

Serotype Identification and Molecular Characterization of Foot and Mouth Disease in and Around Mekelle, Tigray Region

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Abstract: FMD is one of the most economically important disease affecting the livestock sector in most developed and developing countries causing death of calves and morbidity of adult animals. This disease is caused by seven distinct serotypes namely O, A, C, SAT1, SAT 2, SAT3 and Asia 1. The study was conducted in Tigray regional state of Ethiopia from January 2011 to March 2012 with major objective of serotype identification and molecular characterization of foot and mouth diseases virus. A total of 30 samples were collected from clinically sick cattle during the outbreak from Mekelle University Farm, Aynalem, Shibta, Cholekot, Debri and submitted to the National Veterinary Institute (NVI), Debre-Zeit Ethiopia and World Reference Laboratory for FMD (WRLFMD) Perbright, UK, for virus isolation, serotype identification and phylogenetic analysis. The result showed that cytopathic effect (CPE) was observed in 30 samples with BHK-21 cell culture. From this, serotyping of FMD virus was done by cell culture ELISA at Perbright and serotype O was identified. From the 30 samples, 11 samples were sent to Perbright, UK for further molecular characterization and phylogenetic analysis and of this, 1 serotype O FMD (O/ETH/59/2011) virus of Ethiopia was characterized to study phylogenetic relationships with other O type isolates from other countries. O serotype isolates of Ethiopia fell into a single topotype East Africa-3 (EA-3). This isolate (O/ETH/59/2011) was also (94.05-95.31%), 93.11% and 92.96% similarity with Sudan isolates. The study revealed that FMD virus was circulating in the study area. The serotype identified was serotype O and accordingly the vaccination, animal movement control and quarantine were recommended to control the disease.

Key words: Cattle • FMDV • Phylogenetic Analysis • Serotype • Tigray • Topotype

INTRODUCTION

Ethiopia is one of few countries in Africa with huge livestock resources that play a crucial role in the livelihoods of the majority of Ethiopians. Animal rearing is an integral part of the agricultural production in Ethiopia and animals represent the major drought power (95%) for crop production. The country is believed to have the largest livestock population in Africa comprising approximately 49.3 million cattle, 25.02 million sheep and 27.88 goats [1]. The agricultural sector constitutes about 45% of the gross domestic production (GDP), more than 90% of foreign exchange earnings, 85% of employment opportunities and most of the domestic food supply [2].

Animal diseases are currently widespread in all agro-economical zone of the country and annual mortality rates due to diseases is estimated at 8-10% for cattle herd and 15% and 12% for sheep and goats flock respectively. It is estimated that animal diseases reduce the production and productivity of livestock by 50 to 60% per year. Among the livestock diseases hampering productivity of the sector foot and mouth disease is considered as a bottleneck to the livestock production [3].

Foot-and-mouth disease virus (FMDV) is 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. There are seven

immunologically distinct serotypes - O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 and over 60 strains within these serotypes were found [4].

The disease is endemic to most countries in sub-Saharan Africa where six of seven serotypes occur, where virus circulates between wild hosts and domestic animals [5]. The disease could not be eradicated from south and east Africa where infected buffalos are the main reservoirs. Lack of animal movement control within countries and across international borders for both wild life and domestic animals aggravate the problem.

In Ethiopia FMD is one of the major livestock diseases of socio-economic importance. 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 memory [6]. Livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries. The disease occurred frequently in pastoral herds in the low land areas of Ethiopia [7] but in recent years the incidence of this disease has increased and became apparent in the high land areas where 60% of the total livestock population occurs.

In Ethiopia According to Animal Health Division of Ministry of Agriculture (2000), the incidence of FMD has increased between 1.3 to 1.5 times since 1990. The occurrence of FMD in Ethiopia is increasing and in 1999, almost 10% of cattle were under risk of infection [8] and the records of ministry of agriculture and rural development (MOARD) indicated that FMD outbreaks occurred every year from 2000 to 2006 with the highest in 2001 with 88 outbreaks

An understanding of the molecular epidemiology of the disease is critical for the implementation of good control programmers and the eradication of the disease. An important part of combating foot-and-mouth disease (FMD) is virus characterization, which is important to establish the geographic relatedness between isolates, the genetic variation and molecular evolution of viruses in carriers. During outbreaks, it is also important to identify the origin of infection and its relationship to vaccines available for protection which will assist in planning a control program in the country. This year FMD outbreak has been occurred in Tigray region. The current outbreak was responsible for the high death of animals in the region.

Therefore the objective of this study was to identify the serotype circulating in the region and molecularly characterize the Foot and mouth disease virus of the outbreak in the study area.

MATERIALS AND METHODS

Study Area: The study was conducted from January 2011 to March 2012 in Tigray Regional State, northern Ethiopia in and around Mekelle. The Region covers an area of 54,548.32km². The livestock resource of the Region consists of 3,596,649 cattle, 1,646,752 goats, 1,064,501 sheep, 364,940 equines, 13,661 camels and 2,570,833 poultry, representing nearly 10% of the livestock population of the country. Mekelle is the capital city of Tigray Regional State located about 783 km North of Addis Ababa at 38.5° East longitude and 13.5° North latitude at an altitude of 2300 m.a.s.l. The climate conforms to that of the Ethiopian highland.

Study Population and Sampling Method: The study population consists of cattle that were found in the outbreak areas of FMD. The investigation of outbreak was conducted in Mekelle city, shibta, Ayanalem, Deбри and Chelekot. Tissue samples were collected purposely from infected animals.

Study Methodology

Clinical Examination: During the outbreak investigation animals were clinically examined for the presence of typically vesicular lesions in the mouth, lips, tongue, feet and teat.

Sample Collection: A total of thirty epithelial tissues was collected from non ruptured or freshly ruptured vesicles and placed in the bottle with transport medium, equal amount of buffer saline solution. The samples were labeled with animal species identification number, sex, age and type of tissue. The samples were transported using ice box to the National Veterinary Institute (NVI) laboratory, Debrezeit.

Virus Isolation and Serotype Identification: A suspension was prepared by grinding the sample with sterile pistol and mortar with small volume tissue culture medium and antibiotics medium was added, so that the final volume was 10x that of the epithelial tissue, producing

10% suspension [9]. The suspension was centrifuged at 3500 rpm for 10 minutes. The suspension was filtered in universal bottle.

Isolation and characterization of the virus was golden rule for diagnosis of viruses. The supernatant was collected and filtered by Millipore filter of 0.22µm pore size. About 1 ml of filtered tissue suspension was inoculated on baby hamster kidney (BHK-21) monolayer cells grown on 25 cm² tissue culture flask and incubated at 37°C for 1 hour for adsorption of the virus and then flashed with growth media (2% MEM) and incubated at 37°C and 5% CO₂ in a humidified incubator for 24-48 hour. The Monolayer which were shown CPE harvested when 85-100% of CPE was observed. Tissue-cultured FMD virus samples that showed CPE were labeled using the following format: three-letter country code/isolate number/year (e.g., ETH/02/2012). The three letter country codes were designated as outlined by the World Reference Laboratory for FMD. The samples were submitted to World FMD Reference Laboratory, Perbright, UK, for further serotyping, topotyping and phylogenetic analysis.

RESULTS

Virus Isolation: All the collected samples (30) were cultured on BHK and cytopathic effect (CPE) on BHK-21 monolayer cell cultures for FMD virus was observed after 24 hours which indicates the presence of the virus in the collected tissue. The CPE is characterized by a fast destruction of the BHK-21 monolayer cells and infected cells were found singly and the cell becomes round in shape. Complete destruction of the cell sheet was mostly seen within 48 hours of inoculation. From the 30 samples that showed CPE, eleven [9] samples were sent to WRL for FMD, Perbright, UK, for further serotyping, topotyping and phylogenetic analysis.

FMDV Serotype Identification: Serotype O was isolated from CPE positive samples using cell culture ELISA at WRL for FMD, Perbright, UK (Table1).

Phylogenetic Analysis: Phylogenetic analysis was done at WRL for FMD, Perbright, UK. The VP1 gene characterization was used to study phylogenetic relationships between serotype O FMD viruses isolated from Mekelle Tigray and other O type isolates from other countries of the world. The serotype isolated from Mekelle in the current study falls in to topotype East Africa-3 (EA-3).

Table 1: FMDV serotype identified in different sites of outbreaks using cell culture ELISA

Site of out break	No.of sample	CPE Positive	Sero type
Mekelle	6	6	O
Ayanalem	2	2	O
Shibta	2	2	O
Debri	1	1	O

Table 2: Most Closely Related Viruses with the current isolates (ETH/59/2011) from Mekelle

Virus name	Identity (%)	Difference (%)	Topotype
O/ETH/26/2011	99.84	0.16	EA-3
O/ETH/28/2011	99.84	0.16	EA-3
O/SUD/5/2008(GU566061)	95.31	4.69	EA-3
O/SUD/6/2008 (GU566062)	95.15	4.85	EA-3
O/SUD/3/2008(GU566059)	94.05	5.95	EA-3
O/SUD/4/2008 (GU566060)	94.05	5.95	EA-3
O/SUD/1/99 (DQ165076)	93.11	6.89	EA-3
O/SUD/3/99 (GU566043)	93.11	6.89	EA-3
O/SUD/4/99 (GU566044)	93.11	6.89	EA-3
O/SUD/14/2004 (GU566050)	92.96	7.04	EA-3

Serotype O isolated from Mekelle during the study period was also compared with other countries type O isolates. Isolate from Mekelle O/ETH/59/2011 was 99.84% similarity with Ethiopian isolates of 2011 (O/ETH/26/2011 and O/ETH/28/2011). This isolate was 94.05-95.31% similar with Sudan isolates of 2008 (O/SUD/3/2008 (GU566059), O/SUD/4/2008 (GU566060), O/SUD/6/2008(GU566062)), Similarly the current virus isolates has 93.11% of similarity with Sudan isolates of 1999 (O/SUD/1/99 (DQ165076), O/SUD/3/99(GU566043) O/SUD/4/999 (GU5660440)) (Table 2).

DISCUSSION

In the current study serotype O was isolated from the samples collected from Mekelle University farm, Aynalem, Shibta, Cholekot and Debri. Similarly, previous studies also indicated that serotype O was highly prevalent and dominant serotype causing most of the outbreaks in Ethiopia [9].

In addition, the molecular epidemiology of serotype O has been well studied [10]; in Ethiopia on the basis of comparison of sequence data of the VP1 gene, existence of 1 serotypes O EA-3 topotype has been demonstrated within samples collected from Mekelle Tigray region, Ethiopia. This is in agreement with previous study on molecular epidemiology of serotype O by Asfaw and

Sintaro [11] who 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 isolated from Tigray region O/ETH/59/2011 was also genetically most closely related (92.96-95.31%) with Sudanese Isolates (O/SUD/3/2008 (GU566059), O/SUD/4/2008 (GU566060), O/SUD/5/2008 (GU566061), O/SUD/6/2008 (GU566062), O/SUD/1/99 (DQ165076), O/SUD/3/99 (GU566043) and O/SUD/4/99 (GU566044).

This indicates that the disease was endemic in the region and in the country and this might be aggravated because of unrestricted movement of animals. This poses serious problem due to the transmission of various disease causing agents like FMD virus. The molecular epidemiology of the serotype O of FMD virus from the 2001 Ethiopian outbreak suggests that there were transboundary movement of the virus between Ethiopia and the neighboring countries in the past

CONCLUSION AND RECOMMENDATIONS

Foot-and-mouth disease is endemic in Ethiopia. The presence of foot and- mouth disease in the country is a major obstacle to the development of agriculture because of its adverse effects on livestock production and agricultural exports. During the study period only serotype O was recorded throughout the study area. The molecular characterization and phylogenetic analysis indicates that isolates from Mekelle (ETH/59/2011) falls in to a topotype East Africa-3 (EA-3) and this isolate was genetically similar to Sudan type. The current finding indicates that transboundary movement livestock between Ethiopia and the neighboring countries might be the major risk for the occurrence of FMD out breaks in the region.

Therefore the Following Points Are Recommended:

- Restriction of cross border animal movement and establishment of quarantine station around the border area is mandatory.
- Regular surveillance and monitoring is necessary
- Research should be strengthening to produce polyvalent vaccine containing the dominant serotype.
- Vaccination of all susceptible animals against the different serotypes

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