Academic Journal of Animal Diseases 3(2): 08-16, 2014
ISSN 2079-200X

DOI: 10.5829/idosi.ajad.2014.3.2.85169

Molecular Epidemiology of Foot and Mouth Disease Virus Outbreaks in Ethiopia in 2011/2012

[1]Sentayhu Menda, [2]Shiferaw Jenberie, [3]Haileleul Negussie, [2]Gelagay Ayelet and [4]Kassaw Amasalu

[1]College of Agriculture and Natural Resource,
Debre Markos University, P. O. Box 269, Debre Markos, Ethiopia
[2]National Veterinary Institute, P. O. Box 19, Debre Zeit, Ethiopia
[3]College of Veterinary Medicine and Agriculture,
Addis Ababa University, P. O. Box 34, Debre Zeit, Ethiopia
[4]College of Veterinary Medicine, Mekelle University, Mekelle, Ethiopia, P.O. Box 2084

Abstract: The study was conducted in five regional states of Ethiopia: Amhara, Oromia, Southern Nation Nationalities and People's (SNNP), Tigray and Addis Ababa city council from January 2011 to March 2012 with the objectives of identifying the serotypes and topotypes of foot and mouth disease viruses and to determine the genetic relationship of FMD viruses in Ethiopia. From the study animals; cattle and swine, 59 epithelial tissue samples were collected in the FMD outbreak areas of the country and submitted to the National Veterinary Institute (NVI), Debre Zeit, Ethiopia and World Reference Laboratory for FMD (WRLFMD), Pirbright, UK for virus isolation, serotype identification and phylogenetic analysis. Cytopathic effect (CPE) was observed in 43 samples in BHK-21 cell culture. Only serotype O was recorded throughout the country where outbreaks occurred. A total of 36 samples were sent to WRLFMD, Pirbright, UK for further molecular characterization and phylogenetic analysis. Of this, 12 serotype O FMD viruses of Ethiopia were further characterized and phylogenetic relationships with each other and type O isolates from other countries of the world were determined. All serotype O isolates of Ethiopia falls into a single topotype, East Africa-3 (EA-3). More than 98% similarity with each other and approximately 14-15% different to members of the EA-1, EA-2, EA-4 and ME-SA topotypes of other countries of Africa and Asia were found. Serotype O isolated from Mekele University Farm was also 93-95% similarity with Sudan O isolates of 1999, 2004 and 2008. Generally regular investigation of FMD outbreaks to have more detailed information about the serotypes and topotypes circulating in Ethiopia is important for effective vaccine development.

Key words: Ethiopia · FMDV · Phylogenetic Analysis · Serotype and Topotype

INTRODUCTION

Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic losses; resulted from the loss of milk production, retarded growth, loss of draught power, abortion in pregnant animals and deaths in calves, kids and lambs [1].

Foot and mouth disease is probably the most important livestock disease in Ethiopia in terms of economic impact. he disease had become the major constraint hampering export of livestock and livestock products to the Middle East and African countries; the Egyptian trade ban of 2005/2006, in which Ethiopia lost more than US$14 million, being a recent memory [2]. Livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries.

A detailed knowledge of the molecular characteristics/epidemiology of the major antigenic sites of FMDV were helpful to define strains, to identify transmission events, to characterize biodiversity, to effective quarantine measures against reintroduction [3]

Corresponding Author: Sentayhu Menda, College of Agriculture and Natural Resource, Debre Markos University, P.O. Box 269, Debre Markos, Ethiopia.

and the development of specific diagnostic tests and protective vaccine. Most studies of FMDV have concentrated on the highly variable region of the capsid gene VP1, known to be an important antigenic site [4-6]. Phylogenetic analysis of the VP1 region of FMDV has been used extensively to investigate the molecular epidemiology of the disease worldwide. The techniques have assisted in studies of the genetic relationships between different FMDV isolates, geographical distribution of lineages and genotypes and the establishment of genetically and geographically linked topotypes and tracing the source of virus during outbreaks [7, 8].

The molecular epidemiology of FMDV has been studied in some detail in different countries of the world using nucleotide sequencing of the main antigenic determinant of the virus and phylogenetic analysis. In Ethiopia, however, records from the National Animal Health Diagnostic and Investigation Center (NAHDIC) and National Veterinary Institute (NVI) of Ethiopia indicated that serotypes O, A, C, SAT1 and SAT2 were responsible for FMD outbreaks during 1974 – 2008 [9-13]. Therefore, continuous research is needed to establish genetic relationships between different FMDV isolates, geographical distribution of lineages and genotypes, it is also important to identify the origin of infection. Therefore, the objectives of this study were to isolate and identify the serotypes and topotypes of foot and mouth disease viruses causing outbreaks in Ethiopia and to determine the genetic relationship of FMD viruses in Ethiopia

MATERIALS AND METHODS

General Description of Study Areas: The study was conducted from January 2011 to March 2012 in five national regional states of Ethiopia: Amhara, Oromia, Southern Nation Nationalities and People's (SNNP), Tigray and Addis Ababa. A total of thirteen FMD outbreaks; one in Amhara, seven in Oromia, two in SNNP, two in Tigray and one in Addis Ababa city council were investigated as shown in Figure 1.

The Amhara regional state is located in North-western and North central part of Ethiopia. It covers an estimated area of 170,752 km^2 [14] and FMD outbreaks occurred in Debre Berehan Zone. In Addis Ababa, which lies an altitude ranging from 2,000 - 2,800 m.a.s.l. There are about 5,200 dairy farms with some 58,500 cattle and almost 50% are cross breed [14]. FMD outbreak is investigated in Akaki-Kality District. In SNNP region, FMD outbreak

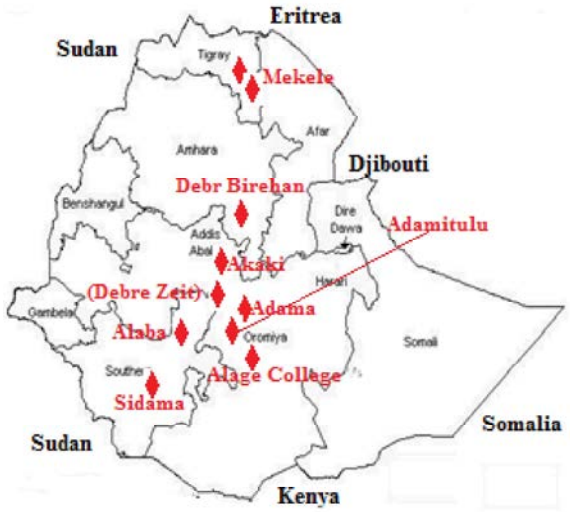

Fig. 1: Map of Ethiopia showing the distribution of FMD outbreaks in Ethiopia during the study period

was occurred in Sidama Zone, Malga woreda (Ketena District) and Alaba woreda (Negele Odisha district). Tigray regional State is located in Northern Ethiopia. The region has common boundaries with Afar and Amhara regional States at the Eastern and Southern parts, respectively and international boundaries with Sudan and Eritrea at the Western and Northern parts, respectively. It covers 54,548.32 km^2. The FMD outbreaks were occurred at Mekele University Dairy Farm and Mekele (Enderta District). FMD outbreaks were also investigated in the Oromia regional state. It covers 366,000 km^2, accounting for 31.17% of the total area of Ethiopia. FMD outbreaks were investigated in Eastern Shewa, Alage Dairy Farm (Alage College, Zeway), Adamitulu (Jido Kombolcha woreda), Debre Zeit Swine Farm (Debre Zeit), Behylu Dairy Farm (Debre Zeit), Tigest Dairy Farm (Debre Zeit), Ethiopian Meat and Dairy Technology Institute (EMDTI) (Debre Zeit) and Adama administrative Zone of Oromia regional State (Figure 1).

Study Population and Sampling Method: The study population consists of cattle and swine that manifest clinical signs of FMD in the outbreaks (Table 1). The outbreaks were occurred in five regions, eight administrative Zones and thirteen areas were included for the occurrence of FMD outbreaks. Sampling was purposive sampling and based on temporal feasibility to investigate. Cattle and swine of all age groups, sex, breeds and different management practices were recorded. Accordingly, a total of 59 epithelial tissue samples were collected.

Table 1: Summery of FMD Outbreaks in Ethiopia 2011/2012

No	Location (Area)	Date of Outbreak	Species
1	Alage Dairy Farm (Alage College)	1/1/2011	Bovine
2	Alaba (SNNP)	6/1/2011	Bovine
3	Adamitulu Jido kombolcha (Oromia)	7/1/2011	Bovine
4	Debre Zeit Swine Farm (Oromia)	15/7/2011	Swine
5	Behylu Dairy Farm (Debre Zeit) (Oromia)	3/9/2011	Bovine
6	Tigest Dairy Farm (Debre Zeit) (Oromia)	19/9/2011	Bovine
7	Malga (Sidam Zone, SNNP)	8/10/2011	Bovine
8	EMDTI (Debre Zeit) (Oromia)	14/10/2011	Bovine
9	Adama (Oromia)	26/10/2011	Bovine
10	Akaki-Kality (Addis Ababa)	25/11/2011	Bovine
11	Mekele Universty Farm (Tigray)	29/11/2011	Bovine
12	Enderta (Tigray)	1/12/2011	Bovine
13	Debre Berehan (Amhara)	30/3/2012	Bovine

Table 2: Primers used for the amplification of FMDV genome

Oligo name	Sequence (5' - 3')	Sense	Location	Serotype	Use PCR/ Sequencing
EUR-2B52R	GACATGTCCTCCTGCATCTGGTTGAT	-	2B	O, A, C	PCR & Sequencing
O-1C244F	GCAGCAAAACACATGTCAAACACCTT	+	VP3	O	PCR
O-1C272F	TBGCRGGNCTYGCCCAGTACTAC	+	VP3	O	PCR & Sequencing
O-1C283F	GCCCAGTACTACACACAGTACAG	+	VP3	O	PCR
A-1C562F	TACCAAATTACACACGGGAA	+	VP3	A	PCR
A-1C612F	TAGCGCCGGCAAAGACTTTGA	+	VP3	A	PCR & Sequencing
C-1C536F	TACAGGGATGGGTCTGTGTGTACC	+	VP3	C	PCR
C-1C616F	AAAGACTTTGAGCTCCGGCTACC	+	VP3	C	PCR & Sequencing

Key to symbol: R=A/G, Y=C/T, B=G/T/C, N=A/C/G/T

Study Design: At the beginning of an investigation proper information channel to Veterinary professionals, District Veterinary officers, Regional and Federal Animal and Plant Health Regulatory Authority, Regional laboratories and NAHDIC were organized. All these were informed to report FMD outbreaks to the NVI. When an active outbreak was reported, a field investigation was conducted at specific site of an outbreak (Table 1). Sample of animals were clinically examined for presence of FMD lesions on the mouth and feet, teat and udder and specimens were collect for diagnostic testing.

Clinical Examination: Cattle and swine were carefully examined for presence of characteristic clinical signs of FMD. In each outbreak, animals manifesting the characteristic signs of FMD like vesicular lesions (ruptured vesicles) in oral cavity and on the feet and teats, salivation, lameness and rise in temperature were considered as clinically affected by FMD. Other animals in the herd without these signs were similarly examined, but sampling of epithelial tissue in such instance was done only when lesions were suggestive of FMD.

Sample Collection: During the study period, epithelial tissue samples were collected from FMD suspected animals in different areas of Ethiopia (veterinary clinics, Institutes and Farms) and submitted to the NVI, Debre Zeit, Ethiopia. Bovine and swine epithelial tissue samples were collected from where outbreaks occurred. The samples were transported from the collection site to the NVI in 0.04 M phosphate buffer saline solution (pH 7.2–7.6) with glycerol and antibiotics at 4°C and stored at –20°C until processed [1]. For those samples which are tested at the NVI, were also submitted to the WRL for FMD, Pirbright, UK for additional studies. A total of 59 epithelial tissue samples were collected from 13 outbreaks during the study periods.

Virus Isolation and Serotype Identification: Virus isolation was established under laminar air flow hood class II on cell layers of baby hamster kidney (BHK-21) inoculated with 1 ml of filtered tissue suspension and incubated at 37°C for 1 hour for adsorption of the virus and then flashed with growth media (2% MEM media) and incubated at 37°C and 5% CO_2 in a humidified incubator for 24 - 48 hours. CPE was observed after 48 hours (or even less) in positive cases. If no CPE was detected, the cells were frozen and thawed, used to inoculate fresh cultures and examined for CPE for another 48 hours before the samples were declared to be negative [15-17]. Samples not exhibiting CPE by 72 hours post-infection on the second pass were considered virus

negative. Serotyping of FMDV were made by applying Agarose gel-based RT-PCR at NVI, Debre Zeit [18, 19] or/and by cell culture ELISA at WRL for FMD, Pirbright, UK [15].

Tissue-cultured FMD virus samples that showed CPE were submitted to World Reference Laboratory for FMD, Pirbright, UK for further serotyping, topotyping and phylogenetic analysis (Table 3). According to [20], specimens were submitted to the WRL for FMD using the following recommended international standards format of three letter country code / isolate number /year (e.g, ETH/02/2012).

Extraction of Virus RNA: The total RNA was extracted from the cell culture isolated by using RNeasy® mini kit (Qiagen, USA) based on the manufacturer protocols. Briefly, 500µl of tissue culture sample was taken and put into a 1.5 ml eppendorf tube, centrifuge at 5000 rpm for 15 sec and add 460µl volume of Lysis buffer RLT (Containing 1% 2-mercaptoethanol) was added to the sample and mixed by vortexing and then 460µl of 70% ethanol was added and mixed by vortexing. The mixture was transferred to RNeasy spin column (700µl maximum loading volume) and spinned in a micro centrifuge for 15 sec at 13,000 rpm. The flow through was discarded and repeated with remaining volume. The RNA was washed with 700µl washing buffer RW1 (centrifuge for 15 sec at 13,000 rpm) and with 500µl RPE buffer subsequently. After the flow through was discarded the column was centrifuged at 13,000 rpm speed for 2 min to dry the membrane. Then, the RNA was eluted with 50µl DEPC-H_2O into a new clean collection tube and stored at -20°C.

Complementary DNA Synthesis (cDNA): The complementary DNA was synthesised based on the manufacturer protocol (Invitrogen, Germany) in 20µl reaction volume. Primarily 1µl oligodT primer, 1µl 10 mM dNTPs, 3µl RNase free water and 5 µl extracted RNA were added to 0.5ml PCR tube and incubated at 65°C for 3 minutes (PCR machine) and chilled on ice for 3 minutes. Then 10x RT buffer, 25mM $MgCL_2$, 0.1M DTT and RNase out were added with the rate of 2µl, 4µl, 2µl and 1µl, respectively and incubated at 42°C for 2 minutes. Finally, 1.5µl Superscript III (Reverse transcriptase) was added and incubated at 42°C for 50 minutes, followed by heating at 85°C for 5 minutes and chilled on ice for 2 minutes then add 1 µl RNase-H and centrifuge and incubate at 37°C for 20 minutes and cDNA was stored at -20°C until needed.

Polymerase Chain Reaction (PCR) and Primers Used: The PCR was performed using (Advantage® cDNA PCR, Novagen, USA) Kit. Primarily master mix containing (5µl 10x PCR buffer, 1.5µl 25mM $MgCl_2$, 1µl 10mM each dNTPs, 0.5µl Taq DNA polymerase and 35µl DEPC-H_2O) was prepared for each reaction. Then, 12µl of the master mix, 1.5µl of each of specific forward and reverse primer (10pmol) and 5µl of the cDNA were added to each tube. Final run on a 2720 Thermal cycler (Applied Biosystem, USA) program cycle of initial denaturation at 95°C for 5 minutes and then 40 cycles of 94°C for 45 seconds, 65°C for 45 seconds and 72°C for 2 minutes, ending with incubation at 72°C for 7 minutes.

Three alternative primer combinations were used for RT-PCR of FMDV serotype O viruses: O-1C244F/EUR-2B52R, O-1C272F/EUR-2B52R and O-1C283F/EUR-2B52R and two primer sets for each of the other serotypes: serotype A (A-1C562F/EUR-2B52R and A-1C612F/EUR-2B52R), serotype C (C-1C536F/EUR-2B52R and C-1C616F/EUR-2B52R) (Invitrogen, Germany) (Table 2).

Agarose Gel Electrophoresis for Serotype Identification: The PCR products were analyzed on a 1.5% agarose gel containing 0.5 µg/ml of ethidium bromide (Sigma, USA). Briefly, 10µl PCR product mixed with 2 µl loading buffer (Invitrogen, Germany) and loaded to wells in pre prepared gel and run at 100 volt for about 1 hour in parallel with DNA molecular weight marker in electrophoresis apparatus. The DNA band was visualized by UV illumination, documented and the size was determined against the DNA molecular weight marker and serotype was identified. For DNA quantification GeneRuler™ 100bp plus DNA ladder (Fermentas, Germany) was used and run at 100 volt for one hour and DNA concentration is determined.

Sequencing Reaction: Selected virus isolates were further characterized by sequencing of the VP1 gene between serotype O FMD viruses in Ethiopia as well as with other O type isolates from other countries of the world (Table 3). The sequencing reaction was performed at World Reference Laboratory for FMD, Pirbright, UK.

Data Analysis: A homologous region of 639 nucleotides corresponding to the whole VP1 gene was used for all phylogenetic analysis. Nucleotide sequences of serotype O isolates from other African countries were included to deduce the phylogeny of this serotype on the African continent as well as isolates from the Middle East and

Table 3: Source of FMD Type O viruses used for genotyping comparisons (Sequencing reaction)

Pos	Virus name	File name	Topotype	Strain
1	O/ETH/9/2011	ETH11-09	EA-3	Unnamed
2	O/ETH/7/2011	ETH11-07	EA-3	Unnamed
3	O/ETH/7/2010	ETH10-07	EA-3	Unnamed
4	O/ETH/59/2011**	ETH11-59	EA-3	Unnamed
5	O/ETH/58/2005 (FJ798141)	ETH05-58	EA-4	Unnamed
6	O/ETH/51/2011**	ETH11-51	EA-3	Unnamed
7	O/ETH/50/2011**	ETH11-50	EA-3	Unnamed
8	O/ETH/5/2011	ETH11-05	EA-3	Unnamed
9	O/ETH/49/2011**	ETH11-49	EA-3	Unnamed
10	O/ETH/48/2011**	ETH11-48	EA-3	Unnamed
11	O/ETH/46/2011**	ETH11-46	EA-3	Unnamed
12	O/ETH/45/2011**	ETH11-45	EA-3	Unnamed
13	O/ETH/45/2009	ETH09-45	EA-3	Unnamed
14	O/ETH/42/2011**	ETH11-42	EA-3	Unnamed
15	O/ETH/4/2011	ETH11-04	EA-3	Unnamed
16	O/ETH/38/2011**	ETH11-38	EA-3	Unnamed
17	O/ETH/34/2011**	ETH11-34	EA-3	Unnamed
18	O/ETH/32/2011**	ETH11-32	EA-3	Unnamed
19	O/ETH/3/2011	ETH11-03	EA-3	Unnamed
20	O/ETH/3/2004 (FJ798109)	ETH04-03	EA-3	Unnamed
21	O/ETH/29/2011**	ETH11-29	EA-3	Unnamed
22	O/ETH/28/2011	ETH11-28	EA-3	Unnamed
23	O/ETH/26/2011	ETH11-26	EA-3	Unnamed
24	O/ETH/2/2011	ETH11-02	EA-3	Unnamed
25	O/ETH/2/2006 (FJ798127)	ETH06-02	EA-3	Unnamed
26	O/ETH/18/2011	ETH11-18	EA-3	Unnamed
27	O/ETH/13/2011	ETH11-13	EA-3	Unnamed
28	O/ETH/11/2011	ETH11-11	EA-3	Unnamed
29	O/ETH/1/2011	ETH11-01	EA-3	Unnamed
30	O/ETH/1/2007 (FJ798137)	ETH07-01	EA-3	Unnamed
31	O/BHU/3/2009	BHU09-03	ME-SA	Ind-2001d
32	O/UGA/5/96 (AJ296327)	UGA96-05	EA-1	Unnamed
33	O/UGA/3/2002 (DQ165077)	UGA02-03	EA-2	Unnamed
34	O/TAN/2/2004	TAN04-02	EA-2	Unnamed
35	O/SUD/6/2008 (GU566062)	SUD08-06	EA-3	Unnamed
36	O/SUD/5/2008 (GU566061)	SUD08-05	EA-3	Unnamed
37	O/SUD/4/99 (GU566044)	SUD99-04	EA-3	Unnamed
38	O/SUD/4/2008 (GU566060)	SUD08-04	EA-3	Unnamed
39	O/SUD/3/99 (GU566043)	SUD99-03	EA-3	Unnamed
40	O/SUD/3/2008 (GU566059)	SUD08-03	EA-3	Unnamed
41	O/SUD/2/86 (DQ165075)	SUD86-02	EA-3	Unnamed
42	O/SUD/14/2004 (GU566050)	SUD04-14	EA-3	Unnamed
43	O/SUD/1/99 (DQ165076)	SUD99-01	EA-3	Unnamed
44	O/MAL/1/98 (DQ165074)	MAL98-01	EA-2	Unnamed
45	O/K83/79* (AJ303511)	KEN79B83	EA-1	Unnamed
46	O/K40/84*	KEN84-40	EA-1	Unnamed
47	O/IRN/61/2001 (DQ164896)	IRN01-61	ME-SA	Irn-2001
48	O/IND/R2/75* (AF204276)	IND75--A	ME-SA	Unnamed
49	O/IND/53/79 (AF292107)	IND79A53	ME-SA	Unnamed

*Not a WRL for FMD reference number

**Samples submitted to WRL for FMD, Pirbright, UK.

South Asia to ensure that all previously identified lineages and genotypes were represented [7]. Phylogenetic tree were constructed using methods of analysis included in MEGA version 5.0 [21] and confidence levels were assessed by 1000 bootstrap replications. Serotypes were distinguished on the basis of nucleotide sequence differences of 30-50% and high bootstrap support (> 70%) while a divergence of 15% to 20% distinguishes topotypes [7].

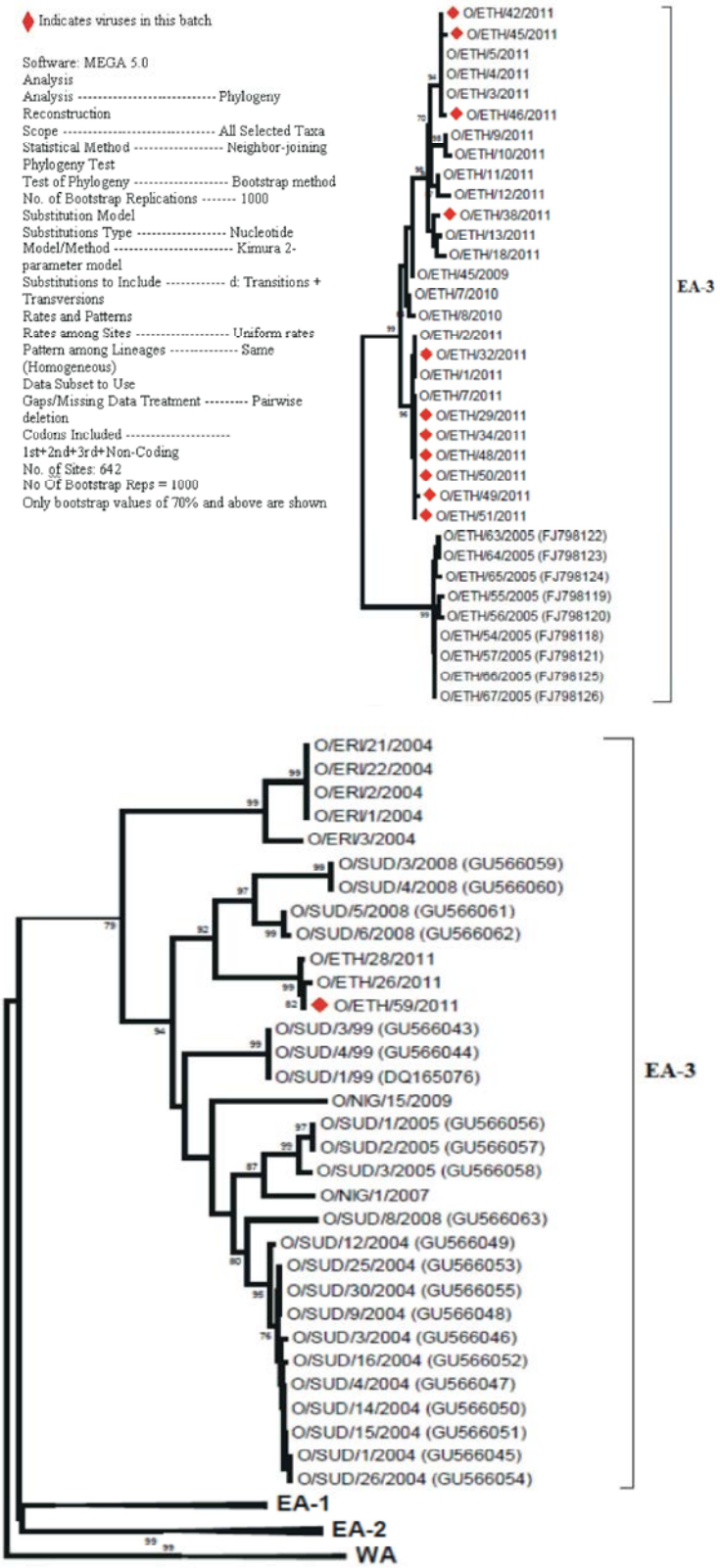

Fig. 2: Phylogenetic tree showing the relationships between VP1 sequences of serotype O FMDV isolates. (A) Isolates from Debre Zeit, Adama and Sidama, (B) Isolate from Mekele University Farm

Table 4: FMDV serotype identified in different Regions of outbreaks

No	Regions of outbreak	No of sample	CPEPositive	Serotype
1	Oromia	28	18	O
2	Southern Nation Nationalities and People's (SNNP)	10	8	O
3	Tigray	11	11	O
4	Addis Ababa	2	2	O
5	Amhara	8	4	O
Total	59	43		

RESULTS

Virus Isolation and FMDV Serotype Identification: Out of the total 59 bovine and swine epithelial tissue cultured samples, 43 samples were showed FMDV CPE on BHK-21 monolayer cell cultures (Table 4). The CPE was characterized by a fast destruction of the BHK-21 monolayer cells and infected cells were found singly and the cell was found round in shape. Complete destruction of the cell sheet was mostly seen within 48 hours of inoculation. Of 43 samples that showed CPE, 36 samples were sent to WRL for FMD, Pirbright, UK, for further serotyping, topotyping and phylogenetic analysis.

On those samples that showed CPE, further examination were done to identify the type of the virus at NVI, Ethiopia and WRL for FMD, Pirbright, UK. Thus, only serotype O was recorded from all samples collected from outbreaks (Table 4).

Phylogenetic Analysis: The VP1 gene characterization was used to study phylogenetic relationships between 12 serotype O FMD viruses in Ethiopia as well as with other O type isolates from other countries of the world. All serotype O isolates of Ethiopia falls it to a single topotype, East Africa-3 (EA-3) as shown in Figure 2 A and B.

Serotype O isolated in different districts of Ethiopia during the study periods were also compared with each other and with other countries type O isolates. Isolates from Debre Zeit, Adama and Sidama were showed 98.75-100% similarity to each other and approximately 14–15% different to members of the EA-1, EA-2, EA-4 and ME-SA topotypes of other countries of Africa and Asia (Figure 2A). Isolate from Mekele University Farm O/ETH/59/2011 was 99.84% similarity with Ethiopian isolates of 2011 (O/ETH/26/2011 and O/ETH/28/2011) which were collected by other person. This isolate (O/ETH/59/2011) was also 94.05–95.31%similarity with Sudan isolates of 2008 (O/SUD/3/2008(GU566059), O/SUD/4/2008(GU566060), O/SUD/5/2008(GU566061), O/SUD/6/2008(GU566062)), 93.11% similarity with Sudan isolates of 1999 (O/SUD/1/99(DQ165076), O/SUD/3/99(GU566043), O/SUD/4/99(GU566044)) and 92.96% similarity with Sudan isolates of 2004 (O/SUD/14/2004(GU566050)), respectively which are genetically homologues at 639nt. sequence level, but it differ 13.62-14.42% with topotypes of EA-1, EA-2, EA-4 and ME-SA of other countries of Africa and Asia (Figure 2B).

DISCUSSION

FMD Virus Isolation and Serotype Identification: In this study FMD virus was isolated from most of the samples collected from outbreaks. Out of the total 59 epithelial tissue-cultured samples, 43 samples showed FMDV CPE on BHK-21 monolayer cell cultures for FMD virus suspected tissue, but the other 16 tissue cultured samples had not yet any CPE. This might be due to improper transportation from the field to the NVI laboratory since some outbreaks were occurred in areas where vehicle is inaccessible and some may be due to death of the virus during transportation.

Serotyping of the virus revealed that serotype O was the dominant serotype identified from bovine and swine samples collected from different district of Ethiopia. Previous studies have also indicated that serotype O was highly prevalent and a dominant serotype causing most of the outbreaks in Ethiopia [10, 13, 22]. Klein [23] indicated that it is the most prevalent serotype worldwide.

Phylogenetic Analysis: The molecular epidemiology of serotype O has been well studied [3, 7]. Samuel and Knowles [3] demonstrated the existence of eight serotype O topotypes within samples collected around the world based on the comparison of sequence data of the VP1 gene. Among those, two topotypes were found in Africa, one in East Africa and one in West Africa.

In this study all serotype O strains had falls within East Africa-3 (EA-3) topotypes. These indicated that EA-3 topotype has wider distribution and highly prevalent in Ethiopia. This is in agreement with previous study on molecular epidemiology of serotype O by Ayelet *et al*. [22] and Haileleul *et al*. [13] demonstrated the existence of

EA-3 and EA-4 topotypes in Ethiopia based on the comparison of sequence data of the VP1 gene with the highest rate of EA-3 topotype.

Serotype O isolates from Debre Zeit, Adama and Sidama were closely related to each other (<2% nt. sequence difference) were observed at 639 nt. sequence. This indicated that outbreaks due to these isolates were the same origin. Furthermore, serotype O isolated from Mekele University Farm O/ETH/59/2011 and Ethiopian isolates of 2011 (O/ETH/26/2011 and O/ETH/28/2011) collected by other person were closely related (< 1% nt. difference) which indicated that these outbreaks have the same origin. This might be due to free movement of livestock and livestock products among various markets in different regions and states which play an important role in the dissemination of the virus. This isolate (Mekele University Farm isolate (O/ETH/59/2011) were also genetically most closely related (~ 4-7% nt. sequence differences) from Sudan isolates in 2008 (O/SUD/3/2008(GU566059), O/SUD/4/2008(GU566060), O/SUD/5/2008(GU566061), O/SUD/6/2008(GU566062)), Sudan isolates in 1999 (O/SUD/1/99(DQ165076), O/SUD/3/99(GU566043), O/SUD/4/99(GU566044)) and Sudan isolates in 2004 (O/SUD/14/2004(GU566050)). These indicated that outbreaks due to these isolates were the same epizootics (common origin). This might be due to free movements of livestock and livestock products among Ethiopian and Sudan borders.

CONCLUSIONS

Foot and mouth disease is endemic in Ethiopia due to the factors such as the presence of high numbers of susceptible domestic animals, free movement of livestock and livestock products in different regions and states across the country, free cross borders between neighboring countries, lack of control of animal movements and lack of effective vaccination contributed to the occurrence of FMD and to the difficulty in controlling the outbreaks. During the study periods only serotype O were identified throughout the Ethiopia where outbreaks occurred. Out the total samples collected, most samples showed CPE in BHK-21 cell culture. Samples were also sent to WRL for FMD, Pirbright, UK for further molecular characterization and phylogenetic relationship with other O type isolates form other countries of the world thus, all O serotype isolates of Ethiopia falls in to a single topotype East Africa-3 (EA-3) and they are related with each other but differ from other O topotypes; whereas isolate from Mekele University Farm was also have similarity with Sudan O isolates. Therefore, Restriction of animal movement across the regions, importation/movement of livestock and livestock products across the border areas and regular investigation of FMD outbreaks should be done to have more detailed information about the serotypes and topotypes circulating in Ethiopia.

ACKNOWLEDGEMENTS

The authors would like to thank the NVI, Ethiopia, for Funding and the provision of laboratory facilities. The molecular Sequencing was carried out at the WRL for FMD, Pirbright UK.

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