Porcine FMD Sero-prevalence in Kenya and its potential effect

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Abstract

The role of pigs in the epidemiology of foot-and-mouth disease in Kenya has not been thoroughly investigated. In order to understand this, a cross sectional study was conducted on serum samples from 15 counties in Kenya in order to determine the seroprevalence of foot-and-mouth disease in porcine species. The study utilized serum samples at FMD laboratory including SERECU project collected in the year 2010. The porcine serum samples were subjected to AniGen® FMD NSP Ab screening ELISA test. The ELISA kit was designed to detect FMDV specific antibodies in serum. From the serology results, the mean seroprevalence of foot and mouth disease in porcines was 54.4% (n=98) on NSP screening while 45.6% (n=82) turned out to be negative. The FMD seroprevalence in porcines was higher compared to similar study done in bovines 52.5%, although the difference between the two was not statistically significant at 95% confidence interval. None of the sampled pigs were vaccinated and therefore the seropositivity was due to the wild virus circulation.

Keywords: Porcines, Foot and Mouth Disease, Kenya, Seroprevalence, Counties

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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 (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, (South African Territories) 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 i.e., O, A, C, SAT1 and SAT2 (Vosloo *et al*., 2002). In addition, within each serotype, a number of genetic and antigenic variants with different degrees of virulence exist. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used (Shale, 2004).

Morbidity can be as high as 100% in susceptible populations but mortality is low in adults. Infected animals show a spectrum of responses to FMD ranging from apparent infection to severe disease and death. In pigs, the initial signs are fever of 40-40.6^{o}C, anorexia, reluctance to move, and squealing when forced to move. These signs are followed by vesicles on the coronary band, heels, inter-digital space, and on the snout. Mouth lesions are not too common and when they occur are smaller and of shorter duration than in cattle and tend to be a "dry"-type lesion; there is no drooling; sows may abort, and piglets may die without showing any clinical sign (Blood *et al*., 1994). Once an outbreak begins, most transmission is by aerosol from one infected animal to another. Pigs produce tremendous amount of aerosol, their exhalations having 30-100 times more virus than those of sheep and cattle (hence termed *amplifier host*).

Nonstructural protein (NSP) 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. Therefore 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.

FMD is endemic in Kenya and even with this fact; the country had insufficient data to show the FMD seroprevalence distribution in pig keeping areas. The understanding of the seroprevalence across the country will be an important step in the establishment of FMD free zones and subsequent eradication in line with FAO/OIE FMD progressive control

pathway (PCP). The aim of the study was therefore to determine FMD porcine seroprevalence in counties where pigs are reared.

Serology results indicated very high FMD seroprevalence in counties within Western and North Rift areas of Kenya in both bovines and porcines. Some counties such as Uasin Gishu, Nandi, Trans-Nzoia, Bungoma and Kakamega recorded 100% prevalence. There were no detectable FMD antibodies in porcine sera from Bomet and Kilifi counties (Table 1). Since there are no vaccinations carried out in pigs in Kenya, the sero-positivity would therefore indicate FMD virus circulation.

MATERIALS AND METHODS

Samples

The sera 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 in counties where pigs were reared with the unit of sampling being the randomly computer generated villages in every district in the year 2010 (collection done between April and May). The sera were collected from a total of ten randomly selected villages in each of the selected district with a total of 3709 bovine sera collected from 39 counties and 180 porcine sera collected from 15 counties. All the 180 porcine samples were individually verified and entered into a data sheet with the details as follows; animal laboratory identification, location and coordinates of the source (district and county), species, sex (either male or female), age

of the animal (<1 year, 1-2 years and >2 years) and vaccination history (either vaccinated, non-vaccinated or unknown). The counties from which porcine sera were collected included; Uasin Gishu, Nandi, Trans Nzoia, Bungoma, Kakamega, Bomet, Nakuru, Siaya, Kisumu, Nyeri, Embu, Meru, Kilifi, Mombasa and Taita Taveta counties (Fig 1).

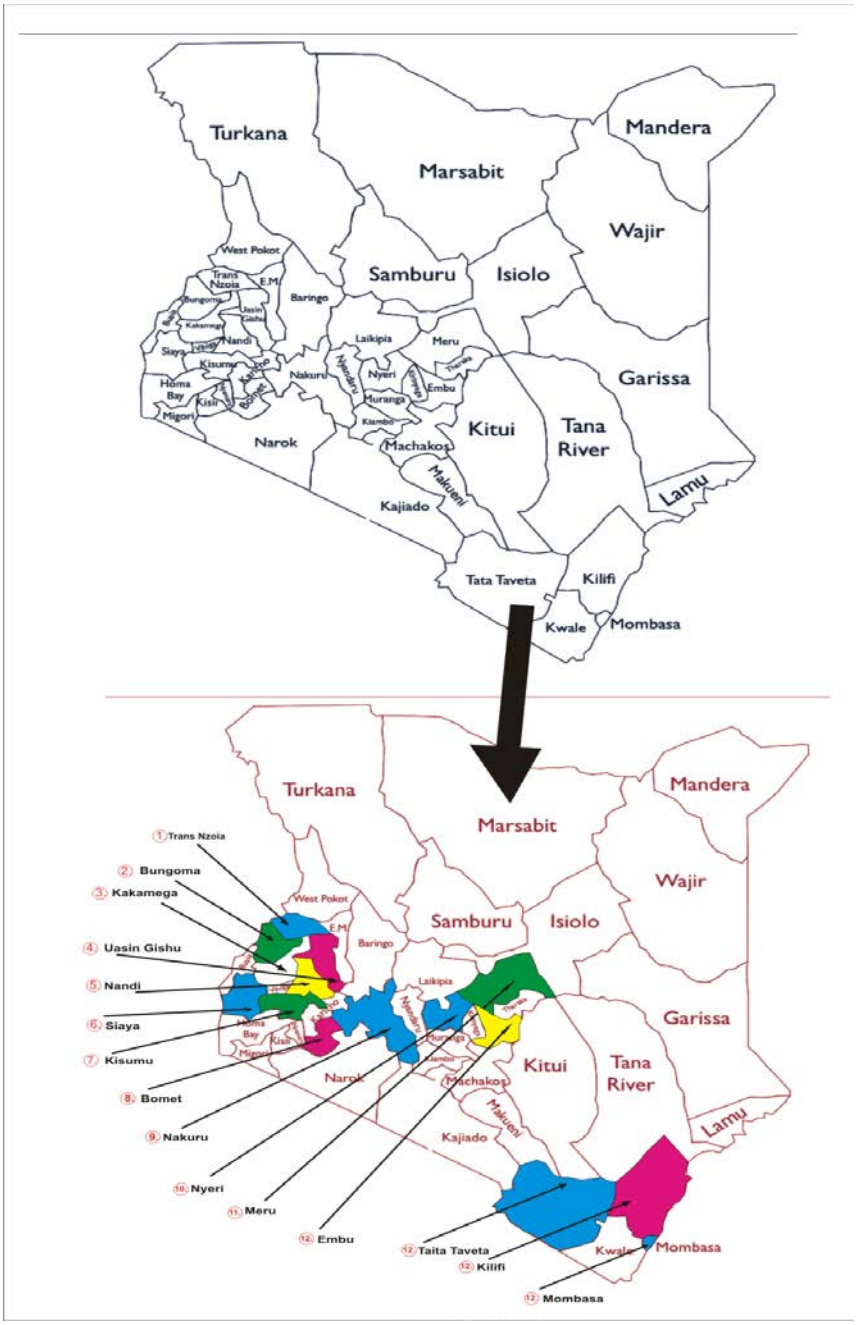


Fig 1: Map of Kenya showing the 15 sampled counties.

Serological tests

All the 180 porcine samples were subjected to FMD screening test (AniGen® FMD NSP Ab ELISA). The AniGen® foot-and-mouth disease virus Ab ELISA kit was designed to detect FMDV specific antibodies in serum. The test kit 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.

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 minutes at 37^0C, the plates were washed 6 times with washing buffer. After the last washing, tetramethylbenzidine (TMB) ready to use substrate was added and plates were incubated at room temperature for another 15 minutes. The reaction was terminated by adding 1M Sulphuric acid stopping solution. The absorbance of the antigen-antibody complex was read using bichromatic spectrophotometer at 450nm with reference wavelength of 620nm. The Percentage Inhibition (PI) was calculated as recommended by the manufacturer {PI = [1-(OD sample/mean OD negative)] X 100%}. A sample with PI value of above 50 (i.e. ≥ 50.0) on AniGen FMD NSP Ab Elisa was regarded as a positive result while a sample of PI value of less than 49 (i.e. <50.0) was regarded as a negative result.

Data analysis

The results of the NSP Elisa were then entered in an excel spreadsheet (Microsoft Corp) with the following information; sampling location, age, sex, species, vaccination history and AniGen results. Descriptive statistical analysis was then done to determine the proportion of positive samples across the 15 counties. Graphs were drawn using microsoft excel.

RESULTS

A total of 180 porcine samples were analyzed using AniGen FMD NSP Ab screening Elisa. The proportion of sampled females was higher than that of males (females 72.8% (n=131) and males 27.2% (n=49)). The proportion of pigs sampled aged between 1-2 years was higher at 91.7% (n=165) compared to the young (<1 years) and the old (>2 years) with 7.2% (n=13) and 1.1% (n=2) respectively. Of the 180 samples subjected to NSP screening test, 54.4% (n=98) were interpreted as positive while 45.6% (n=82) turned out to be negative (Table1).

Serum samples from Uasin Gishu, Nandi, Trans Nzoia, Bungoma and Kakamega counties had the highest levels of seropositivity (100%). Porcine sera from Siaya, Kisumu, Taita Taveta and Embu counties had high prevalence of more than 50% seropositivity while porcine sera from Nakuru, Nyeri, Meru and Mombasa counties had low seropositivity of less than 50%. No foot and mouth disease antibodies were detected in samples from Bomet and Kilifi. It is worth noting that all the porcines sampled in the

above counties did not have any history of vaccination (Table 1). Serum samples from bovine were used for comparative purposes and are shown in Table 2 and 4.

Table 1: Results Summary

RESULTS SUMMARY TABLE														
			Sex		Age			Vaccination History			AniGen Results		% Seropositivity	
No	County	No.of samples	Male	Female	< 1 years	1-2 years	>2 years	Yes	No	Uknown	Positive	Negative	% +VE	% -VE
1	Uasin Gishu	19	6	13	0	19	0	0	19	0	19	0	100	0
2	Nandi	10	4	6	1	9	0	0	10	0	10	0	100	0
3	Trans-Nzoia	8	2	6	0	7	1	0	8	0	8	0	100	0
4	Bungoma	7	1	6	0	7	0	0	7	0	7	0	100	0
5	Kakamega	9	3	6	0	9	0	0	9	0	9	0	100	0
6	Bomet	3	0	3	3	0	0	0	3	0	0	3	0	100
7	Nakuru	19	7	12	0	19	0	0	19	0	7	12	36.8	63.2
8	Siaya	16	7	9	9	7	0	0	16	0	12	4	75	25
9	Kisumu	8	1	7	0	8	0	0	8	0	6	2	75	25
10	Nyeri	33	12	21	0	32	1	0	33	0	7	26	21.2	78.8
11	Embu	8	0	8	0	8	0	0	8	0	4	4	50	50
12	Meru	8	2	6	0	8	0	0	8	0	1	7	12.5	87.5
13	Kilifi	16	2	14	0	16	0	0	16	0	0	16	0	100
14	Mombasa	8	0	8	0	8	0	0	8	0	3	5	37.5	62.5
15	Taita Taveta	8	2	6	0	8	0	0	8	0	5	3	62.5	37.5
	Total	180	49	131	13	165	2	0	180	0	98	82		
	% Proportion		**27.2**	**72.8**	**7.2**	**91.7**	**1.1**	**0**	**100**	**0**	**54.4**	45.6		

Table 2: National FMD prevalence in bovines

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

Table 3: Distribution of pig holdings within the country

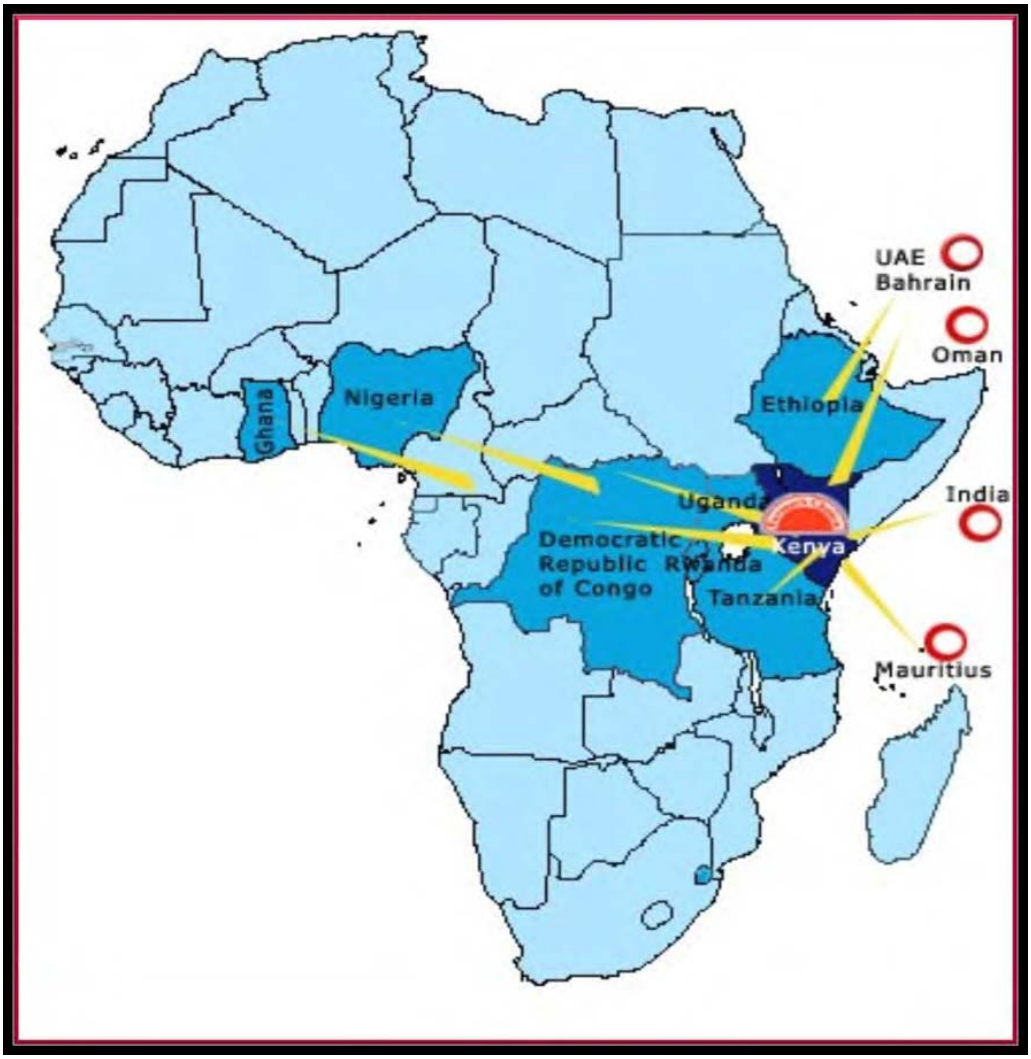
Province	Pig Population	Commercial sector	Traditional/Backyard sector
Western	87,838	3,512	84,325
Rift valley	48,495	14,579	35,654
Nyanza	27,612	900	26,712
North Eastern	68	68	0
Eastern	43,480	35,654	7,826
Coast	5,243	5,243	0
Central	91,977	75,421	16,556
Nairobi	29,976	13,976	16,000
Total	**335,301**	**149,965**	**187,073**

Source: KNBS 2009 Census; MOLD Department of Livestock Production Estimates, 2010

Table 4: County seropositivity

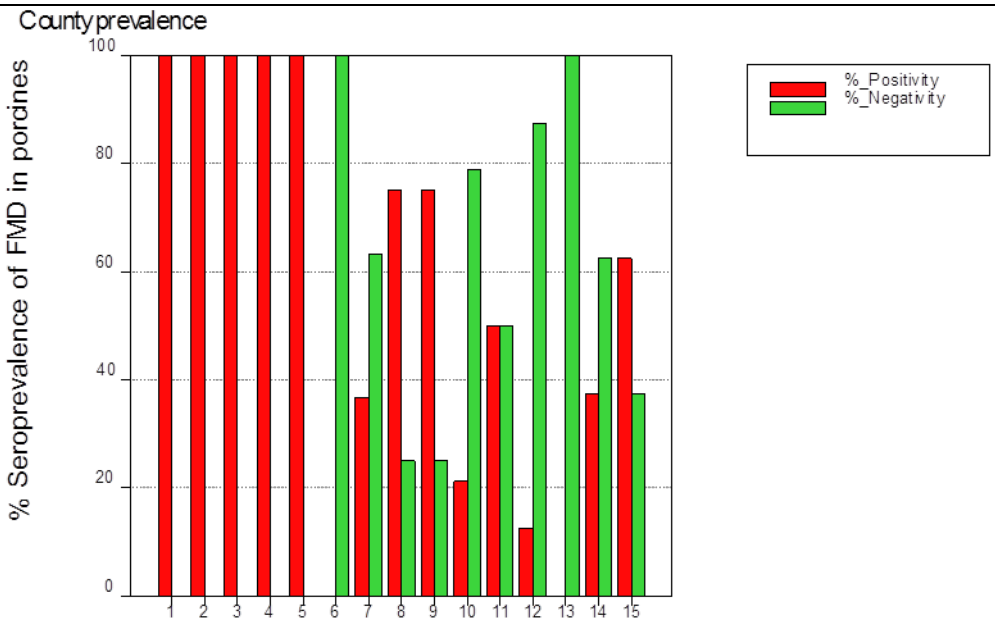
County FMD seropositivity between porcines & bovines			
	County	Porcines (CI95%)	Bovines (CI95%)
1	Uasin Gishu	100	100
2	Nandi	100	100
3	Trans-Nzoia	100	100
4	Bungoma	100	100
5	Kakamega	100	100
6	Siaya	75 (69.4-80.6)	62.1 (59.68-64.52)
7	Kisumu	75 (69.4-80.6)	51.1 (48.68-53.52)
8	Taita Taveta	62.5 (56.9-68.1)	40.2 (37.78-42.62)
9	Embu	50 (44.4-55.6)	82.9 (80.48-85.32)
10	Mombasa	37.5 (31.9-43.1)	42.9 (40.48-45.32)
11	Nakuru	36.8 (31.2-42.4)	22.7 (20.28-25.21)
12	Nyeri	21.2 (15.6-26.8)	5.3 (2.88-7.72)
13	Meru	12.5 (6.9-18.1)	35 (32.58-37.42)
14	Bomet	0	70 (67.58-72.42)
15	Kilifi	0	25.3 (22.88-27.72)

Fig 2: Map showing distribution network of pork and pork products by Farmer's Choice



Source: www.farmerschoice.co.ke

Fig 3: Graph showing county prevalence.



1	Uasin Gishu	5	Kakamega	9	Kisumu	13	Kilifi
2	Nandi	6	Bomet	10	Nyeri	14	Mombasa
3	Trans-Nzoia	7	Nakuru	11	Embu	15	Taita Taveta
4	Bungoma	8	Siaya	12	Meru		

DISCUSSION

The AniGen® foot-and-mouth disease virus Ab ELISA kit was designed to detect FMDV specific antibodies in 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 (vaccine-induced antibodies). Antibody to the NSP is only found in virus-infected animals but not in vaccinated animals (Diego *et al.,* 1997).

The seroprevalence of foot-and-mouth disease in Kenya was found to be higher in porcines at 54.4% compared to bovines at 52.5% (Table 1 and 2) although the difference in seroprevalence between the two species was not statistically significant ($p>0.05$). This could be attributed to very minimal foot-and-mouth disease control efforts that include vaccination in porcines despite being the main source of aerosol infections to cattle. Pig production plays an important role in household income with traditional/backyard production system being highly practiced in Western, Nyanza and some parts of Rift Valley (Table 3). The pigs roam freely, continuously interacting with either wild suidae or cattle therefore acting as a medium of interspecies FMD transmission. In reference to the findings, there was no vaccination undertaken in these areas although it has been shown that FMD seropositivity increases with the absence of vaccination (Kibore *et al.,* 2013).

Research conducted by ILRI around Ruma National Park in Homabay County showed that domestic pigs in free range system in Nyanza and Western Kenya interacted with giant forest hogs, river hogs, warthogs and bush pigs roaming at night. Bush pigs also continuously interact with the African Bufallo in the nearby national parks for example the Mt. Elgon National park. The African Buffalo have been identified as the major carrier wildlife species of SAT serotypes in southern Africa and serological analyses suggest them being the major species infected in East Africa (Vosloo *et al*., 2002). It is generally accepted that FMDV is spread locally in Africa through uncontrolled movements in addition to continous interaction between the domestic and wild animals. The subclinical nature of the disease in wild *S. Caffer* is considered a major challenge in the control of the disease (Ayebazibwe *et al*., 2010; Bastos *et al*., 2000; Vosloo *et al.,* 2009; Thomson *et*

al., 2003). This may explain why serum samples from Uasin Gishu, Nandi, Trans Nzoia, Bungoma and Kakamega counties had very high seropositivity levels (Table 1 and Fig 3).

The porcine FMD seroprevalence findings in the four counties above were consistent with the findings of a similar study in bovines species (Table 4). Pigs produce tremendous amount of aerosol, their exhalations having 30-100 times more virus than those of sheep and cattle. The continuous interaction between cattle and roaming pigs may explain the high seropositivity between these two species in the above counties. Pigs are always allowed to forage in slaughter houses and areas of waste disposal and therefore come into contact with infected materials thereby maintaining the transmission cycle.

Porcine sera from Siaya, Kisumu, Taita Taveta and Embu counties had high seropositivity of more than 50% (Table 1 and Fig 3). Pigs in Kisumu and Siaya counties roam freely in search of swill therefore interacting with either carriers/susceptible hosts. The presence of indirect transmission cannot be ruled out through consumption of contaminated hay and straw. The virus can survive for several weeks in favorable conditions (House and Mebus, 1998). Airborne spread of FMD is also possible under precise meteorological and epidemiological conditions. High relative humidity, low temperature, massive clinical cases and wind favor the airborne route. Pigs are very susceptible and known to shed a large amount of virus favoring aerosol transmission of the virus although the most common transmission mode is the introduction of an infected animal into a population of susceptible animals. The virus can be transmitted directly from one animal to another. Indirect virus spread via infected meat or milk as well as mechanical transmission by

vehicles and people is also likely (Donaldson *et al.,* 2001; Alexandersen *et al*., 2003). The airborne spread of FMDV has been modelled in special models, such as the Gaussian Dispersion model or the Lagrangian Particle model (Gloster *et al*., 2003; Sorensen *et al*., 2000; Mayer *et al*., 2007). Only Harvey *et al.* (2007) incorporated airborne spread into their animal disease spread model. They modelled airborne spread up to 1 Km, although it was likely up to 10 km.

Foot-and-mouth disease seropositivity was found to be higher in porcines compared to bovines in Siaya, Nakuru, Nyeri, Kisumu and Taita Taveta counties. Embu, Meru, Kilifi and Mombasa counties recorded a higher seroprevalence in cattle than in pigs (Table 4). Cattle are kept in largely sedentary set up with less inter-species interaction between them and pigs therefore reducing the level of FMDV transmission, but at the same time, there exists very vibrant livestock markets within these areas that may facilitate transmission between infected and susceptible animals (Kitching *et al*, 2005). Other indirect transmission routes exist which may have been responsible for the high seroprevalence in bovines. Veterinary equipment and vehicles are known to carry foot and mouth disease virus from one premise to another. Unchecked movement of people, milk tankers, animal feed vehicles, veterinarians and livestock buyers can also act as formites that may transmit the FMDV to naïve herds causing the disease. It has been shown that FMDV can reside passively in the human nasal passage for 24 hours and so be carried to new areas (Sellers *et al*., 1971, Donaldson *et al*., 2001).

With increasing globalization, the persistence of transboundary animal diseases anywhere in the world poses a serious risk to the world animal agriculture, food security

and jeopardize international trade and therefore there is need to establish the virus circulation in porcines and make an appropriate control policy. A case example is farmers choice which exports about 2,000 metric tonnes of pork and pork products annually to several destinations in the world including other African countries, Middle East & India and therefore, there exists real danger of disease spread (Fig 2).

The porcine seroprevalence in Nakuru, Nyeri, Meru and Mombasa counties were less than 50% (Table 1). These areas are characterised by presence of commercial farms with intensive pig production system and therefore contact between the carrier and the susceptible host is minimal thus attributable to low seroprevalence. Some counties for example Nakuru, Nyeri, Meru and Mombasa practice intensive foot-and-mouth disease vaccinations in cattle. The use of vaccines during an outbreak of FMD may be suppressive or protective. A suppressive vaccination is used to reduce potential FMD virus production in herds and flocks that may have been exposed to infection, but in which possibly only a few of the animals are incubating the disease (The commission of the European Communities 2001/246/EC). By vaccinating all of the exposed animals, it is hoped that those not already infected will develop sufficient immunity to provide at least partial protection against clinical disease. Protective vaccination is used on herds and flocks that are in the vicinity of an outbreak but are thought not to have been exposed to live virus (The commission of the European Communities 2001/279/EC).

An FMD vaccine stimulates a predominantly humoral immune response in the vaccinated animal. In cattle, there is a good correlation between antibody level and protection against

live virus challenge by the same strain of FMD virus from which the vaccine was produced (Brown, 1999). Therefore, in order to achieve maximum advantage from an FMD vaccine, it is necessary to ensure that the FMD virus strain used to produce the vaccine shares as many antigenic characteristics as possible with the outbreak strain it is intended to protect against (Doel, 1999). Normally, when a number of animals are vaccinated, some animals fail to develop immunity. Should these animals become infected and develop clinical FMD, they can excrete large amounts of virus, which may overcome the vaccinal immunity of other animals in the group. It is almost impossible to provide pigs with complete protection by vaccination if they are in contact with clinically infected animals (Garner *et al*., 1997).

No foot and mouth disease antibodies were detected in samples from Bomet and Kilifi (Table 1). It is worth noting that all the porcines sampled from these counties were unvaccinated. In Kilifi, cattle are largely kept in ranches and therefore there is less contact between them and pigs. Small scale commercial farming is practiced where pigs are kept in a confined area which limits contact between them and other wild reservoirs or with infected cattle. Although the porcine sera were negative of FMD antibodies, there was variation in bovine seropositivity in the two counties (Table 4). The variation may be attributed to the intensity of foot and mouth disease vaccination in the two counties. The vaccination cover in Kilifi was at 81.3% compared to Bomet which had no history of vaccination. The above finding is consistent with the fact that a correlation exists between antibody level and protection against live virus challenge by the same strain of FMD virus from which the vaccine was produced (Brown, 1999). The sub-optimal use of FMD

vaccination in relation to the population size of livestock increases the risk of susceptible animal populations (FAO, 2005-2006).

In Kenya, between 1971 and 1985, there were 17 outbreaks of non- bovine FMD out of which 8 were in pigs (Mbugua *et al*.,1991). These porcine FMD outbreaks were four in Kiambu (48/71, 89/82, 180/82, 11/84) and one each in Nakuru (291/79), Nyandarua (196/82) Kericho (101/83) and South Nyanza (97/83). In Kiambu pigs are reared in isolation from other animals and the high incidence of FMD here could be related to contaminated feeding of pigs with restaurant swill, cabbages and potato peels which were occasionally mixed with pig feed. The outcome of this study is inconsistent with the findings of Kanyari & Wandaka (2005), who reported that the Porcine FMD was not common in Kenya with the last case reported being in the year 1997.

In conclusion, FMD is endemic in Kenya with seroprevalence in porcines higher than in cattle (Table 1 and 2). Porcines act as a store and source of infection to other susceptible animals like cattle. Clinically infected pigs shed high doses of foot-and-mouth disease virus that act as source of infection. Since pigs are never vaccinated in Kenya, seropositivity would indicate that there is FMD virus in circulation. It is prudent therefore to develop a holistic approach in the control of foot-and-mouth disease. In the face of the move toward globalization and highly mobile human populations, each country is facing threats of outbreaks due to emerging or reemerging infections. There is need to critically evaluate the vaccination regime currently in place to take into consideration the dynamics in spread and circulating serotypes as well as role of porcines in the spread of FMD. This

may be the right time to consider vaccinations with recombinant based vaccines to retain immunity level sufficiently high to resist outbreaks. There is also need to enlighten the farmers, taking advantage of the county governments structures, on the dangers posed by the free range type of pig production as well as enforce the animal disease act (cap 364) and uplands bacon factory act (cap 362) to fully curb roaming pigs. 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.

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