

Characterisation of foot and mouth disease virus isolates from the Somali ecosystem in Kenya

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Abstract

Foot and mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals characterised by fever and vesicular eruptions in the mouth, nares, muzzle, and feet and on the mammary glands. Although well documented in the highlands of Kenya, it is not described in the Somali eco-system (SES) which is a porous zone neighbouring Somalia and Ethiopia. In this study, outbreaks of FMD in the SES were closely monitored between 2008 and 2009. Three outbreaks were encountered, one in Wajir and two in Mandera all of which occurred in the dry season. From these outbreaks antigen detection by enzyme linked immunosorbent assay (ELISA) test was positive for serotype "O" on samples taken in the Wajir outbreak and in the second Mandera outbreak FMD nucleic acid was detected by Polymerase Chain Reaction(PCR). There was no positive laboratory result from Mandera samples in the first outbreak but the clinical signs strongly resembled those of FMD.

In a carrier status survey 105 oesophageal-pharyngeal fluid were collected from apparently healthy cattle in Garissa market out of 589 cattle present and 4 out of 40 from Mandera. Out of

the total 109 samples, six were positive for serotype O by antigen detection ELISA test giving an overall FMD carrier status of SES cattle of 5.5%. Testing using PCR (a more sensitive test) at FMD World Reference Laboratory (WRL), Pirbright gave the actual percentage carrier status to be 14.89%. All positive samples were adult cattle over 3 years of age. No carrier status was detected in calves and yearlings.

Keywords: Characterisation; Foot and mouth disease; Kenya; Somali ecosystem

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Introduction

Foot and mouth disease (FMD) is an extremely acute, highly contagious viral disease of both domesticated and wild cloven-hoofed animals and camelids (bactrians) characterised by fever and vesicular eruptions in the mouth, nares, muzzle, and feet and on the mammary glands (Blood and Radostits, 1989). In domestic animals it is associated with productivity losses such as milk production, loss of weight and death of young stock. It is an endemic disease in Kenya affecting animals such as cattle, sheep, goats, pigs, buffaloes, deer and antelopes (Ngichabe *et.al.*,) with four serotypes currently in circulation i.e., O, A, SAT1 and SAT2.

Of all the eight provinces in Kenya, seven provinces indicate that FMD is prevalent, while epidemiological maps indicate that North Eastern Province which is in the Somali Eco-system is

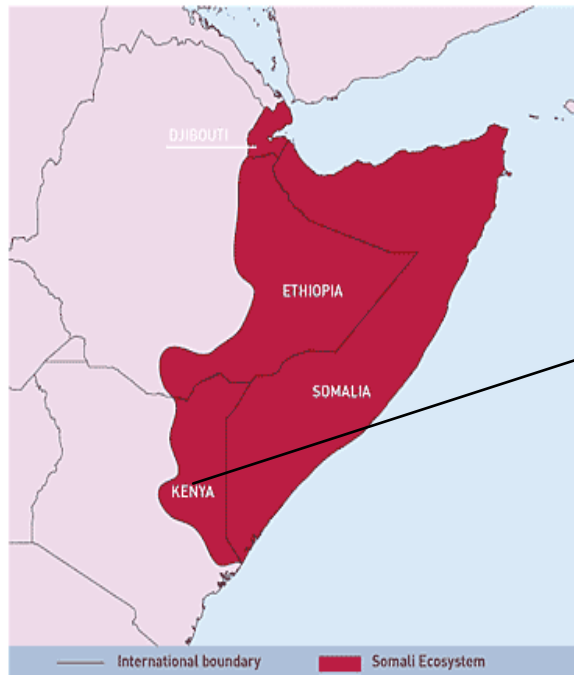
free of the disease. This study was therefore carried out to determine the prevalent FMD serotypes in the SES, as well as describe the clinical manifestation of FMD and characterise the FMD virus found in the SES in Kenya.

Materials and Methods

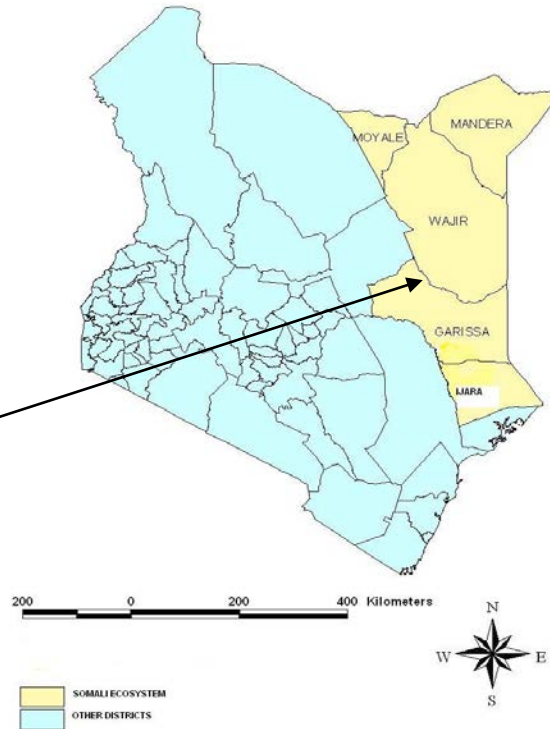
Area of study

The Somali ecosystem in Kenya is part of the greater Somali ecosystem whose other constituent parts are South Eastern Ethiopia and Somalia, where the Somali ethnic group inhabits and the area is characterised by porous borders. The Somali Ecosystem in Kenya is mainly in the North Eastern Province in Kenya which is one of the eight administrative provinces in Kenya with an overall population of 2,385,572 (in 2009), and a land area of 126,902 km². It lies within Agro-Ecological zones -IV to VI with a Mean annual rainfall range of 300 to 700 mm per annum and a mean evapo-transpiration of 2600 mm. The province lies within an altitude range of 70 to 300 meters above sea level. The mainstay of the population is livestock rearing in a nomadic lifestyle. According to a 2009 census report (Kenya Bureau statistics, 2009) they keep all classes of livestock which include indigenous cattle (2,694,786), Camels (1,700,893) Indigenous Goats (7,886,586) Sheep (4,264,155) poultry (382,345) and donkeys (71,313). The districts within this Province which formed the focus of this study are Mandera, Wajir, Garissa and Ijara. Tana-River at the Coast and Moyale in Eastern Province were also included as they form part of the SES. Extensive farming and pastoral systems are practiced in this area with livestock production constituting the major economic activity of the zone (Figs 1a and 1b).

THE SOMALI ECOSYSTEM



Source: www.fao.org/ag/againfo/.../en/grep/Somali_ecosystem



Source: *Epidemiology and Economics System Kabete, Kenya 2005*

Sampling methods

Prior to beginning the field work, a questionnaire was administered to 47 Veterinary Officers and other Veterinary service providers working in the SES Kenya to find out FMD occurrence, recognition and very importantly whether FMD vaccinations are usually carried out. From the analysis of their responses, no vaccinations are carried out for FMD in the SES.

The SES, Kenya was closely monitored for FMD outbreaks through direct communication with the District Veterinary officers manning each district between November 2008 and October 2009. FMD outbreak foci were the site of choice to collect epithelial samples for virus isolation

but where there were no classical lesions, oesophageal-pharyngeal fluid samples were taken to isolate the FMD virus.

Epithelial sampling

Herds with FMD outbreaks were selected and epithelial samples obtained, kept in low temperatures until they reached the laboratory for virus isolation. About 1 g of epithelial was collected from un-ruptured or recently ruptured vesicles. Five epithelial scrapings were taken from each outbreak but in the second Mandera outbreak additionally four samples of oesophageal-pharyngeal fluid were also taken from apparently healthy animals because the lesions were too mild. One challenge experienced during sampling is that the lesions in indigenous cattle are not as classical as those seen in exotic breeds and again they heal quickly compared to those of exotic breeds.

Epithelial samples were placed in a transport medium (50% glycerol and 0.04M Phosphate buffer, penicillin 1000 I.U, neomycin sulphate 100 I.U, mycostatin 100 I.U and polymyxin B 50 I.U. The final pH of 7.2-7.6 with phenol red as an indicator (4). Samples were kept frozen at -20 °C and on the day of transportation to the FMD Laboratory they were kept in cool boxes with icepacks and transported in the shortest time possible. For the Mandera samples due to the long distance to the FMD Laboratory and poor infrastructure they were flown to Nairobi with the help of Eco-flight air services.

Sample analysis

In the laboratory, samples were kept refrigerated and processed as soon as possible. The epithelium sample was taken from the PBS/glycerol transport media, blotted dry on absorbent

paper to reduce the glycerol content, and weighed. A suspension was prepared by grinding the sample in sterile sand using a sterile pestle and mortar with a small volume of tissue culture medium and antibiotics. Further medium was added until a final volume of nine times that of the epithelial sample to give a 10% suspension. This was clarified on a bench centrifuge at 2000 g for 10 minutes. This made the original suspension which is also tested directly by ELISA test. Once clarified, the suspensions of samples were inoculated onto BHK-21 (baby hamster kidney) cell cultures to passage the virus and to check presence of virus by cytopathic effects after 24 -48 hours. Virus isolation was done using BHK- 21 cells and where there was no success Calf thyroid cells were used.

The cell cultures were examined for cytopathic effect (CPE) for 48 hours and where no CPE was detected, the cells were frozen and thawed, used to inoculate fresh cultures and examined for CPE for another 48 hours. Where there was cythopathic effect the sample was clarified by centrifugation and aliquoted into three. One was used for ELISA testing, another to be preserved for sending to WRL, Pirbright for ELISA and PCR tests (with extra epithelium sample where available) and the third portion for storage to be used for repeat tests where necessary and research.

Indirect Sandwich ELISA

An Indirect Sandwich ELISA kit used was procured from Institute for Animal Health, Pirbright Laboratory Surrey, United Kingdom. Indirect sandwich ELISA was performed following standard procedures (Roeder *et. al.*, 1987). Briefly rabbit anti-sera specific for serotypes O, A C SAT 1 and SAT 2 were passively adsorbed to polystyrene ELISA plates (Nunc-Maxisorp Denmark) by overnight incubation at 4 °C. Samples and control antigen were added and plates

incubated at 37 °C for 1 hour on a rotary shaker. Detection of a captured virus was performed by adding specific guinea pig antisera to serotypes O, A C SAT 1 and SAT 2 followed by addition of rabbit anti-guinea pig peroxide conjugate. Plates were washed after each incubation step. Chromogen/substrate mixture was added and plates were incubated for 15 min. in the dark at room temperature. The reaction was stopped with 1.25 M sulphuric acid and the plates were read on an ELISA reader (BDSL Immunoskan, Finland) at a wavelength of 492 nm. A mean corrected optical density value of >0.1 indicated a positive result and the serotype was read. Tests were always done on the original sample and the sample passaged, the results were double checked by comparing the results from original suspension and doing another ELISA test on passaged sample after 24 -48 hours of incubation.

Detection of FMD carrier status

Garissa market was chosen as the site for collection of oesophageal-pharyngeal fluid samples because this was a site where many adult animals expected to be carriers of the FMD virus and secondly because animals arriving at this market converge from the whole region of the SES. The other very important reason was that this market is in close proximity to the Garissa Regional Veterinary Investigation Laboratory with the required facilities to freeze the samples soon after collection and thus improve the chances of successful viral isolation.

One hundred and nine (109) oesophageal--pharyngeal fluid samples were collected from apparently healthy cattle of various ages. One hundred and five (105) were from healthy cattle on sale on a market day in Garissa and 4 out of 40 were from apparently healthy cattle in a farm in Mandera after an outbreak. In the Garissa market 589 cattle were in the market on the sampling

date and a total of 105 were randomly selected from different herd groups. Of all the 109 samples a total of 18 were from calves less than 1 year of age, 23 from yearlings aged > 1-2 years, 30 aged >2-3 years and 38 from adult cattle > 3 years. The sample was collected by inserting a probang cup over the tongue into the oesophageal-pharyngeal area and passing it vigorously backwards and forwards about 5 times. Each of this sample was put in universal bottles containing 2mls composed of special media containing 0.08M phosphate buffer with 0.01% bovine serum albumin, 0.002% phenol red and antibiotics 1000 I.U penicillin, 100 I.U neomycin 100 I.U mycostatin and 50 I.U polymyxin (pH 7-2). The samples were held in cool boxes with ice packs for a brief period then taken to Garissa Veterinary Laboratory to be kept in a deep freezer of -20 °C. They were removed the day of transportation and put in cool-boxes with ice packs in their frozen state. They were then transported directly to FMD Laboratory, Embakasi for analysis. At the laboratory the samples were thawed and processed by ELISA test. Where no virus was detected the sample was passaged for 24-48 hrs in BHK-21 cells and the ELISA test repeated where cytopathic effect was seen to have taken place in the monolayer cells.

Confirmation of diagnosis and Nucleic acid detection

An assortment of both negative samples and positive samples on antigen detection were sent to World Reference Laboratory, Pirbright for confirmation of diagnosis (proficiency testing) and nucleic acid detection and characterisation. A total of 47 oesophageal-pharyngeal fluid samples and one epithelium sample were sent.

Results

Outbreak serotyping results

In the period of this study 3 outbreaks were encountered, one in Wajir sampled in mid December 2008 in a farm with a herd size of 40 and 2 outbreaks in Mandera. The outbreaks in Mandera occurred in February in a farm with 170 cattle and in March 2009 it affected three herds with a herd size of 40, 20 and 30 all totalling 90. All the outbreaks were therefore in the dry season of the year. Five epithelial scrapings were taken from each outbreak but in the second Mandera outbreak 4 oesophageal-pharyngeal fluid samples were also taken from apparently healing animals apart from the epithelial scrapings because the lesions were too mild in some cattle and in others healing (Figs 2a, 2b 2c, 2d and 2e). From these three outbreaks antigen detection by ELISA test was positive for serotype ‘O’ in the Wajir outbreak though in the second Mandera outbreak only FMD nucleic acid was detected by Polymerase Chain Reaction. The results obtained on ELISA tests and from the samples sent to WRL, Pirbright are given in the Table I.

No positive result was obtained by antigen detection ELISA from Mandera samples in the first outbreak but pictures taken of healing animals show lesions strongly resembling those of FMD (Figs 2a, 2b, 2c,2d and 2e). In the second outbreak in the same district an oesophageal-pharyngeal sample was positive for Foot and mouth disease virus.

Table I – FMD outbreaks encountered and investigated

District	Type of sample	Tests carried out and results	Serotype detected/isolated
Wajir	Epithelium	1. Ag-ELISA +ve 2. VI –BHK21 +ve VI- CTY +ve 3. PCR + ve	“O” (Also “O” in WRL and characterised)
Mandera	Epithelium	1. Ag-ELISA -ve 2. VI –BHK21 -ve VI- CTY -ve 3. PCR - ve	NVR
Mandera	Epithelium and oesophageal-pharyngeal fluid	1. Ag-ELISA -ve 2. VI –BHK21 -ve CTY -ve 3. PCR + ve for one oesophageal-pharyngeal fluid	Positive for FMDV by PCR but serotype was undetermined

KEY:

Ag –ELISA: Antigen detection ELISA

VI-CTY: Virus isolation on Calf thyroid primary cells

VI-BHK21: Virus isolation on Baby Hamster Kidney cells

NVR: No virus Recovered



Fig. 2a



Fig. 2b



Fig.2c



Fig.2d



Fig. 2e

Fig. 2. Healing lesions in the mouth and feet of affected cattle in Mandera District.

Oesophageal-pharyngeal samples test results

From the 109 oesophageal-pharyngeal fluid collected 6 samples were positive for serotype O by antigen detection ELISA test giving an overall carrier status of 5.5%. Passage on BHK-21 was done to isolate the virus. All those which tested positive were adult cattle over 3 years of age. No carrier status was detected in calves and yearlings. A total of 47 oesophageal-pharyngeal samples were selected from these samples by taking all the positives by ELISA and randomly selecting the negative samples for further testing in WRL, Pirbright. Six samples which tested positive by antigen detection ELISA in FMD Laboratory also tested positive in WRL. By Polymerase Chain Reaction test 7 samples were positive. Since PCR is a more sensitive test the actual percentage FMD carrier status of cattle in the Somali ecosystem is estimated 14.89% (7/47). The findings are summarised in Table II.

Table II – World Reference Lab serotyping results for oesophageal-pharyngeal fluid samples

Number of samples	Tests at FMD Lab. Kenya/WRL UK	Serotype/ Number of positives	%Carrier status
109	Ag ELISA } FMD Lab Virus Isolation }	‘O’ 6 positive ‘O’ 6 positive	5.5
47	Ag ELISA } WRL Lab PCR }	‘O’ 6 positive 7 positive for FMDV	14.89 (PCR)

Sequencing Results from WRL Pirbright

On the basis of genotyping report, the serotype O positive epithelium sample sent to FMD World Reference Laboratory Pirbright, the isolate was found to be closely related to vaccine strain currently in use in Kenya ‘O’K77/78. Of the top five closely related isolate four were historic isolates collected in Kenya which caused outbreaks in various regions of the country in the year 1995 namely ‘O’K 10/95, ‘O’K29/95 and ‘O’K38/95 by 99.37%, 99.37% and 99.06% respectively. This means the other serotype O isolate causing outbreaks in Kenya in 2008-2009 and included in the analysis are not very closely related to this isolate. It also indicates that there are different strains of serotype O in circulation in the country. The isolate was also closely related to isolates collected in Burundi in 1984 and 1986.

From the FMD genotyping analysis Kenyan O isolates fall into two main topotypes namely East African topotype 1(EA-1) and East African topotype 2 (EA-2). This particular isolate belongs to EA-1 together with OK77/78 (vaccine strain). The other serotype O viruses collected from other parts of Kenya belong to EA-2.

Other relationships were compared with viruses from outside Africa and significantly high percentage identity was seen with viruses from India (86.38% - 87.45%), Turkey 85.76% and Israel 84.82%.

Discussion

Serotype O was isolated from Wajir in an outbreak that occurred in 2008. Characterisation revealed that the serotype was closely related to the vaccine strain produced in Kenya, K ‘O’ 77/78 by 99.06% on the VP1 protein responsible for infection and protection in vaccination. This

means that vaccination with this strain is likely to give good protection against outbreaks by this field strain though for confirmation vaccine matching tests would have to be done. Currently no FMD vaccinations are carried out in the SES according to the questionnaires responses from the Veterinary service providers obtained before the field work was carried out.

About 50% of infected animals in other studies have been found to remain carriers after the acute phase of the disease (Doel *et al.*, 1994) but this percentage continues to drop with time and with no repeated challenge may eventually decline to undetectable levels. Of the total animals that were sampled to check on carrier status in Garissa and Mandera, 5.5% were positive for serotype O on typing by ELISA and the same serotype O was detected from the same samples sent to WRL for confirmation by ELISA test. On PCR the carrier status was higher at 14.89%, which is expected since PCR is more sensitive. This finding is in agreement with other studies by Brooksby (1982) who confirmed persistent infection in cattle for up to 2-3 years. It may not be possible to determine when these sampled animals were acutely infected but certainly by isolation of the virus in oesophageal-pharyngeal fluid samples from apparently healthy animals is an indication of carrier status.

All the cattle positive cases for carrier status were adults over 3 years of age. Earlier studies by Bashiruddin *et al.* (2004) demonstrated that young cattle especially less than 6 months of age appeared to resist persistent infection but those older than 7 months were more likely to become carriers. Younger cattle eliminated the virus more efficiently than older cattle meaning that host factors (including maternal antibodies) and viral factors may be more important in establishment of persistence (Zhang *et al.*, 2004). In addition, lameness and vesicular lesions were observed in animals between 2- 4 years old.

All the outbreaks encountered occurred during the dry season of December 2008 to March 2009 which is similar to studies in Ethiopia where outbreaks were related to animal movement in search of pastures and water during the dry season (Tesfaye, 2006).

Four of the districts in the SES Mandera, Wajir, Garissa and Ijara border Somalia to the west and the border are porous with frequent border passage of people and livestock. There has been no surveillance of FMD in Somalia since 1991 due to state failure. However, in 2010 out of six epithelial samples submitted from different parts of Afmadow district Lower Juba region three (50%) were positive for type O indicating the presence of type O across the border (Jabra and Hassan, 2010). Cattle movement from one place to another, sometimes involving the crossing of international borders, has been a long tradition and is considered as an indispensable activity in the daily life of people in the region. This is exacerbated by insecurity and lack of statehood in Somalia. An effective foot and mouth disease control strategy that may be developed for the region when security improves may therefore involve vaccination with a quadrivalent vaccine containing serotypes O, A, SAT 1 and SAT 2 on a regional basis.

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