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Foot and mouth disease sero-prevalence in cattle in Kenya

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A cross sectional study was conducted on serum from 39 counties in Kenya in order to determine the prevalence of foot and mouth disease in bovine species. The study utilized serum samples at Foot-and-Mouth Disease (FMD) laboratory including Somali Ecosystem Rinderpest Eradication Coordination Unit (SERECU) project collected in the year 2010. From the serology results, the national prevalence of foot and mouth disease in bovines was 52.5% (CI = 95). Of the 3709 samples subjected to Nonstructural protein (NSP) enzyme linked immunosorbent assay (ELISA) screening test, 1,947 of those were interpreted as positive representing 52.5% (1947/3709) while the other 1,762 samples turned negative representing 47.5% (n = 1,762). There was significant association between seropositivity and age groups (p = 0.002) and vaccination status (p = 0.048) but no association between the seropositivity and sex (p = 0.063).

Key words: Cattle, foot and mouth disease (FMD), Kenya, seroprevalence.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious acute viral infection of cloven hoofed animals including domesticated ruminants and pigs and more than 70 wildlife species, and is one of the most important economic diseases of livestock (Coetzer et al., 1994; Broonsvoort et al., 2004). The disease is characterized by fever, loss of appetite, salivation and vesicular eruptions in mucosa of the mouth, skin of the inter-digital spaces and coronary bands of the feet and teats. It is also characterized by high morbidity and low mortality (Coetzer et al., 1994). It is caused by virus of genus *Aphthovirus*, in the family Picornaviridae (Belsham, 1993), of which seven distinct serotypes O, A, C, SAT1, SAT2, and SAT 3 and Asia 1 are known. The disease is endemic in Kenya, and five of these serotypes have been in circulation that is, O, A, C, SAT1 and SAT2 (Vosloo et al., 2002), although no type C has been recorded in the world since 2004 (FAO, 2005, 2006).

Foot-and-mouth disease is one of the major Trans-boundary animal diseases (TADs) that impact negatively on trade in livestock and livestock products in the region. In order to control and/or eradicate this disease in the targeted areas, a good understanding of disease epidemiology is important and this can only happen if the disease is traced and regular, and effective surveillance is done together with vaccination regimes being put in place (FAO, 2005, 2006).

FMD is a global disease that through the years has affected most of the countries. FMD is endemic in most countries in sub Saharan Africa (Vosloo et al., 2002) with six of the seven serotypes reported to occur in East Africa namely O, A, C, SAT 1, SAT 2 and SAT 3 thus

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complicating the epidemiology and control of the disease in the region. Serotype SAT 3 has been recorded only in Uganda (Vosloo et al., 2002). Infection with any one serotype does not confer immunity against the other serotypes. Within serotypes, many strains can be identified by biochemical and immunological tests. The disease spreads rapidly by movement of infected animals or mechanically on formites such as clothing, shoes, vehicles, and veterinary instruments. The reasons for the rapidity of spread to fully susceptible populations is due to the highly infectious nature of the virus, the production of high titer in respiratory secretions and the large volumes of droplets and aerosols of virus shed by infected animals, the stability of virus in such droplets, the rapid replication cycle with very high virus yields and the short incubation period (Sellers, 1971).

Nonstructural protein (NSP) enzyme linked immunosorbent assay (ELISA) test is useful because it is able to discriminate animals that have been infected from those that have been vaccinated. Such test would be able to detect continued viral circulation and would therefore be extremely useful for serological surveys with a view to eradication. As NSP ELISA detects antibodies to the non-structural proteins of FMDV and is therefore able to differentiate between vaccinated and convalescent animals where purified vaccine is used. Previous serology results indicated a very high FMD prevalence in counties within Western and North Rift areas of Kenya in both bovines and porcines. Some counties such as Uasin Gishu, Baringo, Elgeyo Marakwet, Tranzoia, Bungoma, Pokot and Kakamega recorded 100% prevalence. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3, 7.5 and 5.3%, respectively.

The aim of this study was therefore to determine the prevalence of antibodies against foot and mouth disease virus in Kenya using NSP ELISA, to determine the prevalence of antibodies in each county and lastly to determine the prevalence of FMD in disaggregated units including age and sex.

MATERIALS AND METHODS

Sample source

The serum samples were obtained from the collection at the Embakasi laboratory assembled from various activities including the Somali Ecosystem Rinderpest Eradication Coordination Unit (SERECU) project. The samples were collected throughout the country, with the unit of sampling being the computer generated divisions in every district, in the year 2010 between April and May. All the 4,262 samples were individually verified and entered into a data sheet with the details below; animal laboratory identification, location and coordinates of the source (district and county), species, sex (either male or female), age of the animal (stratified as follows < 1 year, 1 to 2 years and > 2 years) and vaccination history (either vaccinated, non-vaccinated or unknown).

Study area

Serum samples analyzed were collected from the 39 counties out of the 47 counties. The counties that did not feature included; Kiambu, Murang'a, Nairobi, Nyamira, Busia, Kirinyaga, Kericho and Kisii (Figure 1).

Sampling method

The sampling unit was considered to be the county. The sample size was calculated using the following formula (Dohoo et al., 2003).

$$n = \frac{(1.96)^2 p(1-p)}{L^2}$$

Where L is the required precision, (+ or - error around the estimate) and was assumed at 95%, p is the anticipated prevalence or proportion of attribute. The anticipated prevalence used was 50%. The sample size obtained was 384 and in order to improve on the precision, the sample size was increased by four fold to 1,383 samples. The sample size was also informed by the number of test kits that were available. On average, 34 samples were randomly selected from each county. Some counties had fewer samples as to enable random selection. In that scenario, all the samples were considered for analysis. For counties that had slightly above the average number of samples required, systematic random selection was done.

Serological tests

All the 3,709 bovine samples were subjected to FMD screening test; NSP-ELISA (AniGen® FMD NSP Ab ELISA). The AniGen® Foot and Mouth Disease Virus Ab ELISA kit was designed to detect FMDV specific antibodies in bovine serum. The test was useful because it was able to discriminate animals that had been infected (wild virus induced antibodies) from those that had been vaccinated with purified vaccine (vaccine induced antibodies). Some of the test materials including NSP-ELISA test (AniGen FMD NSP Ab ELISA/Anigen/South Korea), chemical (washing and stopping solutions) and biological (negative and positive control, enzyme conjugate and substrate) reagents were availed by the FMD Reference Laboratory while others were sourced from Pirbright through the assistance from National Council for Science and Technology.

Briefly, the test sera, negative and positive reference sera were added to all the 96 well ELISA plate coated with 3ABC antigen. Following addition of the diluted enzyme conjugate and incubation for 90 min at 37°C, the plates were washed 3 times with washing buffer. After the last washing, tetramethylbenzidine (TMD) ready to use substrate was added and plates were incubated at room temperature for another 15 min. The reaction was terminated by adding 1 M sulphuric acid stopping solution. The optical density of the samples were measured at 450 nm and the result was expressed as an index derived by dividing the absorbance value of the test serum by that of the cut-off control (OIE, 2004). A sample with a percentage Inhibition (PI) value of above 50 (that is, ≥ 50.0) on AniGen FMD NSP Ab Elisa was regarded as a positive result while a sample of PI value of less than 49 (that is, < 50.0) was regarded as a negative result.

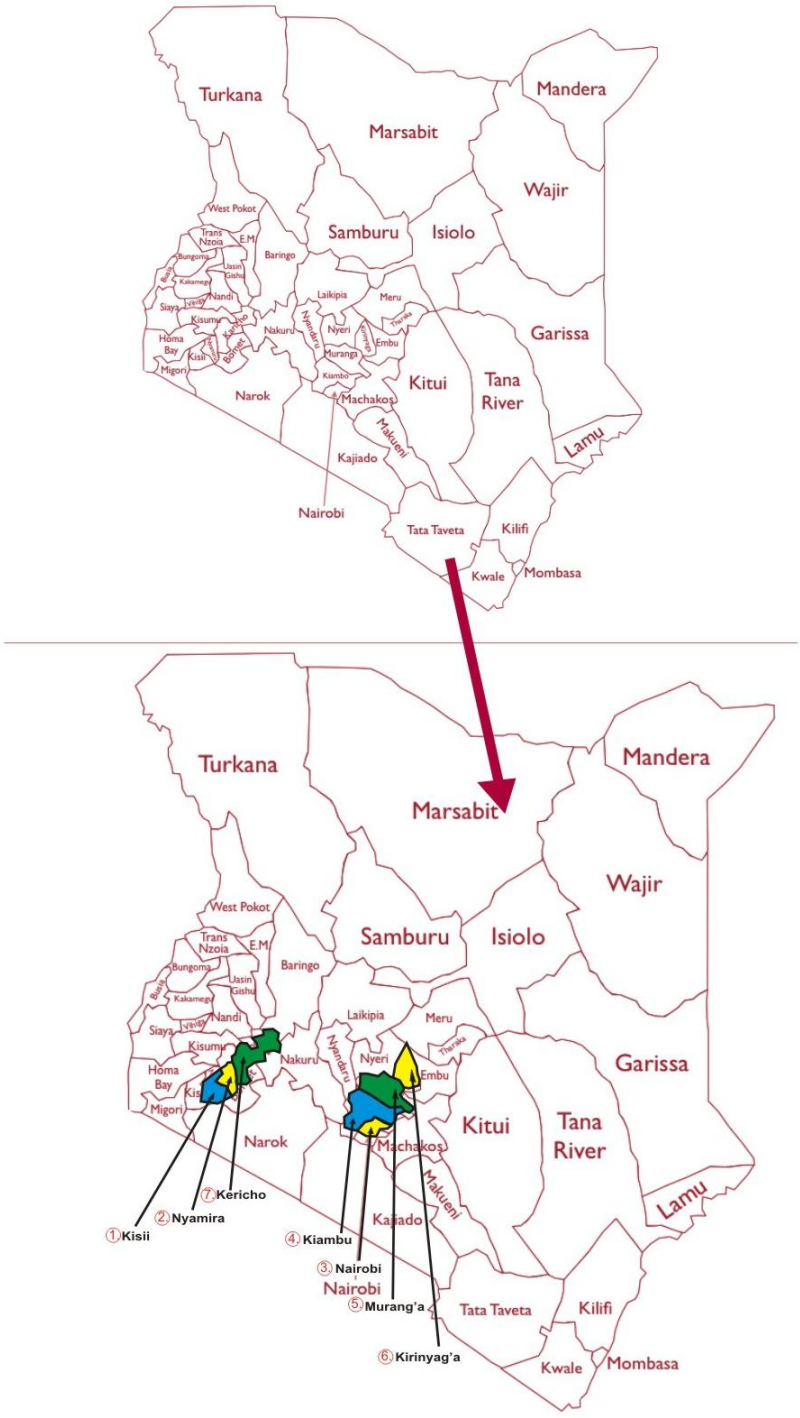

Figure 1. Map showing the new 47 administrative units of Kenya and the missing counties.

Data analysis

The results of both the NSP Elisa were then entered in an Excel spreadsheet (Microsoft Corp) with the following information; sampling location, age, sex, species, vaccination history, AniGen results. The data was imported to statistical package for social sciences (SPSS 20 version) for analysis. Descriptive statistical analysis was then done to determine the proportion of positive samples and

Table 1. The overall county prevalence.

No. of counties sampled	No of animals sampled	Results			
		Positive	Negative	Prevalence	95%CI
39	3709	1947	1762	52.5	49.97-55.03

Table 2. The seropositivity & distribution of different serotypes within sexes (M/F).

Number of serotypes			Sex		Total
			Female	Male	
	0	Count within sex	98	49	147
0		% within sex	22.0	19.0	41.0
		% of total	13.3	6.6	19.9
1	1		83	52	135
		% of total	11.2	7.0	18.2
2	2		123	45	168
		% of total	16.7	6.1	22.8
3	3		112	40	152
		% of total	15.2	5.4	20.6
4	4		70	27	97
		% of total	9.4	3.7	13.1
5	5		32	7	39
		% of Total	4.3	1	5.3
Total count			518	220	738
% of Total			70.2	29.8	100.0

serotype distribution across the country and at county level. Graphs were drawn using Microsoft excel.

RESULTS

NSP results (AniGen)

Of the total of 3,709 bovine serum samples subjected to NSP screening 70.4% (n = 2611) were females while 29.6% (n = 1098) were males. Of the 738 serum samples that were positive on NSP and subjected to LPBE, 29.8% (220/738) were males while the rest 70.2% (518/738) were females. 78% (171/220) of all males tested positive for at least one serotype where as 22% (49/220) tested negative (Table 2). Samples from adult animals (> 2 years) accounted for 44.0% (n = 1635). Those of 1 to 2 years accounted for 28.4% (n = 1,052) while serum samples belonging to age group less (< 1 year) were 1,022 representing 27.6% of the total samples. Of the total positive animals that tested positive to at least one serotype of FMD virus 181/591 (30.6 %) were of the age group <1 years. Those of between 1-2 years were 26.6 % (157/591) whereas as 253/591 of cases 42.8% were those of >2 years age (Table 3). Of the 3,709 subjected to NSP screening test, 1,947 of those were interpreted as positive representing 52.5% while the other 1,762 samples turned negative representing 47.5% (Table 1).

County prevalence

The national bovine FMD prevalence stood at 52.5%. From among the counties that had the highest FMD prevalence are those in Western part of the country and included: Baringo, Elgeyo Marakwet, Uasin Gishu, Nandi, Tranzoia, Bungoma, Kakamega and West Pokot counties, all of which had 100% seropositivity (Table 4). Bovine sera from Narok, Embu, Turkana, Migori, Garissa and Bomet counties showed high seropositivity of > 70%. Serum samples from Kajiado, Lamu, Tana River, Siaya, and Kisumu counties had medium to high seropositivity of between 50 and 70%. Counties that had low seropositivity of between 30 and 50% included Laikipia, Machakos, Kwale, Taita Taveta, Samburu, Tharaka Nithi, Nyandarua, Meru, Isiolo and Wajir. The counties that had low

Table 3. The seropositivity and distribution of different serotypes within age groups.

Serotype level	<1 year	1-2 years	>2 years	Total
0	46	46	55	147
1	49	41	45	135
2	52	40	76	168
3	50	37	65	152
4	22	25	50	97
5	8	14	17	39
≥ 1 serotype	181	157	253	591
% level	30.60	26.60	42.80	80.00
TOTAL	227	203	308	738
% Total	30.80	27.50	41.70	100

seropositivity of < 30% included Kitui, Makueni, Malindi, Kilifi, Marsabit and Nakuru. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence. 30 counties had seropositivity of more than 30% (> 30%). This accounted of 76.9% of the total serum samples (Table 4).

DISCUSSION

Serology results indicated a very high FMD prevalence in counties within Western and North Rift areas of Kenya. Some counties such as Uasin Gishu, Baringo, Elgeyo Marakwet, Tranzoia, Bungoma, Pokot and Kakamega recorded 100% prevalence. This may be attributable to the sub-optimal vaccination carried out in these areas, the presence of highly susceptible breeds in sedentary system, and the very high intensity of exposure because of the closeness to the national park (Mt. Elgon) with subsequent interaction with wild life (bufallos). The African Buffalo have been identified as the major carrier wildlife species of SAT serotypes in southern Africa and serological analyses suggest they are the major species infected in East Africa (Vosloo et al., 2002). In Pokot county for example, there is unrestricted high herd mobility, continuous contact and intermingling of different herds at water points and communal grazing areas.

Bovine sera from Narok, Embu, Turkana, Migori, Garissa and Bomet counties showed high prevalence of ≥ 70% (Table 4). The high seroprevalence in these areas may still be attributable to the sub-optimal vaccination together with cross border movement. Narok, Garissa and Turkana counties lie within borderlands where intense unrestricted border movements in search of pasture and water occur. These counties also harbor very vibrant livestock markets where contact among cattle from different sources is high therefore acting as one of the key factors of exposure.

Serum samples from Kajiado, Lamu, Tana River, Siaya, and Kisumu counties had medium to high FMD prevalence of between 50 to 70% (Table 4). Counties which had medium seropositivity of between 30 to 50% included Laikipia, Machakos, Kwale, Taita Taveta, Samburu, Tharaka Nithi, Nyandarua, Meru, Isiolo and Wajir. The moderately high seroprevalence may be associated with the sub-optimal vaccination carried out in these areas together with the livestock production system in which people are largely pastoralists and therefore high incidence of exposure. The distance to the national parks with subsequent mixing with wild ungulates may also explain the relatively high prevalence. In some counties like Taita Taveta, Machakos, Laikipia, Tana River and Kajiado, several animal ranches exist. Animals bought straight from the markets are taken here for fattening. These animals trek from markets for long distances and therefore disseminate the virus along the way.

Other counties had low prevalence of < 30% and included: Kitui, Makueni, Malindi, Kilifi, Marsabit and Nakuru. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3, 7.5 and 5.3%, respectively. Very robust vaccination regime is carried out in the counties of Nyeri, Nakuru, Kitui and Makueni explaining the low prevalence of FMD in these areas. The indigenous type of cattle kept in Moyale, Kilifi, Mandera, Marsabit, Malindi, Kitui and parts of Makueni are resistant to the FMD virus infection.

The counties that make up the Somali ecosystem

Table 4. FMD prevalence in different counties.

Range	Counties	% Prevalence
Very High	Baringo	100
	Elgeyo Marakwet	100
	Uasin Gishu	100
	Nandi	100
	Trans nzoia	100
	Bungoma	100
	Kakamega	100
	West Pokot	100
High	Narok	90.4
≥70%	Embu	82.9
	Turkana	80
	Migori	75.6
	Garissa	72.9
	Bomet	70
Medium	Kajiado	67.6
50-70	Lamu	65.7
	Tana River	65.7
	Siaya	62.1
	Kisumu	51.1
Low	Laikipia	49.2
30-50	Machakos	43.2
	Kwale	42.2
	Taita Taveta	40.2
	Samburu	40
	Tharaka Nithi	40
	Nyandarua	37.5
	Meru	35
	Isolo	33.9
	Wajir	33
Very Low	Kitui	28.8
<30%	Makueni	26.8
	Malindi	26.7
	Kilifi	25.3
	Marsabit	18
	Nakuru	22.7
	Mandera	8.3
	Moyale	7.5
	Nyeri	5.3

namely Mandera, Wajir and Garissa had a mean prevalence of 38.1%, which was slightly lower than the national mean prevalence. The prevalence was highest in Garissa at 72.9% and lowest in Mandera at 8.3%. The above prevalence findings in the Somali ecosystem slightly varied with the findings of Chepkwony et al. (2012) which found mean prevalence of 45.3%. Garissa county had the highest FMD seroprevalence prevalence at 72.9% as compared to the other counties within SES. The main market for cattle in the SES is located in the town of Garissa and receives animals from Somalia, Ethiopia and other parts of the SES. About 1,500 to 4,000 are sold every wednesday and then trucked to Nairobi or Mombasa for the beef market or to the coastal ranches.

During trekking, animals meant for the market interact with local cattle and this may explain why the prevalence rates are high. There is need to enforce this practice of preventing interaction

Association between seropositivity, sex and age

The number of females sampled were higher at 70.2% (n = 518) compared to males 29.8% (220/738). There was no significant association between seropositivity and sex ($p \leq 0.05$). This finding was consistent with previous findings elsewhere (Esayas et al., 2009; Megersa et al., 2009), where sex appeared not to have a significant effect on seropositivity of FMD. On the contrary, in their report on the incidence of FMD among dairy cattle in northwest of Ethiopia, Hailu et al. (2010) documented a higher incidence rate in female (16.63%) cattle compared to that of males (1.37%).

The study revealed a significant association between seropositivity and age. There was great variation in seropositivity among the three age groups. The significantly higher seroprevalence in adults > 2 years compared to that of calves < 1 year and mid age 1 to 2 years was consistent with other previous findings largely done in Ethiopia which includes studies by Rufael et al. (2008) in Borena pastoral area and Molla et al. (2010) in Gamo Gofa and Sidama zones. On the other hand, the results were not in agreement with the findings of Esayas et al. (2009) who carried out a research in Bench Maji zone, southern of Ethiopia and documented no significant association. The high seropositivity of adults > 2 years may be associated with high frequency of exposure in addition to movement of animals especially in pastoral areas in search of water and pasture and intermingling with wildlife especially the African bufallo. In high potential counties, in some instances with sub = optimal vaccination cover, adult dairy animals kept for long time because of milk and with history of previous infection act as chronic carriers. Young cattle are herded around

homesteads and hence have less chance of exposure.

Conclusion

FMD is endemic in Kenya with variation in prevalence across several counties. Movement of the herds, sometimes across the borders, in search of water and pasture is one of the major factors that contribute to contact of cattle and subsequent spread of FMD. The movement and intermingling of domestic and wild animals is a risk factor for FMD and should be addressed in programmes to prevent and control FMD in the affected counties. It is recommended that sero-prevalence surveys be undertaken for wildlife and small stock so as to determine the role that these animals may play in the transmission of FMD in Kenya. Such surveys should be part of a systematic disease surveillance and data collection. It is recommended that strategic vaccination programmes using quadrivalent vaccines would reduce the risk posed by cattle obtained from these areas and which are transported and marketed freely for beef production.

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