

Full Length Research Paper

## Molecular serotyping of foot and mouth disease outbreaks in Ethiopia

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This study was conducted in five regional states of Ethiopia from January 2011 to March 2012 with the objective of identifying the serotypes of foot and mouth disease by molecular technique in Ethiopia. Epithelial tissue samples were collected from cattle and swine found in the foot and mouth disease outbreak areas of the country and submitted to the National Veterinary Institute, DebreZeit, Ethiopia and World Reference Laboratory for Foot and Mouth Disease, Pirbright, UK. Thus, virus isolation and serotype identification were performed. From a total of 59 samples, cytopathic effect was observed in 43 (72.88%) samples in BHK-21 cell culture. Serotyping of foot and mouth disease viruses were done by applying agarose gel-based RT-PCR at the National Veterinary Institute, and by cell culture ELISA at World Reference Laboratory for Foot and Mouth Disease. Serotype O was recorded throughout the country where outbreaks occurred. Regular investigation of foot and mouth disease outbreaks is important to have more detailed information on the serotypes and topotypes circulating in Ethiopia and for effective vaccine development.

**Key words:** Ethiopia, foot and mouth disease (FMD), serotype.

### INTRODUCTION

Foot and mouth disease (FMD) is a severe, highly contagious viral disease of livestock with significant economic impact. The main effect of the disease is its economic losses resulting from the loss of milk production, retarded growth, loss of draught power, abortion in pregnant animals, and deaths in calves, kids and lambs. In areas of the world where food and draft animals are essential for subsistence agriculture, FMD can affect nutrition. In countries with highly developed animal industry and free trade, outbreaks are responsible for economic devastation (OIE, 2007).

Foot and mouth disease virus (FMDV) was identified by Loeffler and Frosch in 1898 as the first filterable viral agent to cause animal disease. The virus responsible for FMD is a member of the *Aphthovirus* genus in the *Picornaviridae* family (Alexandersen and Mowat, 2005). There are seven immunologically distinct serotypes: O, A, C, South African Territories 1 (SAT-1), SAT-2, SAT-3 and Asia 1 and over 60 strains within these serotypes. New strains occasionally develop spontaneously. Early indications of the disease include fever, excessive salivation and vesicles on the tongue especially in small ruminants

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in which clinical signs are often milder, depending on the strain of the virus. The disease spreads rapidly among non-immunized animals, because of very high morbidity rates, whilst mortality is low except in young animals (Cameron et al., 1999; Geering and Lubroth, 2002; Ryan et al., 2007).

The disease affects cattle, swine, sheep, goats and other cloven hoofed ruminants. Furthermore, elephant and giraffe are susceptible to FMD (Kitching, 2005; Mahy, 2005). Depending on the conditions, FMDV can become aerosol and spread to susceptible animals. FMD is a notifiable disease because the exports of infected livestock and animal products could easily cause outbreaks in countries currently free from FMD. Recently, FMD has been endemic in several parts of the world, particularly in Asia, South Africa, the Middle East, and South America (Mahy, 2005).

FMD is probably the most important livestock disease in Ethiopia in terms of economic impact. Recently, the 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 reminiscence (Leforban, 2005). Livestock are at risk of endemic strains as well as antigenic variants prevailing in neighbouring countries.

Serotype identification of FMDV in Ethiopia were done mostly by 3ABC ELISA, but the recent detailed knowledge of the molecular characteristics of FMDV major antigenic sites have been helpful to identify serotype, strains and transmission events, to characterize biodiversity and effective quarantine measures against reintroduction (Samuel and Knowles, 2001), and to develop specific diagnostic tests and protective vaccine. Genetic analysis of the viral protein 1 (VP1) region of FMDV has been extensively used to investigate the molecular epidemiology of the disease worldwide. The techniques have assisted in studies of the genetic relationship 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 (Knowles and Samuel, 2003; Sangare et al., 2003)

Molecular techniques to identify FMDV have been studied in details in different countries of the world. In Ethiopia, however, records from the National Animal Health Diagnostic and Investigation Center (NAHDIC) and the National Veterinary Institute (NVI) indicated that serotypes O, A, C, SAT-1 and SAT-2 were responsible for FMD outbreaks during 1974-2008 (Sahle et al., 2004; Gelaye et al., 2005; Legess, 2008; Gelagay, 2009; Haileleul et al., 2010). Continuous research is needed to identify FMDV isolates using molecular methods. Therefore, the objective of this study was to identify the serotypes of FMD viruses causing outbreaks in Ethiopia by a molecular technique.

## MATERIALS AND METHODS

### General description of study areas

This 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.

The Amhara regional state is located in North-western and North central part of Ethiopia, with an estimated area of 170,752 km<sup>2</sup> (Central Statistical Authority, CSA, 2012). 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 (CSA, 2012). In SNNP region, FMD outbreak occurred in Sidama Zone. 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<sup>2</sup>. FMD outbreaks were also investigated in the Oromia regional state, which covers 366,000 km<sup>2</sup>, accounting for 31.17% of the total area of Ethiopia.

### Study population and sampling method

The study population consisted of cattle and swine that manifested clinical signs of FMD in the outbreaks. Five regions, eight administrative zones, and thirteen areas were included for the occurrence of FMD outbreaks. Sampling was purposive 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.

### Study methodology

#### *Clinical examination*

Cattle and swine were carefully examined for the presence of characteristic clinical signs of FMD. In each outbreak, animals manifesting 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. Samples were transported from the collection site to the NVI in 0.04 M phosphate buffered saline solution (pH 7.2-7.6) with glycerol and antibiotics at 4°C and stored at -20°C until processed (OIE, 2007). Samples which were tested at the NVI were also submitted to the World Reference Laboratory (WRL) for FMD, Pirbright, UK. 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

**Table 1.** Summary of cytopathic effect (CPE) observed on tissue cultures.

Animal species	No. of tested samples	No. of CPE positive samples	Percentage of CPE positive samples
Bovine	54	38	70.37%
Swine	5	5	100%
Total	59	43	72.88%

**Table 2.** FMDV serotype identified in different outbreaks of Ethiopia.

Site of outbreak	No. of sample	CPE positive	Serotype by		Final result
			Agarose gel-based RT-PCR	Cell culture ELISA	
Alage Dairy Farm (Oromia)*	7	1	O	-	O
Alaba (SNNP)*	3	1	O	-	O
AdamituluJidokombolcha (Oromia)*	1	1	O	-	O
Debre Zeit Swine Farm (Oromia)	5	5	O	O	O
Behylu Dairy Farm (DebreZeit) (Oromia)	2	1	O	O	O
Tigest Dairy Farm (Debre Zeit) (Oromia)	4	3	O	O	O
Malga (Sidam Zone) ( SNNP)	7	7	O	O	O
EMDTI (Debre Zeit) (Oromia)	5	5	O	O	O
Adama (Oromia)	4	2	O	O	O
Akaki-Kaliti (Addis Ababa)	2	2	O	O	O
Mekele Universty Farm (Tigray)	8	8	O	O	O
Enderta (Tigray)	3	3	O	O	O
Debre Berehan (Amhara)*	8	4	O	-	O
Total	<b>59</b>	<b>43</b>			

\*Not sent to the World Reference Laboratory for FMD, Pirbright, UK.

on baby hamster kidney-21 (BHK-21) cell layers inoculated with 1 ml of filtered tissue suspension and incubated at 37°C for 1 h for virus adsorption, then flashed with 2% Modified Essential Medium and finally incubated at 37°C and 5% CO<sub>2</sub> in a humidified incubator for 24-48 h. Cytopathic effect (CPE) was observed after 48 h (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 h before the samples were declared to be negative (Buxton and Faser, 1977; OIE, 1990; Yoseph et al., 1991). Samples not exhibiting CPE by 72 h post-infection on the second step were considered virus negative. Serotyping of FMDV was made by applying Agarose gel-based RT-PCR at the NVI (Vangrysterre and De Clercq, 1996; Mehran et al., 2006) or/and by cell culture ELISA at the WRL for FMD (Buxton and Faser, 1977). According to Kitching and Donaldson (1987), specimens were submitted to the WRL for FMD using the recommended international standard format of three letter, indicating the country code, isolate number and year of isolation (for example, ETH/02/2012).

## RESULTS

### Virus isolation

Forty-three (72.88%) out of the total 59 bovine and swine epithelial tissue cultured samples showed FMDV CPE on

BHK-21 monolayer cell cultures (Table 1). The CPE was characterized by a fast destruction of monolayer cells, and infected cells were found singly and round shaped. Complete destruction of the cell sheet was mostly seen within 48 h of inoculation. Of the 43 samples that showed CPE, 36 samples were sent to the WRL for FMD for further serotyping analysis.

### FMDV serotype identification

Only FMDV serotype O was found both by agarose gel-based RT-PCR at the NVI and by cell culture ELISA at the WRL for FMD on samples collected from outbreaks that showed CPE (Table 2).

## DISCUSSION

In this study, FMDV was isolated from most of the samples collected from outbreaks. Forty-three (72.88%) out of the total of 59 epithelial tissue-cultured samples showed FMDV CPE on BHK-21 monolayer cell cultures for FMD virus suspected tissue, while the other 16 tissue cultured samples had no CPE. This might be due to im-

proper transportation from the field to the NVI laboratory since some outbreaks occurred in areas where vehicle is inaccessible and some may be due to death of the virus during transportation.

As previously reported (Gelaye et al., 2005; Ayelet et al., 2009; Haileleul et al., 2010), our results confirmed that serotype O, the most prevalent serotype worldwide (Klein, 2009), was the dominant serotype from bovine and swine samples collected from different district of Ethiopia.

## Conclusions and recommendations

FMD is endemic in Ethiopia due to factors such as the presence of high number of susceptible domestic animals, free movement of livestock and livestock products in different regions and states across the country and free cross borders between neighbouring countries. Moreover, lack of control of animal movements and ineffective vaccination measures may contribute to the occurrence of FMD and the difficulty in controlling the outbreaks. Only serotype O was identified during the study period throughout the Ethiopia where outbreaks occurred. Most of the samples collected showed CPE in BHK-21 cell culture. Restriction of animal movement across the regions, importation/movement of livestock and livestock products across the border areas, regular investigation of FMD outbreaks and further phylogenetic analysis should be done to have more detailed information on the serotypes and topotypes circulating in Ethiopia.

## Conflict of Interest

The authors have not declared any conflict of interest.

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