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Out Break Investigation and Epidemiology of Foot-and-Mouth Disease Virus Circulating in Central Areas of Ethiopia

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Abstract: A cross sectional study was conducted on the sample collected from different sites of Addis Ababa and its surroundings from September 2009 to April 2010 during FMD outbreaks and submitted to NVI and to the FAO World reference laboratory for foot and mouth disease (WRLFMD), Institute for animal health (IAH), Pirbright, UK. The study was conducted with the aims of outbreak investigation of FMD and identifying of FMD virus circulating in the study area. The samples were collected from swine and bovine. From 23 samples submitted, FMD virus was detected in 13 (56.5%) samples in BHK cells. Identification of the serotypes was done and two serotypes of the virus (O from 2 and SAT2 from 11 samples) were identified. In general, the diversity of FMD virus identified in the study areas indicated the need of polyvalent vaccine for the control measure of the disease.

Key words: Central Ethiopia · Foot-and-Mouth Disease Virus · Outbreak · Serotype

INTRODUCTION

Ethiopia is one of the countries in Africa with huge livestock resources that play a crucial role in the livelihoods of the majority of its population. Animal rearing is an integral part of the agricultural production in Ethiopia with the livestock population comprising approximately 47.57 million cattle and 47.9 million sheep and goats [1, 2]. Ethiopia is a country whose agricultural sector is the biggest to its grand domestic product (GDP) and the major contributor to its export earning is 48% and 90%, respectively [3]. Animals also represent the major draught power (95%) for crop production. Livestock ownership currently contributes to the livelihood of an estimated 80% of the rural population. In the highland, livestock are kept under settled or transhumance system utilizing common pastures, many of which have high clover content and crop residues. Such livestock include some 9.3 million oxen providing draught power for the mixed forming system that prevails. In the arid and semi-arid extensive grazing areas of the eastern, western and semi-arid extensive grazing areas of the eastern, western and southern lowland cattle, sheep, goat and camels are managed in migratory pastoral production system [4].

The livestock marketing authority [5] estimated the annual potential for annual export at 72, 000 metric tons meat equivalent to USD 136 million over the last few years. Due to advantage our proximity, the Middle East countries (Saudi Arabia, Egypt, United Arab, Emirates, Bahrain, Yemen, Jordan, Kuwait, Oman, Qatar, Iran and Syria) increased their demand to estimate USD 1.1. Billion consisting of 205, 846 tons of meat and 12 million head of cattle and shoats. The demand of African countries is estimated at USD 572.3 million consisting of 86.04 tons of meat and 3.2 million heads of cattle and shoat [5]. However, due to the high prevalence of different diseases like FMD and poor management system, the country is not utilizing this huge potential [3].

In Ethiopia, many of the known infectious disease of animals occur commonly. There are different endemic disease causing frequent loses through mortality and productivity. Further outbreak of some of the diseases in the country is one of the major obstacles in export market development resulting infrequent bans from importing countries [6]. Foot-and-mouth disease (FMD) is one of the contagious viral diseases that have great impact on economic development both interims of direct and indirect losses in the country. FMD is highly contagious

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viral disease of cloven-hoofed domestic and wild animal species established [7]. According to the Office International des Epizooties (OIE), FMD ranks first among the notifiable infectious disease of animals [9]. The disease is caused by the genus, Aphtho virus, family Picornaviridae which has seven distinct serotypes, namely: A, O, C, SAT1, SAT2, SAT3 and Asia1 [10].

The disease has an incubation period of 3-14days and excretion of the virus from the infected animals in all secretions and excretion usually begins before the appearance of visible clinical signs [11]. Initial virus multiplication occurs mainly in the pre-pharyngeal area and the lung [12]. Irrespective of the portal of entry, once infection gains access to the blood stream, the virus shows a predilection for the epithelium of the mouth and feet and to less extent, the teat. The characteristic lesions develop at this site after an incubation period. Predilection for lesions to occur on the oral mucosa is attributed to the hyperplasic state of the epithelium caused by persistent local irritation [13]. Clinically FMD is characterized by the appearance of vesicles in and around the mouth, feet and sometimes on the udder and teats. Loss of appetite, severe lameness, sudden drop in milk production and abortion are common. Hyper salivation, loss of condition, pyrexia, which persists for about 2 days, are the major clinical signs identified. The development of vesicle and ulcers on the tongue, hard palate, dental pad, lips and muzzle which is followed by rapid healing (<1-2weeks). Compared to feet lesions that last over 2 weeks further forming necrotic epithelium or scabs [14, 15]. The lesions are susceptible to primary bacterial infection. At this stage, the animals are reluctant to eat and move. Death in calves may occur due virus infection of the developing heart muscles recover within 2 weeks [16]. Morbidity is up to 100% in susceptible animal population but mortality is low in adults.

The disease spreads rapidly by movement of infected animals or mechanically on fomites such as clothing, shoes, vehicles and veterinary instruments. The reasons for the rapidity of spread to fully susceptible population is due to the large volumes of droplets and aerosols of virus shed by infected animals, the stability of viruses in such droplets, the rapid replication cycle with very high virus yields and the short incubation period[17]. FMD is transmitted by a variety of methods between herds, countries and continents but spread from one animal to the other is by inhalation or ingestion. In the tropics, the most important method of spread is believed to be by direct contact between animals moving freely across the national boundaries as trade or nomadic cattle [10].

FMD is endemic to most countries in sub-Saharan Africa and will not be eradicated from southern East Africa while infected buffalo are present with the exception of few countries Southern Africa, where the disease is controlled by the separation infected wild life from susceptible livestock as well as by vaccination [18]. Largely due to the endemicity of the disease and the fact that FMD does not normally cause high rates of mortality in adults animals (2%) and 20% in young animals. FMD outbreaks are not perceived as important and are not reported or investigated further to determine the causative serotypes. However, a number of countries now realize that FMD is one of the transboundary diseases that should be controlled to ensure economic stability and access to lucrative international export markets for animal and animal products. Furthermore, they recognize that a regional approach would be needed to succeed [18]. Lack of movement control within countries and across international borders for both wildlife and domestic animals aggravates the problem and gives credence to the face that FMD will remain a problem on the sub-continent for the foreseeable future [19, 20]. Countries free of FMD impose strict import regulation on animals and animal products and potential viral contaminated fomites from FMD free countries [21]. So greater loss can result from refusal from FMD free countries to import livestock and livestock products from endemic regions [22].

FMD has considerable economic consequences and can be attributed to both direct and indirect costs. The direct effects of the diseases are loss of milk production, loss of drought power, retardation of growth, abortion in pregnant animals, death in calves and lambs while indirect losses can be attributed to the disruption in trade of animals and derivative products. Its sequelae are found to be more important than the acute illness [16]. A striking example is the recent outbreak of serotypes O (the Pan Asia Strain) in Great Britain, a country which had been free of FMD since 1981. This devastating epidemic of 2001 spread to Ireland, France and the Netherlands where the United kingdom alone were forced to slaughter about 4 million infected and in contact animals. The cost of this epidemic in the UK was estimated to be more than USD 29 billion [23].

Statement of the Problem: FMD in Ethiopia was first recorded by food and Agricultural organization and world reference Laboratory (FAO/WRL), which indicated that FMD serotypes O, A SAT2 and C were responsible for FMD outbreaks during the period of 1957 to 1979 [24]. The current situation of FMD in Ethiopia is alarming.

There is no national control strategy; no legislation exists for making FMD notifiable to the veterinary authorities or for animal movement restrictions to be imposed. Therefore, livestock are at risk from endemic strains as well as from antigenic variants prevailing in neighboring countries.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted from October, 2009 to April, 2010 in and around Addis Ababa. Addis Ababa is the capital city and the administration center for the federal democratic Republic of Ethiopia. It lies in the central highlands of the country at an altitude of 2050 meters above sea level and has an estimated population of about 3 million. Geographically Addis Ababa is located at 9°2'N latitude and 30°42'E longitude with the average minimum and maximum temperatures of 10.7 °C and 20 °C respectively [25]. The city receives an annual rainfall of 1800mm in bi modal pattern. The long rainy season extends from June to September followed by a dry season ranging from October to February. The short rainy season lasts from March to May. Addis Ababa has a relative humidity varying between 40% to 50% during the dry month and 70% to 80% during the rainy season [26, 27]. The total cattle population of Addis Ababa is estimated to be 97, 215. Out of this population, the female cattle constitute about 50.9% (49, 487) and the remaining 49.1% (47.728) are male cattle [28] .

The second outbreaks were occurred in Debre-Birhan Prison farm and Tebasie Veterinary Clinic coming from the kebeles and surrounding locals in December 2009. The area is located in North Shewa zone of Amhara regional state, in central highland of Ethiopia, about 130kms North East of Addis Ababa at an altitude of 2780 meters above sea level. Geographically, it is situated between 9°36'N latitude and 39°38'E longitude. The climate of the area is characterized by a bi modal rainfall and a short rainy season from June to September, which accounts for about 72% of the total annual rainfall and a short rainy season from October to February. The area has a relatively cool temperature throughout the year and a mean relative humidity of 68.2%. The annual rainfall varies from 900-1200mm. the monthly temperature ranges from 10 °C in September to 23 °C in June [29].

The total population of the zone is estimated to be 1.82 million of which 1.63million is rural and 0.19 million urbans with an annual growth rate of 2.7%. The predominate mode of agricultural production is subsistence, small holder mixed crop-livestock, population is estimated to comprise 1.2million cattle, 1.4 million sheep, 0.4million goats, 0.3million equines and 0.1million poultry. Livestock in general serve multiple purpose providing draft power for cultivation, milk, meat, wool, manure and others [29].

The third outbreak was occurred in Debre-Zeit on February 21 to 25. Debre-Zeit is located at 9°N' 40°E with an altitude of 1880 meters above sea level in the central highlands of Ethiopia, 47km south east of Addis Ababa. It has annual rainfall of 1151.6mm of which 84% falls down during the long rainy season that extends from June to September and March to May. The minimum and maximum temperatures are 8.5°C and 30.7°C, respectively and the mean relative humidity is 61.3% [28].

Another outbreak was investigated at Sululta, small town which is located 24 kms North West of Addis Ababa. The altitude of the surrounding area lies between 1600 and 3310 m.a.s.l. The temperature of the area ranges from 15-18 °C.

Study Population: The study population were included all susceptible domestic animals (particularly swine and cattle) where the out breaks investigated.

Study Design: The study was conducted using purposive sampling during disease outbreaks from October 2009 to April 2010 to determine the serotypes of FMD virus circulating in clinically affected animals.

While an active outbreak of FMD reported, a field investigation was conducted at the outbreak area. In each sub city of Addis Ababa and surrounding district, information of outbreak was gathered from concerned bodies (sub city outbreak investigation laboratories, district veterinary clinic workers). Clinical information was recorded on redesigned forms and animals were clinically examined and specimens were collected for diagnostic testing. The tissue samples were processed by cell culture laboratory at NVI, Debre Zeit. Positive samples were sent to WRL, Pirbight, UK for serotyping topotyping and phylogenic analysis.

Clinical Examination: In each outbreak, animals were clinically examined from a distance for evidence of salivation and lameness. Salivating and/ or limping animals were restrained in a crush pen for thorough examination and sampling. The mouth cavities of salivating animals were widely opened and examined for evidence of intact and/or ruptured vesicles, erosions and ulcers on the tongue, dental pad and mucosa of the oral cavity.

The hooves of lame animals were thoroughly washed with water and then carefully examined for similar lesions particularly on the coronary bands and inter digital spaces of the hooves. 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.

Epithelial Tissue Samples Collection: Epithelial tissue samples were collected from unruptured vesicles and placed in a bottle with transport medium composed of equal amount of glycerol and 0.04M of phosphate buffer saline solution (PBS) at pH 7.2-7.6 with antibiotics [30]. Species, identification number, sex, age, village and type of tissue were labeled and samples were immediately placed in a cooler containing ice for transport to National Veterinary Institute (NVI), Debre Zeit. Once the samples arrived at NVI, they were stored at +4 °C until processed and placed at -20 °C until analysis.

Virus Isolation: The epithelial tissue samples collected and stored in freezer were thawed at room temperature and washed three times using sterile PBS at a PH of 7.2 under laminar air flow hood class II. About 1 gm of epithelial tissue sample was grounded using sterile mortar and pestle by adding 10ml of sterile PBS containing antibiotic. The tissue suspension was centrifuged at 5000 rpm for 15 min. The supernatant was collected and filtered by Millipore filter of 0.22μm pore size. About 1ml of filtered tissue suspension was inoculated on baby hamster kidney (BHK-21) monolayer cells grown on 25cm^2 tissue culture flask and then flashed with growth media and incubated at 37°C and 5% CO_2 in a humidified incubator for 48hrs. Cells were monitored for cytopathic effect (CPE) daily and frozen when CPE was exhibited. A second pass was performed on those samples not presenting CPE following the same procedure as the first pass. Samples not exhibiting CPE by 72 hours post-infection on the second pass was considered as negative [30].

Serotype/ Topotype and Phylogenetic Analysis: The samples were submitted to World FMD Reference Laboratory, Pirbright, UK, for further molecular characterization (serotype, topotype, phylogenetic analysis and r-value determination). The VP1 gene characterization was used to study phylogenetic relationships of FMD viruses in Ethiopia as well as with other isolates from East, South and West Africa, the Middle East, Asia and Europe.

Data Analysis: The result was analyzed according to[23], where < 1% nt sequence difference indicated that virus isolated from the same epizootic; < 7% nt sequence difference indicated that virus belonging to the same epizootics (common origin); virus of the same genotype differ up to 15% and viruses from different genetic lineages differ by >20 % nt sequence.

RESULTS

Serotype Identification

Virus Isolation: Out of the 23 outbreak samples collected and examined for FMD virus (CPE) was observed in 13 (56.5%) samples in BHK21 cell line culture (Table 1). The CPE was characterized by a fast destruction of the cell monolayer and infected cell were round and formed singly.

On those samples that should CPE molecular analysis (RT-PCR) using serotype specific primers was conducted to identify the serotype of the virus responsible for the outbreak. Thus, serotype O and SAT2 were identified. Serotype SAT2 was the dominant serotype identified while serotype O was identified only from sample collected from Addis Ababa. Serotype SAT2 was isolated from both bovine and swine samples which were collected from Debre Birhan prison farm, Sululta and from Debre zeit (Table 1).

Topotype Identification: Further molecular characterization was conducted on the 13 identified FMD virus isolates to determine the responsible topotype of the virus. Serotype O was under topotype East Africa-3 (EA-3) and serotype SAT2 was under topotype XIII.

Fig. 1: Map of Ethiopia indicating study sites/outbreak area

Table 1: Serotypes of FMD virus identified in the study areas

				Serotype				
Area	Species /breed	No of Sample	No of virus isolated (positive)	O	A	C	SAT1	SAT2
Addis Ababa	Bovine/cross	6	2	2	-	-	-	
Debre Birhan	Bovine/cross	8	6	-	-	-	-	6
Debre Birhan	Swine	2	1	-	-	-	-	1
Debre zeit	Bovine/cross	3	1	-	-	-	-	1
Sululta	Bovine/cross	4	3	-	-	-	-	3

Fig. 2: Cattle that show typical clinical signs of FMD and gross lesion observed in the oral cavity

DISCUSSION

In most parts of Africa, FMD is enzootic and only a few countries in the continent have managed to control the disease to allow access to lucrative export markets for live animals and animal products [1]. Ethiopia has a large livestock population including both domestic and wild animals [3] and FMD has been reported every year. Vaccination against FMD remains largely not being practiced except only in few dairy herds containing exotic animals. In this study FMD virus was isolated from most samples collected from outbreaks. Serotyping of the virus revealed that serotype SAT2 was the dominant serotype, while the other serotypes occurred at much lower levels. The finding of this study disagrees with previous studies which indicated that serotype SAT2 is less prevalent in Ethiopia [31] and also the recent study conducted by Ayelet *et al.* [32] on FMD samples collected between 1981 to 2007 throughout the country from different species of animals showed that serotype O, A, C, SAT1 and SAT 2 were recorded. Type O was the dominant serotype identified with the degree of 73.3% and widely distributed throughout the country, while the rate for serotype A was 19.5%, C 1.4%, SAT 2 was 4.1% and SAT 1 was 1.8 % with limited distribution in the country. This study did not include any samples from outbreaks area due to facility access to the peasant association inaccessibility to the area and lack of information of the outbreaks.

SAT2 was recorded in 2007, after a gap of 16 years, from a bovine sample collected from Bambas, Benshangul-Gumuz, Western Ethiopia, bordering the Sudan [32]. This has two possible explanations: The 1st is that the virus is present in Ethiopia, however have not been detected as all the outbreaks are not reported or investigated, the other explanation is that there might be sub clinical circulation of type SAT2 viruses in Ethiopia which may involve wild life. The association of SAT serotypes with wild life particularly with African buffalo was indicated by various researchers [1].

The 2007 Ethiopian SAT1 and SAT2 isolated are from Mezan Teferi and Benshangul-Gumz, respectively are known to have large number of wild life including African buffalo; particularly Mizan-Teferi borders to both the Mago and Omo National parks. Therefore, the possibility of these new viruses being transmitted to domestic animals from wild animals cannot be eliminated.

This study revealed that the outbreaks of the disease were relatively higher in females than in males in urban areas. Age specific outbreaks study revealed an increasing the occurrence of the disease as the age increases. This may indicate the cumulative experience of the population with the agent [33]. Therefore, those animals aged greater than 4 years might have acquired the infection from multiple serotypes and could produce antibodies against all serotypes of FMD.

A small-scale vaccination practice against FMD is realized in occasions like FMD outbreaks in different parts of the country. However, FMD control by vaccination does not seem cases, animals vaccinated using bivalent

A and O vaccine were found to be affected by sever outbreaks. By virtue of these facts and given the mode of livestock movement without restriction, FMD virus is maintained in the country making the disease endemic.

Movement of livestock is not limited among the different administrative regions of the country as well as the neighboring countries. This is posing a serious problem due to the exchange of various disease causing agents, like FMD virus. The molecular epidemiology of serotype O of FMD virus from the 2001 Ethiopian outbreak suggests that there were transboundary movement of the viruses between Ethiopia and the neighboring countries in the past [1].

From the study result and visit of outbreaks, the owner described well most of the local perceptions of the disease signs, the indigenous epidemiological knowledge that the FMD occurs usually during dry season when feed is not available. The clinical signs listed were consisted with what is indicated in veterinary literatures [34]. In Ethiopia, similar signs have been reported in pastoral cattle of Afar [35], Somalia [36] and Oromia [3] and throughout the country [32].

Although it is not statistically significant, animals that have previous history of FMD were highly affected than those that have no history of FMD. Despite statistical insignificant due to lack of power, this difference antigenically novel viruses. This might also be because the vaccine did not contain all the types and sub types of the viruses and to the lack of proper study of the vaccinal strains.

CONCLUSION

The circulating FMDV serotypes in and around Addis Ababa was tried assessed and found that multiple serotypes of the virus (O and SAT2) were involved that affected Cattle and Swine. In addition, the lack of prophylactic vaccination and veterinary infrastructure to handle the outbreak on a large-scale greatly contributes to the frequent occurrence of the disease and also makes the FMD control extremely challenging.

Since FMD is a highly contagious and capable of easy distribution of multi directional tentacles, the animal health centers of the city need to take the necessary disease control and prevention measures before and after the occurrence of an outbreak. In spite of the face that vaccination has been the main strategy of control in endemic area. The strategy should a need for a revision of the service delivery.

Regular monitoring FMD outbreaks to have more detailed information of the situation to formulate an efficient vaccine-based FMD control strategy for Ethiopia are needed. Control program like vaccination should always be backed up by continuous and complete epidemiological surveillances and laboratory investigations designed to identify the serotypes and subtypes of FMDV involved even in a single outbreak so as to make compatible vaccine to circulating viruses.

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REFERENCES

1. Sahle, M., 2007. An Epidemiological study of the Genetic Relationships of Foot and Mouth Disease viruses in East Africa, Phd Thesis.
2. CSA, 2008. Central statistical Authority. Ethiopia's livestock Estimates. *Bulletin 52.* Addis Ababa, Ethiopia.
3. Tesfaye, R., 2008. Participatory Appraisal and Seroprevalence study of FMD in Borana Pastoral System, Southern Ethiopia. Msc thesis, FVM, AAU, Debre Zeit, Ethiopia.
4. FAO/WFP, 2005. Food and Agriculture Organization and world food program; FAO Global information and Early warning system on food and agriculture. Special warning system on food and agriculture. Special report of FAO/WFP crop and food supply assessment mission to Ethiopia, pp: 1-10.
5. Livestock marketing Authority (LMA), 2004. Brief Baseline information on Ethiopian Livestock Recourse Base and its Trade. Addis Ababa, Ethiopia.
6. OIE, 2000. Office international desEpizooties. Manual of standards for Diagnostic Tests and Vaccines, 4th ed., pp: 77-91
7. Brooksby, J.B., 1982. Partraits of viruses: foot-and mouth disease virus. Virology, 18:1-23.
8. Castro, E.A. and P.W. Heuschole, 1992. Veterinary Diagnostic Virology. U.S.A., Mosby-year Book Inc., pp: 100-103.
9. Alexandersen, S., Z. Zhang, A.I. Donaldson and A. J.M. Garland 2003. Pathogenesis and Diagnosis of foot-and mouth disease. J. Comp. Path, 129: 1-36.

10. Radositis, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine: A Text Book of the Disease of cattle, sheep, pigs, Goats and Horses. FMD, 9th ed. Elsevier Ltd. China, pp: 1059-1064.
11. Kitching, R.P., 2002. Clinical Variation in Foot and Mouth disease: cattle. OIE Scientific and technical Review, 21(3): 499-517.
12. Burrows, R., J.A. Mann, A. Greig, W.C. Chapman and D.Groodridge, 1982. The pathogenesis of natural and simulated natural foot and mouth disease infection in cattle. Journal of Comparative Pathology, 91: 599-609.
13. Blood, D.C. and O.M. Radostitis, 1989. Veterinary Medicine, A Textbook of the disease of Cattle, Sheep, Pigs, Goats and Horses. FMD, 7th ed., The University press Ltd., London U.K, pp: 824-831.
14. Kitching, R.P., 1992. FMD. In: Andrews andrews, A.H., Blowey, R.W., Boyd, H. and Eddy, R.G. Bovine Medicine: Diseases and Husbandary of cattle, Blackwell Science Ltd., U.K, pp: 537-543.
15. Reid, S.M., N.P. Ferris, G.H. Hutchings, Z.G.J. Belsham and S. Alexandersen, 2002. Detection of all seven serotypes of foot and mouth disease virus by real time, Flourogenic Reverse Transcription polymerase Chain Reaction Assay, J. Virol. Meth., 105: 67-80.
16. Woodbury, E.L., 1995. A review of the possible mechanisms for the persistence of foot-and mouth disease virus. Epidemiology and Infection, 114: 1-13.
17. Sellers, R.F. and M.S. Daggupaty, 1990. The epidemic of foot and mouth disease in saskatchewan, Canada 1951-1952. Canadian Journal of veterinary *Research,* 54: 457-464.
18. Vosloo, W., A.D.S. Bastos, O. Sangare, S.K. Hargreaves and G.R. Thomson, 2002. Review of the status ad control of foot and mouth disease in sub-Saharan Africa. Rev. Sci. Tech., 21: 437-449.
19. Bruckner, G.K., W. Vosloo, B.J.A. Duplessis, P.E.L.G. Kloeek, L. Connoway, M.D. Ekron, D.B Weaver, C.J Dickason, F.J. Schreuder, T. Marais and M.E. Mogajane, 2002. Foot and Mouth disease: the experience in South Africa. Rev. Jech. OIE., 21: 751-764.
20. Thomson, G.R., W. Vosloo and A.D.S. Bastes, 2003. Foot and mouth disease in wild life. Virus Res., 91: 145-61.
21. Hirsh, D.C. and Y.C. Zee, 1996. Foot and mouth disease In: Tropical animal health. Kluwer Academic Publisher, Netherlands, pp: 425-431.
22. Kahrs, F.R., 2001. Foot and mouth disease In: Viral disease of cattles. IOWA state university press, Elsevier, pp: 271-275.
23. Samuel, A.R. and N.J. Knowles, 2001. Foot-and-mouth disease type) viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes). J. Gen. Virol., 82: 609-621.
24. Martel, J.L. and C. Gallon, 1975. Comparative serolodical study of the principal foot and mouth disease virus strains isolated in Ethiopia, 1969-1974. Revued' Elevage et de Medecine Veterinare des Pays Tropicaux, 28: 287-295.
25. AACA, 2004. Addis Ababa City Administration for establishment of sub city and kebele. Addis Ababa, Ethiopia.
26. CSA, 2007. Agricultural Sample Survey 2006/07: Report on Livestock and Livestock Characteristics. Statistical Bulletin: Addis Ababa, 2: 1-30.
27. NMSA, 2003. National meteorological statistical Agency. Rainfall, Humidity and temperature data. Addis Ababa. Ethiopia.
28. CSA 2001/02. Central statistical Authority. Ethiopia's Livestock Estimates. Bulletin 52. Addis Ababa.
29. CSA, 2004. Central statistical Authority. The 2001/2002 Ethiopian Agricultural sample Enumeration (EASE), Executive summary, Addis Ababa, Ethiopia.
30. OIE, 2004. Office international desEpizooties. Manual of standards for Diagnostic Tests and Vaccines, 4th ed., pp: 77-91.
31. Gelaye, E., B. Beyene and G. Ayelet, 2005. Foot and mouth disease virus serotypes identified in Ethiopia. Ethiop. Vet. J., 9(1): 75-80.
32. Ayelet, G., M. Mana, E. Gelaye, B.G. Egziabher, T. Rufeal, M Sahle, Nigel, P. Ferris, W. Jemma, Geoffrey H. Hutchings and Nick J. Knowles 2009. Genetic Characterization of Foot-and-Mouth Disease Viruses, Ethiopia, 1981–2007. Emerging Infectious Diseases www.cdc.gov/eid, 15: 1409-1417.
33. Murphy, F.A., E. P.J. Gibbs, M.C. Horzinek and M.J. Studdert, 1999. Veterinary Virology, 3rd edition. USA< Academic Press, pp: 412-421.
34. Catley, A., R.T. Chibunda, E. Ranga, S. Makungu, F.T. Magayane, G. Manoma, M.J. Madege and W. Vosloo, 2004. Participatory diarnosis of intorance syndrome in cattle in Tanzania and Association with foot and mouth disease. Prev. Vet. Med., 65. 1730.

35. Tadesse, G., 2003. Participatory Studies on Heat Intolerance Syndromes Associated with foot and mouth disease in indigenous cattle in Afar Pastoral area of Ethiopia. Faculy of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM, Thesis, pp: 18-21.
36. Eshetu, T., 2003. Participatory studies on heat intolence syndrome associated with foot and mouth in indigenous cattle of Somali pastural area Shinille zone, Ethiopia.Eshetu, T. (2003): Participatory Studies on Heat Intolerance.
37. Syndrome Associated with Foot and Mouth Disease in indigenous cattle of Somali pastoral area in Shinille Zone,
38. Ethiopia. Faculy of Veterinary Medicine, Addis Ababa University, Debre Zeit, DVM, Thesis, pp: 10-15.