

Research Article

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Prevalence of Foot and Mouth Disease Virus Serotypes in Kilifi County of Kenya

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Abstract

Five of the seven known Foot and mouth disease (FMD) serotypes are endemic in Kenya namely; O, A, C, SAT type 1, and SAT type 2. Presence of five serotypes in Kenya has complicated the epidemiology and control of the disease. This study evaluated the most prevalent of FMD serotypes in Kilifi County, Kenya, using three new and one gold standard serological tests for FMD detection. The serum samples were obtained from blood collected from cattle in four constituencies of Kilifi County; Bahari, Kaloleni, Ganze, Malindi and Magarini. A retrospective study design based on qualitative data was conducted using animal sera samples. 421 serum samples from cattle were selected from Kilifi County. A multi stage sampling method was applied. The serum samples were subjected to Anigen © FMD NSP Ab ELISA - Korea, NSP Priocheck®, Liquid Phase Blocking Enzyme Linked Immunosorbent Assay (LPBE), and the viral neutralization test (gold standard) which identified positive from negative serum samples. LPBE assays detected Serotype O 16/56(28.57%), SAT1 16/56(28.57%) SAT2 12/56 (21.43%) and serotype C 12/56 (21.43%). However Serotype A was not detected in any of serum sample. This study reveals that four FMD Serotypes (O, SAT1, SAT2 and C) were found to be circulating within Kilifi County.

Keywords

Foot and mouth disease virus.

Introduction

Foot and Mouth Disease (FMD) is endemic in Kenya with five of the seven serotypes namely; O, A, C, SAT types 1, and 2 reported to occur thus complicating the epidemiology and control of the disease in the country (Vosloo, 2002). It has been suggested that pastoralist livestock keeping areas in Kenya form an ecosystems in which FMD is maintained (FAO, 2012).

These ecosystems also play an important role in wildlife-livestock reservoir. In Kenya, FMD control measures which include vaccinations and restriction of animal movement which has not fully managed to control the disease. Control measures in the country are being enhanced with the focus on improving the diagnostic and surveillance capacities as well as the improvement of the

efficiency of vaccinations through better quality and relevant strain vaccines.

During the period 2004-2006, circulating FMD serotypes in Nakuru included types O, A, C, SAT1 and SAT2. In the recent past, the majority of the outbreaks in Kenya have been caused by serotypes O and SAT2. Serotype A occurs on a lesser frequency while serotype C has been rare with only one outbreak last reported in 2004.

Recently, an upsurge of SAT1 and SAT2 outbreaks was recorded. In the year 2004, one outbreak of SAT1 and nineteen of SAT2 were recorded while three of SAT1 and ten of SAT2 occurred in 2005. At the end of September 2006, seven outbreaks of SAT1 and four of SAT2 have been reported in Nairobi and Kajiado counties. The upsurge of these outbreaks particularly SAT1 after a long absence needed further examination on the possible sources of the virus.

The identification of some new genotypes of SAT1 and SAT2 as causing these epidemics which has led to prevalence of 52.5% of FMD in Kenya (Kibore *et al.*, 2012). These viruses sources as suggested by their inability to cause persistent outbreaks in livestock. Sequences of the SAT2 isolates indicate that the viruses fall into distinct groups. A group of 2004/2005 viruses from the Central (KEN/10/2004, and KEN/13/2004), Eastern (KEN/17/04) and Rift Valley (KEN/5/2004, KEN/8/2004, KEN/22/2004 and KEN/8/2005) provinces are closely related to viruses isolated from outbreaks of FMD in Tanzania and Malawi in 2004 (98.44 to 99.23% nucleotide identity) suggesting a sweeping regional epidemic. Rwanda 2000-2004 isolates are distinct suggesting a different source for the outbreaks in Rwanda. Similarly the identification of additional genetic types in Kenya represented by 1999 isolates and SAT2/KEN/7/2005 also suggests additional sources of outbreaks such as wildlife. Two genetic lineages of SAT2 have been identified in East Africa with one group involving Kenya, Rwanda and Uganda while the other involves Kenya and Tanzania (Bastos *et al.* 2003). These viruses are also suggested to evolve independently. The existence of multiple lineages in Kenya is suggestive of introductions from the cross-border animal movements prevalent in the country (Ndiritu, 1984). Serotype SAT 1 isolate SAT1/KEN/1/2005 from Nyeri District in Central province which was sequenced was not closely related to any other SAT1 viruses in the data base. Through identification of specific serotypes, it will enable proper management and control of FMD in Kenya, where vaccines

are produced according to the known circulating serotypes.

The objectives of the study was to determine the positives and negatives of Anigen © FMD NSP Ab ELISA –Korea, NSP Priocheck® kits and Liquid Phase Blocking Enzyme Linked Immunosorbent Assay and to determine the most common foot and mouth disease virus serotypes in Kilifi County.

Materials and Methods

Blood Specimens for this study were collected from cattle in Kilifi County at the coastal region of Kenya where outbreaks of FMD frequently occur. Blood samples were collected from Bahari, Kaloleni, Ganze, Malindi and Magarini constituencies between March and September 2012. The sample size was calculated from the previous prevalence rate of 52.5%. Kilifi County is located on an estuary of River Rare that provides water for domestic and agricultural purposes. During this feeding time transmission may occur by direct or indirect contact (droplets). Farmers practice dairy farming which accounts to a significant quantity of milk consumed in Kilifi town and other towns such as Mombasa and Lamu hence animate vectors (humans, live animals & products etc.) are possible suggested method of FMDV transmission. Roads passing all the areas where livestock are kept are passed by inanimate vectors like vehicles when transporting goods and people to their destinations; hence they may carry the FMDV.

This was a cross-sectional study design. The blood samples were collected in non EDTA Vacucontainer tubes to allow for serum collection. Blood sample collection bottles were labeled and stored in a cool box where the samples were triple packaged. The samples were transported to the laboratory in Nairobi and stored in a deep freezer at -20°C . In the FMD laboratory, four hundred and twenty one blood samples were selected from One thousand six hundred and twenty four collected from Bahari, Kaloleni, Ganze, Malindi and Magarini constituencies using multistage process.

The stored sera were removed from the freezer and was put on the bench to thaw. A multi stage sampling method was applied. At the first Stage, five Constituencies in the Kilifi County were selected to form clusters. At the second stage, samples were sorted according to the constituency of origin. At the third stage, serum was sampled from each cluster to represent the animals using systematic random sampling

technique. At the fourth stage, the table of random numbers was used to select the first and then every Kth serum preservation bottle in the serial list until the sample size was realized. Data analysis was done with SPSS version 16.0. Criteria for sensitivity, specificity and predictive values Reference / gold standard test was used. Exact 95% confidence limits for binomial proportions were calculated from F-distribution. Kappa statistics (coefficient) and Chi-square test were employed for parameters analysis and calculating this extent for purposes of Comparing sensitivity agreement levels of the tests. Any value above 0.75

and above was established as excellent agreement, between 0.40 and 0.75 intermediate agreement and below 0.40 poor agreements. The samples were either expressed as either negative or positive. The data was presented in frequency data tables.

3.10. Criteria for determining positives and negative samples.

Negative, positive and predicative values were calculated as represented in table below.

Table 1: Criteria for determining positives and negative samples. Reference / gold standard test

TP	FP	TP +FP
FN	TN	FN+ TN
TP+FN	FP+TN	TP+FP+FN+TN

T- True
 P -positive
 F-False
 N-negative
 True positives= TP / (TP+FP)
 True negative= TN / (FN+TN)

3.12 Ethical considerations

Scientific and Ethical approvals letter was obtained from Kenya medical research institute and ministry of agriculture, livestock and fisheries Scientific and steering committee and ethical review committees. Authorization letter was also being sought from the officer in charge FMD national reference laboratory, industrial area who grants the permission to access the serum samples. All procedures were carried out in

accordance to FMD Bio safety guidelines and waste disposal.

Results

The three serological assays were used with VNT as gold standard. These include; Anigen © FMD NSP Ab ELISA – Korea, NSP pricocheck© and LPBE.

Table 2: positives and negatives by NSP Ab ELISA - Korea against virus neutralization test

TEST	Positives	Negatives	Total
Screening test: NSP Ab ELISA – Korea Positives	17(TP)	1(FP)	18
Negatives	15 (FN)	388 (TN)	403
TOTAL	32	389	421

Table 2 Indicates positives and negatives serum as identified by NSP Ab ELISA –Korea and VNT. 17(53.13%) serum were identified as positive and 388(99.74%) as true negatives samples, 15(46.88%) as false negatives and 1 (0.028%) false positive. The

results show that NSP Ab - Korea ELISA assay identified 53.13% of serum as positives and 99.74% as negatives.

**Table 3 positives and negatives serum screened by NSP priocheck© Netherlands and virus neutralization test.
VNT (Reference / gold standard test)**

TEST KIT	POSITIVES	NEGATIVES	TOTAL
SCREENING TEST: NSP priocheck© :Positives	17 (TP)	20(FP)	37
Negatives	4 (FN)	380 (TN)	384
TOTAL	21	400	421

Table 3 Show positive and negative serum samples as screened by NSP priocheck© assay. FMDV antibodies were found present in 17(80.95%) of the serum and found absent in 388(96.28%). NSP priocheck©

indicated that 80.95% of the animals were of serum was drawn from, had or developed FMD. The absence of FMDV antibodies in serum indicated the absence of the FMDV

**Table 4. Positives and negatives serum screened by LPBE and virus neutralization test
VNT (Reference / gold standard test)**

TEST	POSITIVES	NEGATIVES	TOTAL
SCREENING TEST: LPBE : positives	23(TP)	0 (FP)	23
Negatives	0 (FN)	398 (TN)	398
TOTAL	23	398	421

Table 4 show the positives and negatives serum screened by LPBE as screening tests and confirmed by VNT. Twenty two (22) (5.22 %) of the serum samples were identified to have FMDV antibodies and 399(94.77 %) contained no FMDV antibodies. Presence of antibody serum indicated that animals had FMDV with a possibility of developing FMD.

However negative antibody for FMDV means that 100% of animals are FMD free. Presence of FMDV antibodies in serum indicates that the animal FMDV has the virus and may develop FMD. A negative antibody serum means that FMDV is absent and the animal does not have FMD.

Table 5 positives and negatives serum screened by Virus Neutralization Test

TEST	POSITIVES	NEGATIVES	TOTAL
Positives	22 (TP)	0 (FP)	22
Negatives	0 (FN)	399 (TN)	398
TOTAL	22	399	421

Table 5 show the positives and negatives serum screened by VNT. 22(100%) of serum sample were identified as true positive and 399 (100%) as true negatives. Presence of antibodies in serum indicates that FMDV is present in serum and the animals may develop FMD. Absence of FMDV antibodies means that the animal does not have FMD.

This study determined prevalent serotypes of FMDV using Liquid Phase Blocking Enzyme Linked Immunosorbent Assay and virus neutralization Test in Kilifi County. The results are presented on table 6.

Table 6: FMDV Serotypes

Test	O	A	C	SAT 1	SAT 2	TOTAL
LPBE	16(28.57%)	0	12 (21.43%)	16(28.57%)	12(21.43%)	56
VNT	16(28.57%)	0	12(21.43%)	16(28.57%)	12(21.43%)	56
	32	0	24	32	24	112

Table 6 shows prevalent serotypes of FMDV in Kilifi. LPBE and VNT assays detected Serotype O 16/56(28.57%), SAT1 16/56(28.57%) SAT2 12/56 (21.43%) and serotype C 12/56 (21.43%). However Serotype A was not detected in any of serum sample. The two assays are used in identification of FMDV because they are specific for FMDV serotypes.

Discussion

From the analysis of this study it is showed that NSP Ab ELISA assay identified 53.13% of serum as positives and 99.74% as negatives. NSP prioncheck© contradicted the results of NSP Ab ELISA Korea which indicated 80.95% of the serum were positive and 96.28% negatives. The results of the two non-structural assays were further differed with the structural (LPBE) which indicated that 22(5.23%) of the serum samples were identified to be positive and 399(94.77%) to be negatives. The results concur with frame work analysis of FAO/IAEA Programme which showed that viral neutralization test and LPBE are the assays which can be used to detect true positives and true negatives (FAO/IAEA, 2007). This is because they are specific for serotypes. Anigen © FMD NSP Ab ELISA -Korea and NSP prioncheck© Netherlands kits which were used to detect viral nonstructural proteins (NSPs) and LPBE and virus neutralization test were used to detect viral structural proteins (SP).

Results of the serological serotyping analysis indicate the identification of some new serotypes types of SAT1 and SAT2 as causing these epidemics. These viruses could also be from non-livestock sources as suggested by their inability to cause persistent outbreaks in livestock. Serology results obtained from this study indicated that four main serotypes of FMD virus are in circulation namely O, A, SAT 2 and SAT 1 in order of frequency. These results concur with the study done in 2011 an epidemiological survey of the serotypes of foot and mouth disease virus in circulation in the Somali ecosystem in Kenya (Chepkwony, 2011). Still these analyses concur with Paul Freeman study on FMD outbreak in Kenya from 1995 to 2010 (Freeman, 2010).

The results shows that serotype O and SAT1 are the dominant serotype in Kenya at present. This concurs with studies done previously on FMD outbreak in Kenya which showed that SAT1 unlike serotype O is the dominant serotype in Kenya at present (Freeman, 2010). These results also concur with the study done on Foot-and-Mouth disease serotypes SAT1 and SAT2 Epidemiology in East Africa which indicated that SAT 1 is still the most prevalent serotype in Kenya,

followed by SAT 2. Analysis From this study shows that only serotype A was not isolated from blood sample. These results contradict with the previous study by (Chepkwony, 2011) which indicated that it was isolated in the samples collected from clinical cases of FMD during an epidemiological survey of the serotypes of foot and mouth disease virus in Kenya.

Conclusion

The study concluded that some samples of serum was detected containing more than one type specific antibodies to FMDV. It can be concluded from the study that NSP assays are not specific for the serotypes despite them detecting the presence of FMDV antibodies. They are only used to detect presence or absence of the FMDV antibodies. These findings are concurring with the earlier studies which showed that the LPBE and VNT are reliable tests which can clearly give the differences and specify serotypes besides being used as indicators of the infection.

The study can conclude that Serotype O and SAT1 are the most prevalent serotypes of FMDV and were found to be highly occurring among the animals population in Kilifi County. SAT2 and serotype C were also found in circulation. Though Serotype A was not detected it doesn't mean that it is complete absent from the general population.

Recommendations

1. The study found that some serotypes of FMD were not detected therefore there is need for regular screening from the general population in order to find out whether other serotypes are present.

Recommendations for further research

This work provides a base line for further studies on evaluation of antibody and antigen sensitivity of tests kits. Therefore genotyping should be done including various enzymes used in genotyping of random primers in FMD diagnosis.

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