presence of FMD lesions on the mouth, teats, nostril, vagina, and feet and samples were collected. Accordingly, a total of 33 epithelial tissue samples were collected from animals that showed typical clinical signs of FMD. Epithelial tissue was collected from unruptured or freshly ruptured vesicles and placed in a bottle with transport medium composed of equal amount of glycerol and 0.04 M of phosphate-buffered saline solution pH 7.2 to 7.6 with antibiotics (OIE, 2004). Breed, identification number, sex, age, village, and type of tissue were labeled and samples were transported in cold-chain and stored at -20°C until processed.

Cell culture for virus isolation

About 1 g of epithelial tissue sample was grounded using sterile mortar and pestle by adding 10 ml of sterile phosphate-buffered saline containing antibiotics. The tissue suspension was centrifuged at 1,500 rpm for 15 min. The supernatant was collected and filtered by Millipore filter of 0.22 µm pore size. About 1 ml of filtered tissue suspension was inoculated on baby hamster kidney (BHK-21) monolayer cells grown on 25 cm^2 tissue culture flask and then flashed with MEM media (2%) and incubated at 37°C and 5% CO_2 for 48 h. Cytopathic effects (CPE) were noted after 24 to 48 h in positive samples (OIE, 2004).

Serotype and topotype identification

Tissue-cultured FMDV samples that showed CPE were submitted to World Reference Laboratory for FMD (WRLFMD), Pirbright, UK, according to recommended international standards for serotype and topotype characterization, phylogenic analysis and vaccine matching (r1-value) study.

Phylogenetic analysis

The VP1 gene characterization was used to study phylogenetic relationships of FMD viruses. FMD viruses that differ between 2 to 7% from each other are generally believed to originate from the same epizootic (Samuel et al., 1997, 1999).

Vaccine matching study (r_1-value determination)

Serotype A and O FMDV isolated strains within this study were matched with some of reference vaccine strains of serotype A and serotype O FMDV isolated from different countries of the world and archived in the WRLFMD in order to select an appropriate vaccine strains to control the disease. Two serotype A viruses (A/ETH/9/2008 and A/ETH/7/2008 strains in Yabello and Sinana districts, respectively) and four serotype O FMDV (O/ETH/15/2008 strain from South Achefer, O/ETH/24/2008 strain from Yilmana Densa, as well as O/ETH/24/2009 and O/ETH/28/2009 strain from Haromaya University dairy farm) were used for vaccine matching study.

The formula for r1 determination was:

$$r1 = \frac{\text{Reciprocal titre of reference serum against field virus}}{\text{Reciprocal titre of reference serum against vaccine virus}}$$

In case of liquid phase blocking ELISA (LPBE) test, r_1-values were interpreted as proposed by Samuel et al. (1990) and OIE (2004), where values between 0 to 0.19 indicated highly significant antigenic variation from the vaccine strains that the vaccine is unlikely to protect; values of 0.20 to 0.39 suggested the field strain were genetically related to the vaccine strain; while r_1-values of 0.40 to 1.0 demonstrated that the vaccine and field strains were similar and the vaccine would provide good protection. In case of neutralization, the r_1-values were interpreted as values $\geq$ 0.3 suggested that there was a close relationship between field strain and vaccine strain and likely conferring protection, but $<$ 0.3 suggested that the field strain was so different from the vaccine strain that the vaccine is unlikely to protect.

Data management and analysis

The antigenic relationship between a field strain and a vaccine virus (r_1-values) was determined by 2dmVNT and LPBE test. The r_1-values were calculated as followed: (r_1 = serum titre against heterologous virus/ serum titre against homologous virus). The molecular sequences generated in this study were done at WRLFMD, Pirbright, UK. Neighbor-joining method included in the MEGA 4 program was used to construct gene trees and the confidence levels were assessed by 1000 bootstrap replications.

Table 1. Serotype and topotype identified in eight districts of the three regional states of Ethiopia.

No.	Site of outbreaks	No. of samples	Showing CPE	Serotype identified	Topotype identified	Strain
1	Bahirdar zuria	5	2	O	EA-3	-
2	Yilmana Densa	5	3	O	EA-3	-
3	South Achefer	7	3	O	EA-3	-
4	Dangela	4	1	O	EA-3	-
5	Akaki	4	2	O	EA-3	-
6	Haremaya	5	5	O	EA-3	-
7	Sinana	2	2	A	Africa	G-VII
8	Yabello	1	1	A	Africa	G-VII

Table 2. Antigenic characterization of FMD field isolates of serotype A by matching with vaccine strains.

Filed isolate	r1-value by 2dVNT							r1 value by LPBE	
	A/Eritrea RZ pool	A/Tur06 Arriah 2nd	A22 Irq23-32 pool	A/Sau41 91 SI94	A/sau95 bvs	A/Irn87 VQ pool	A/Irn 96 UZ pool	A/Eri3/98	A/Sau95
A/ETH/9/08	012	0.46	0.07	0.04	0.3	0.04	0.07	0.1	0.25
A/ETH/7/08	-	-	-	-	-	-	-	0.31	-

- Not done.

RESULTS

FMD virus isolation and serotype and topotype identification

From the total of 33 bovine epithelial tissue cultured samples, 19 (57.57%) samples were showed CPE on BHK-21 monolayer cells culture for FMDV (Table 1). The CPE was characterized by a fast destruction of the mono-layer cell and infected cells were round and formed singly. Complete destruction of the cell sheet was mostly seen within 48 h of inoculation.

Out of the 19 samples that showed CPE, three samples were serotype A, Africa topotype, and G-VII strain, while 16 tissue cultured sam-ples were found serotype O and East Africa-3 topotype.

Vaccine matching

The vaccine matching result revealed that A/ETH/9/2008 was antigenetically close to A/Tur06/Arriah2nd, and A/Sau95/Bvs strain with r_1-values of 0.46 and 0.3, respectively, using 2dmVNT. Although, this field strain was related with A/Sau95 in which r_1-value was 0.25 using LPBE. Highly significant antigenic differences were observed to A/Eritrea RZ pool, A22/Irq23-32 pool, A/Sau41/91/SI94, A/Irn87/VQ pool, and A/Irn96/UZ pool with r_1-values of 0.12, 0.07, 0.04, 0.04 and 0.07, respectively, using 2dmVNT. A/ETH/7/2008 was antige-nically similar with A/Eri3/98 where r_1-value was 0.31 as shown in Table 2. Field strain O/ETH/15/2008 was antigenetically close to O1/Manisa, O/BFS/VP pool, O/Ind/R2/75, and O/Kaufbeuren (O/Kauf/VQ) in which r_1-values were 0.46, 0.70, 0.69 and 0.58, respectively, using 2dmVNT. This was also close related with O/3039 and O/4174 in which r_1-values were 0.75 and 0.25, respec-tively, using LPBE. Field strain O/ETH/24/2008 strain from Yilmana Densa was antigenetically related to O1/Manisa, O/BFS/VP pool, and O/Ind/R2/75 in which r_1 values were 0.44, 0.70 and 0.65, respectively, but there was no antigenic relation with O/Kauf/VQ in which r_1-value was 0.23 using 2dmVNT. In addition, it was also antigenetically matched with O/3039 and O/4174, in which r_1- values were of 1.00 and 0.25, respectively, using LPBE.

O/ETH/24/2009 was antigenetically related with O/manisa, O/Bfs, and O/Ind/R2/75, in which r_1-values were 0.32, 0.43, and more than 1, respectively, using 2dmVNT. In addition, this strain was antigenetically close to O/manisa (r_1-value = 0.75), but different from O/Bfs/1860, O/4174, O/Hkn/6/83, and O/k77/78 in which r_1-values were 0.13, 0.11, 0.25, and 0.13, respectively, using LPBE. O/ETH/28/2009 was also antigenetically matched with O/manisa, O/Bfs, and O/Ind/R2/75 in which r_1-values were 0.32, 0.74, and more than one, respec-tively, using 2dmVNT. Moreover, this strain was antigenetically related with O/manisa (r_1-value = 0.46), but there was no antigenic similarity with O/Bfs/1860, O/4174, O/Hkn/6/83, and O/k/77/78 in which r_1-values were 0.13, 0.16 and 0.50, respectively, using LPBE as shown in Table 3.

Table 3. Antigenic characterization of FMD field isolates of serotype O by matching with vaccine strains.

Filed isolate	r1-value by 2dVNT				r1 value by LPBE					
	O/Manisa	O/Bfs	O/Ind R2/75	O/Kauf/ VQ pool	O/Manisa	O/Bfs	O/4174	O/3039	O/Hkn/6/83	O/k/77/78
O/ETH/15/08	0.46	0.70	0.69	0.58	-		0.25	0.75	-	-
O/ETH/24/08	0.44	0.70	0.65	0.23	-		0.25	1	-	-
O/ETH/24/09	0.32	0.43	>1.0	-	0.75	0.13	0.11	-	0.25	0.13
O/ETH/28/09	0.32	0.74	>1.0	-	0.46	0.13	0.16	-	0.50	-

Key: - Not done.

Figure 2. phylogenetic tree of FMDV serotype A.

Phylogenetic analysis

The 1D gene characterization was used to study phylogenetic relationships between 16 serotype O and 3 serotype A FMD viruses in Ethiopia as well as with other O-type and A-type isolates from other countries of the world. Serotype O isolated in this study falls within East Africa-3 topotype while serotype A was identified as Africa topotype and G-VII strains. Serotype A recovered from Sinana (A/ETH/7/2008 and A/ETH/8/2008) and Yabello (A/ETH/9/2008) districts were compared based on the complete 1D sequence (639 nuclotide) data of VP1 gene. Viruses isolated at Sinana A/ETH/7/2008 and A/ETH/8/2008 were shared > 99% identity with each other and > 94% identity with Yabello isolate A/ETH/9/2008. Also the serotype A isolates were antigenetically homologues (> 94% identity at 630 nt. sequence level) with viruses isolated in Egypt in 2006 (A/EGY/1/2006, A/EGY/2/2006, A/EGY/3/2006, A/EGY/4/2006, A/EGY/7/2006 and A/EGY/9/2006) and Kenya isolated in 2005 (A/KEN/29/2005) as shown in Figure 2. Serotype O from Yilmana Densa O/ETH/24/2008, O/ETH/25/2008, and O/ETH/26/2008 were showed > 99% nucleotide sequence similarity. Isolates recovered from Bahirdar zuria O/ETH/20/2008 and O/ETH/21/2008 were 100% nucleotide sequence similarity. Similarly, O/ETH/19/2008, O/ETH/13/2008 and O/ETH/15/2008 isolated from South Achefer showed 100% nt. sequence similarity. In addition, O/ETH/24/2009, O/ETH/25/2009, O/ETH/26/2009, O/ETH/27/2009 and O/ETH/28/2009 isolated from Haromaya University dairy farm were shared more than 99% antigenetic identity with each other.

These isolates were also antigenetically homologues (more than 98% nt. identity) with virus isolated in Yemen in 2009 (O/YEM/6/2009, O/YEM/16/2009, O/YEM/18/2009, O/YEM/19/2009, O/YEM/20/2009 and O/YEM/21/2009) at 92% bootstrap support as shown in Figure 3.

DISCUSSION

Serotype A (Africa topotype) and serotype O (East Africa-

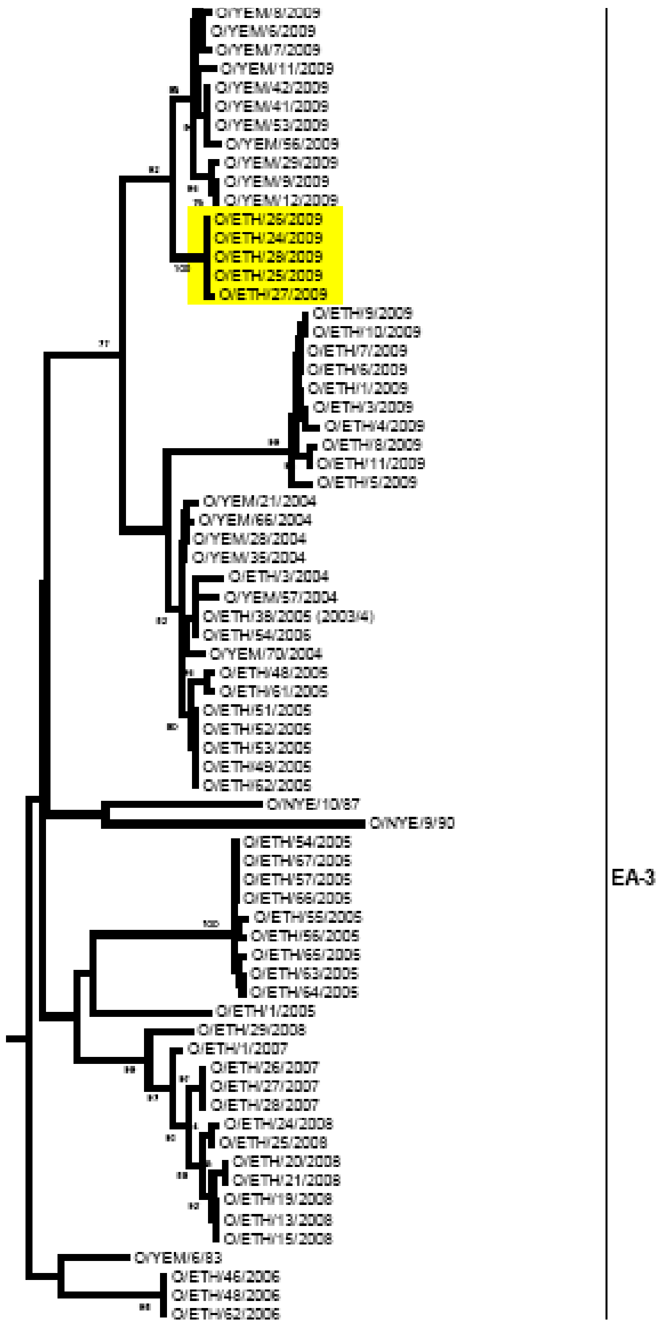

Figure 3. Phylogenetic tree of FMDV serotype O.

3 topotype) of FMDV was isolated in this study and serotype O was the dominant serotype circulating in Ethiopia. Most of the outbreaks were responsible by serotype O followed by serotype A. This showed that serotype O was highly prevalent and a dominant serotype causing outbreaks in Ethiopia. Gelaye et al. (2005), Ayelet et al. (2009) and Klein (2009) reported that serotype O was a dominant FMD virus serotype circulating in Ethiopia. Serotype A isolated from Sinana district (A/ETH/7/2008 and A/ETH/8/2008) and Yabello districts (A/ETH/9/2008) were closely related (< 6% nt. difference) and this indicated that they are antigentically related. In addition, these isolates were antigenetically related (< 6% nt. difference) with viruses isolated in Egypt in 2006 and in Kenya in 2005, indicating that they share a common ancestor. This similarity might be due to the presence of uncontrolled transboundary animal movement. This statement is supported by Samuel et al. (1999) who demonstrated that closely related viruses could either be from the same outbreak or from viruses temporally closely related. All serotype O strains had falls within East Africa-3 topotypes. These indicated that EA-3 topotype has wider distribution and highly prevalent in Ethiopia. This is in agreement with Ayelet et al. (2009) who demonstrated the existence of topotypes EA-3 and EA-4 in Ethiopia with the highest rate of EA-3 topotype. African topotype of serotype A and G-VII strains were also recorded in this study.

Serotype O isolated from Yilmana Densa (O/ETH/24/2008, O/ETH/25/2008 and O/ETH/26/2008), Bahirdar zuria (O/ETH/20/2008 and O/ETH/21/2008), and South Achefer (O/ETH/19/2008, O/ETH/13/2008, and O/ETH/15/2008) were closely related and < 2% nt. sequence difference. This indicated that outbreaks due to these isolates were from the same origin. These might be due to free movement of livestock and livestock products among various markets in different regions and states and this plays an important role in the dissemination of the virus. Furthermore, serotype O isolated from Haromaya University dairy farm (O/ETH/24/2009, O/ETH/25/2009, O/ETH/26/2009, O/ETH/27/2009, and O/ETH/28/2009) were closely related with each other (> 98% nt. sequence similarity), which indicated that these viruses isolated from the same outbreaks. These isolates were also antigenetically more closely related (< 2% nt. sequence) with viruses isolated from Yemen in 2009 and it indicated that they belong to the same epizootics (common origin). The antigenic relationship of serotype A isolated during this study revealed that serotype A (A/ETH/9/2008) isolated from Yabello had an antigenic similarity and could provide protection to A/Tur06/Arriah2nd, (A/Sau95/Bvs), and A/Sau95 isolates where r_1-values were 0.46, 0.3 and 0.25, respectively. However, highly significant antigenic differences were observed with A/Eritrea RZ pool and A/Eri3/98, A22/Irq23-32 pool, A/Sau41/91/SI94, and A/Irn87/VQ pool and A/Irn96/UZ pool in which r_1-values were less than 0.12. This suggested that the vaccine strain might be suitable for use if no closer match could be found provided that a potent vaccine was used and animals were preferably immunized more than once.

Serotype O/ETH/15/2008 was highly significant antigenic similarity to reference vaccine strain of Turkey (O1/Manisa), United Kingdom (O/BFS/VP pool), Indian (O/Ind/R2/75), and Germany (O/Kauf/VQ) where r1-values were 0.46, 0.70, 0.69 and 0.58, respectively. Field isolated strain O/ETH/24/2008 was antigenetically related

to O1/Manisa, O/BFS/VP pool, and O/Ind/R2/75 where r_1 values were 0.44, 0.70 and 0.65, respectively. These suggested that there was a close relationship between field isolate and vaccine strain and a potent vaccine containing the vaccine strains were likely to confer protection. O/ETH/24/2009 was antigenetically related with O/manisa, O/Bfs, and O/Ind/R2/75, in which r_1-values were 0.32, 0.43, and more than 1, respectively. O/ETH/28/2009 was also antigenetically matched with O/manisa, O/Bfs/ and O/Ind/R2/75 in which r_1-values were 0.32, 0.74, and more than one, respectively, using 2dmVNT. Generally, despite the genetic variation observed for serotype O virus worldwide, the antigenic variation is not extensive and the current vaccine strains can protect against most outbreaks.

In conclusion, serotype O and A was identified with highest prevalence of serotype O. The O serotype isolated in Ethiopia lies on East Africa-3 topotype, while serotype A was laid in Africa topotype. The antigenic variation was not diverse for serotype O and most vaccine strains found at WRLFMD can protect against this strain in the study areas while serotype A has highest antigenic variation and only few vaccine strains found at WRLFMD can provide protection against this strain.

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REFERENCES

Asfaw W, Sintaro T (2000). The status of FMD in Ethiopia, a growing concern. Ethiopian Veterinary Epidemiology Newsletter. Addis Ababa, Ethiopia, 1:1-5.

Ayelet G, Mahapatra M, Gelaye E, Berhe GE, Rufeal T, Sahle M, Ferris NP, Wadsworth J, Hutchings GH, Knowles NJ (2009). Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981-2007. Emerging Infectious Diseases, 15(9):1409-1417.

Bronsvoort BMC, Radford AD, Tanya VN, Nfon C, Kitching RP, Morgan KL (2004). Molecular epidemiology of foot-and-mouth disease viruses in the Adamawa province of Cameroon. J. Clinical Microbiol. 42:2186 -2196.

Gelaye E, Beyene B, Ayelet G (2005). Foot and mouth disease virus serotype identified in Ethiopia. Ethiopian Vet. J. 9:7-9.

Klein J (2009). Review on understanding the molecular epidemiology of foot-and-mouth disease virus. Infection, Genet. Evol. 9:153-161.

Martel JL (1974). Foot-and-mouth disease in Ethiopia. Distribution of serotypes of foot-and-mouth disease virus. Rev. Elev. Med. Vet. Pays. Trop. 27:169-75.

Megersa B, Beyene B, Abunna F, Regassa A, Amenu K, Rufael T (2009). Risk factors for foot and mouth disease seroprevalence in indigenous cattle in southern Ethiopia: the effect of production system. Tropical Animal Health Production, 41:891-8.

OIE (2004). Foot and mouth disease. In: Manual of Standards for Diagnostic Tests and Vaccines. 5th Edition, Office international des Epizooties (OIE), Paris, France, pp. 111-128.

Roeder PL, Abraham G, Mebratu GY, Kitching RP (1994). Foot-and-mouth disease in Ethiopia from 1988 to 1991. Trop. Anim. Health Prod. 26:163-167.

Sahle M (2004). An epidemiological study on the genetic relationships of foot and mouth disease viruses in East Africa. Unpublished PhD thesis, University of Pretoria, South Africa, Pretoria.

Sahle M, Venter EH, Dwarka RM, Vosloo W (2004). Molecular epidemiology of serotype O foot-and-mouth disease virus isolated from cattle in Ethiopia between1979-2001. Onderstepoort J. Vet. Res. 71:129-138.

Samuel AR, Knowles NJ, Kitching RP, Hafez SM (1997). Molecular analysis of type O foot-and-mouth-disease viruses isolated in Saudi Arabia between 1983 and 1995. Epidemol. Infect. 119:381-389.

Samuel AR, Knowles NJK, Mackay DKJ (1999). Genetic analysis of type O viruses responsible for epidemics of foot-and-mouth disease in North Africa. Epidemiol. Infection 122:529-538.

Samuel AR, Ouldridge EJ, Arrowsmith AEM, Kitching RO, Knowles NJ (1990). Antigenic analysis of serotype O foot-and-mouth disease virus isolates from the Middle East, 1981-1988. Vaccine 8:390-396.

Yoseph L, Yilkal A, Mesfin S, Gelagay A, Shiferaw J, Haileleul N (2013). First confirmation of foot and mouth disease virus serotype SAT-1 in cattle and small ruminants in Ethiopia in 2007/08. Trop. Anim. Health Prod. 45:1265-1267.