DETERMINATION OF FOOT-AND-MOUTH DISEASE VIRUS SEROPREVALENCE IN KENYA USING THE LIQUID PHASE BLOCKING AND NONSTRUCTURAL PROTEIN ELISA TESTS

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DEDICATION

I dedicate this thesis to my dear family: my wife Monicah J. Kipkew and children Alvin Kipchirchir Kibore and Sasha Chebet Kibore. Thank you for your prayers, encouragement and support throughout the long journey of this study. Not forgetting my parents John K. Kiboiywo, Hellen J.Kiboiywo and my brothers and sisters, I sincerely appreciate your support.

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LIST OF ABBREVIATIONS AND ACRONYMS

Ab-Antibody

AEZ - Agro-Ecological zones

ASALS - Arid and Semi-Arid lands

AU-IBAR-African Union Inter-Bureau of Animal Resources

DVS – Director of Veterinary Services

ELISA - Enzyme Linked Immunosorbent Assay

DFZ-Disease Free Zones

FAO -Food and Agriculture Organization

FMD - Foot-and-Mouth disease

FMD LAB - Foot-and –Mouth disease laboratory

FMDV - Foot-and- Mouth disease virus

GDP-Gross Domestic Product

GF-TADS - Global Framework for progressive control of Trans-boundary Animal Diseases

HMPLS - High and Medium potential lands

KOH-Potassium Hydroxide

LPBE-Liquid Phase Blocking Elisa

Na_2CO_3-Sodium Carbonate

NaOH-Sodium Hydroxide

NSP-Non-structural Protein

OIE- Office Internationale des Epizooties

PACE-Pan-African Programme for the Control of Epizootics

PCP-Progressive Control pathway

RNA-Ribonucleic Acid

SAT-South African Territory

SES-Somali Ecosystem

TAD-Trans-boundary Animal Disease

VP-Virus Protein

WRL-World Reference Laboratory

ABSTRACT

Foot and mouth disease, a trans-boundary animal disease of major economic importance, is endemic in Kenya. Even with this fact, there is limited data to comprehensively show the prevalence and serotype distribution of FMD. The aim of this study was to determine the prevalence of antibodies against foot-and-mouth disease virus in Kenya using NSP and LPB Elisa, to determine the foot and mouth disease virus serotype distribution in the country using the antibody prevalence, to determine the prevalence of FMD in disaggregated units (age and sex) and to estimate the proportion of FMD vaccination cover in the country. The study utilized sera samples available at FMD laboratory in Embakasi including Somali ecosystem rinderpest coordination and eradication unit project collected in the year 2010. The serum samples were randomly selected except in counties that had less number of samples, in which case, all the sera were considered for analysis. The samples (both porcine and bovine) were screened using commercial non-structural protein antibody Elisa kit with the positive samples subjected to liquid phase blocking Elisa test. The serology results were extrapolated in order to determine FMD seroprevalence along the various trade routes, borderlands, proposed disease free zones, national parks and games reserves in addition to pastoral and non-pastoral areas. The national prevalence of FMD in bovine species was 52.5% while that of porcine species was 54.4%. The reported vaccination cover was low at 14.1%. Using chi-square statistical test, there was significant association between seropositivity and age groups ($p=0.002$) and vaccination status ($p=0.048$) but no association with sex ($p=0.063$). All the five serotypes SAT 1, SAT 2, type O, A and C were found to have circulated in the

country with serotype SAT 1 being the most prevalent (50.9%) serotype. A high number of animals were exposed to between two (11.9%) and three (11%) serotypes. The Kenya/Uganda borderland had the highest exposure to FMD with 95% prevalence while the Lokichogio/South Sudan/Lodwar-Pokot-Tranzoia-Uasin Gishu-Nakuru-Nairobi stock route in the Northern corridor had high prevalence of 80.5%. FMD viral prevalence was higher in non-pastoral areas at 58.6% as compared to pastoral areas that had 53%. Therefore, there is need to employ adequate and effective FMD control measures in line with progressive control pathway.

Keywords: Cattle, Pigs, Foot and mouth disease (FMD), Seroprevalence, Kenya, Counties

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Kenya is a country in East Africa that borders with the Indian Ocean to the south-east, Tanzania to the South, Uganda to the west, South-Sudan to the north-west, Ethiopia to the north and Somalia to the north-east. It has a land mass of about 580,000km^2 and lies between latitudes 5^{o}N and 5^{o}S and longitudes 34^o and 42^{o}E.

The country has seven distinct agro-ecological zones (AEZ) that are based on various agro-climatic factors particularly moisture and temperatures (Jaetzold and Schmidt, 1983). Dairy cattle, exotic chicken and pigs are reared in the high and medium potential lands (HMPLS) in agro-ecological zones (I-IV), which comprise 18% of the country's total area (Sombroek *et al*., 1982). These areas also have intensive mixed crop farming systems. The Arid and Semi-Arid lands (ASALs) of the country lie in the agro-ecological zones V-VII with rainfall of less than 700 mm per year and comprise 74% of the total landmass in Kenya. In these areas, pastoral and nomadic livestock production system is practiced.

The country has an estimated population of 38,610,097 (Kenya Bureau of Statistics, 2010) of whom 90% are employed in agriculture of which 10% are pastoralists. The Livestock sub-sector in Kenya contributes about 10% of the gross domestic product (GDP) and accounts for over 30% of farm gate value of agricultural commodities (Muthami, 2010). Livestock production is a major economic and social activity for the communities living in the high rainfall areas

and in the arid and semi-arid areas (ASALS). The sub-sector faces the greatest threat from diseases especially the foot-and-mouth disease.

1.2 Foot-and-mouth disease

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). It is caused by an RNA 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 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). 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 the country.

Foot-and-mouth disease is a listed disease by the World Animal Health Organization (OIE). The disease causes greatest production losses in cattle and pigs and in particular in intensive dairy and pig production systems. It is a major constraint to international trade in livestock and livestock products and acts as a barrier to accessing good markets in the world. Kenya is home to about 17.4 million cattle, 17.1 million sheep, 27.7 million goats, 2.9 million camels, 1.8 million donkeys and 334,689 pigs (Kenya National Bureau of Statistics, 2010).

Foot-and-mouth disease is one of the major transboundary animal diseases (TADs) that impact negatively on trade in livestock and livestock products in the east African region. The Ministry of Agriculture, Livestock and Fisheries development is in the process of developing disease free zones to act as disease control centers for livestock designated for export. 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 with regular and effective surveillance instituted in addition to strict vaccination measures being put in place (Chepkwony *et al.*, 2012).

FMD is a global disease that through the years has affected most of the countries. It occurs in most parts of the world especially in Asia, Africa, the Middle East, and parts of South America. North America, Central America, Australia, New Zealand, Chile, Japan, and most of European countries have been recognized as free of the disease (Sahle, 2004). Due to poor reporting, FMD is considered endemic in most of the African countries (OIE, 2009). 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).

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 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.

During the period 2004-2006, circulating serotypes in Kenya were mainly O, A, C, SAT1 and SAT2. However, an upsurge of SAT1 and SAT2 outbreaks has been recorded recently, though most of the outbreaks in Kenya have been caused by serotypes O and SAT 2 (Sangula, 2006). Serotype A occurs at a lower frequency while serotype C has been rare with only one outbreak last reported in 2004 (OIE, 2009). Between 1995 -1999 the most prevalent serotype in outbreaks recorded was SAT 2, but serotype O has dominated and SAT 1 incidence rose steadily from 2009 causing increased and severe outbreaks in 2010 (FMD laboratory annual report, 2009/2010).

Nonstructural protein (NSP) ELISA test is useful because it is able to discriminate animals that have been infected by wild virus from those that have been vaccinated using either purified/semi-purified vaccines. The non-structural proteins can only be induced by the wild virus. Such test would be able to detect

continued viral circulation and would therefore be extremely useful for serological surveys with a view to eradication.

The liquid Phase Blocking Elisa (LPBE) test used for the detection of antibodies against foot-and-mouth disease virus is a novel, reliable and reproducible test (Hamblin *et al.,* 1986a). The LPBE is an assay based on the specific blocking of a defined amount FMDV antigen by antibodies in the test sample during the liquid phase (Hamblin *et al.,* 1987). It detects antibodies due to structural proteins and is therefore not able to differentiate between vaccinates and natural virus.

1.3 Justification of the study

FMD is endemic in Kenya and even with this fact; the country had insufficient data to show the distribution of FMD serotypes. The study aimed at showing the distribution of FMD virus serotypes across the country which in turn would be of great benefit to the Director of Veterinary Services and service providers in distribution of the vaccines across the administrative units for ease in control. The study would also assist the state veterinary officers to understand the circulating serotypes with a view of acquiring the correct vaccine strain for control. The study would also be beneficial to the FMD laboratory through providing information on the type of serotypes circulating within each county for the purposes of tailoring the vaccine to conform to the circulating virus. The understanding of the prevalence and distribution of FMD strains 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).

As vaccination is widely used in the control of FMD in Kenya, the extent of application of this strategy needed to be established. This study therefore aimed at determining the vaccination coverage both at the national level and at the counties. At a conference held in Bangkok, Thailand between 27th and 29th June 2012 attended by high-level officials from regional and international organizations, adoption of a global strategy on FMD control and eradication was discussed (FAO/OIE, 2012). The strategy that will culminate in global disease eradication would be jump started by having countries determine their own disease prevalence. A good understanding of disease prevalence is therefore important and this can only happen if the disease is traced and regular and effective surveillance is done. The data generated will also be of benefit to the FMD Laboratory in assessing the usefulness and validation of LPB and NSP-Elisa as important and sensitive tools in the diagnosis and vaccine performance monitoring.

1.4 Overall objective of the study

To determine the distribution of foot-and-mouth disease virus in Kenya using the structural and non-structural protein Elisa tests.

1.4.1 Specific objectives

1. To determine the seroprevalence of antibodies against foot-and-mouth disease virus in Kenya.
2. To determine the FMDV serotype distribution in Kenya
3. To determine the seroprevalence of foot-and-mouth disease virus in the disaggregated units (age and sex) and to estimate the proportion of FMD vaccination in the country.

1.5 Problem statement

Foo-and-mouth disease remains a major transboundary disease of cattle in Kenya and yet limited data are available on the circulating FMDV serotypes, prevalence (along borderlands, stock routes, around national parks and game reserves) and vaccination coverage. This study therefore aimed at bridging this knowledge gap by providing relevant information on the circulating serotypes nationally and at each county; FMD prevalence along several disaggregated units such as borderlands, game reserves and major stock routes in addition to providing information relating to vaccination cover at national and county levels.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Definition of foot-and-mouth disease

FMD is the most contagious viral disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. It is characterized by fever, loss of appetite, salivation and vesicular eruptions on the feet, mouth and teats (Thomson, 1994).

2.2 Aetiology of foot-and-mouth disease

2.2.1 Taxonomy, serotypes and subtypes

FMD virus was defined in 1963 by the International Committee on Taxonomy of Viruses (ICTV) as belonging to the genus *Aphthovirus*, family *Picornaviridae* (OIE, 2004). At present, seven immunologically distinct serotypes of FMD viruses are known based on the fact that there is no cross protection between these serotypes. The seven serotypes are O, A, C, Southern African Territories (SAT) 1, 2 and 3, and Asia 1 which infect cloven-hoofed animals (Brooksby, 1982a). In addition, within each serotype, a number of genetic and antigenic variants with different degrees of virulence exist (Vallee and Carree, 1922; Pereira, 1977; Chenug *et al*., 1983; Kitching *et al.,* 1989). 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 (Sahle, 2004).

2.2.2 Physico-chemical properties

Picornaviruses are small RNA viruses that are enclosed within a non-enveloped protein shell (capsid) and the capsid consists of polypeptides, which are devoid of lipoprotein and hence is stable to lipid solvents like ether and chloroform (Cooper *et al.,* 1978). The FMD virus is pH sensitive and is inactivated by a number of chemical substances at the acidic and alkaline pH ranges, however, the virus is stable between pH 7 and 9 and 4^{o}C and -20^{o}C (Mann and Sellers 1990, Wilks 1992).

In meat, the virus can survive for long periods in chilled or frozen bone marrow and lymph nodes (Mckercher and Callis, 1983). Chemicals such as 2% NaOH or KOH and 4% Na_2CO_3 act as effective disinfectants for FMD contaminated objects, but the virus is resistant to alcohol, phenolic and quaternary ammonium disinfectants (Russell and Edington, 1985, Sahle, 2004). The sizes of droplet aerosol also play an important role in the survival or drying out of the virus; droplet aerosol size of 0.5 - 0.7 μm is optimal for longer survival of the virus in the air, while smaller aerosols dry out. In dry conditions the virus also survives longer in proteins for example in epithelial fragments (Donaldson, 1987).

2.2.3 Virus morphology, genome organization and virus replication

The virus is composed of an icosahedral protein coat (capsid) with no envelop and contain one molecule of infectious, positive sense, single stranded RNA (ssRNA), ranging from 7-8.5 kb in length (Melnick *et al.,* 1975, Cooper *et al.,* 1978, Robert and Bruce, 1981). The diameter of 22 - 25 nm capsid is composed of 60

capsomers each consisting of four virus proteins (VP1-4). VP1-VP3 is exposed on the capsid surface, whilst VP4 is located internally (Robert & Bruce, 1981). VP1 is the most antigenic protein (Logan *et al.,* 1993). The sedimentation coefficient (S) of the intact virus particle is 146S (Barteling, 2002).

Acquisition of infection is mainly through oral or by inhalation and the primary site of infection is nasopharynx (Artz *et al.,* 2010). The primary replication of FMDV following aerogenous exposures occurs in the epithelial cells of the pharyngeal mucosa-associated lymphoid tissue crypts and subsequent widespread replication in pneumocytes in the lungs, which coincides with the establishment of viremia (Artz *et al.,* 2010). The virus persists particularly in the basal epithelial cells of the pharynx and dorsal soft palate where, unusually, the infection does not result in the lysis of cells (Zhang and Kitching, 2001).

2.2.4 Serotypes of foot-and-mouth disease virus

Globally, there are seven immunologically distinct serotypes of FMDV: O (Oise), A (Allemagne), C (Island Riems), SAT1, SAT2 and SAT3 (South African Territories) and Asia 1 (OIE, 2004). This serological classification is based on the inability of virus serotypes to induce cross-protection in animals (Pereira *et al.*, 1977). Within each one of these serotypes, there are a large number of strains with their own antigenic characteristics and hence there may be only partial cross-immunity between strains of the same serotype or no cross-protection at all between serotypes (Brooksby, 1982a). Furthermore, within each serotype, genetic and antigenic variants occur with different degrees of virulence (Chenug *et al.*,

1983; Radostits *et al.,* 2000; Kitching *et al.,* 1989). Therefore recovery from one or vaccination against one serotype does not confer immunity against another or may not confer immunity within the same serotype (Grubman and Mason, 2002). Similarly, a single dose of a monotypic vaccine fails to protect against heterotypic challenge (Cartwright *et al*., 1982). The highly contagious nature of FMDV and the associated productivity losses make it a primary animal health concern worldwide.

FMD is endemic in sub-saharan African countries, except for Madagascar (Kitching, 1998; Vosloo *et al.,* 2002) with six serotypes O, A, C, SAT-1, SAT-2 and SAT-3 circulating but thought to have marked differences in the distribution and prevalence (Pereira 1981; Anderson 1981; Abu Elzein 1983; Abu Elzein *et al.,* 1987; Kitching 1998; Vosloo *et al.,* 2002). Serotypes A and O are widespread throughout sub-saharan Africa, whilst type C appears to have disappeared from the world as a whole with the exception of Kenya (Kitching, 2002a; Sangula *et al.,* 2011). All the three SAT serotypes have circulated in southern and eastern Africa, SAT1 and SAT2 have made incursions into the West Africa and Middle East while SAT-3 has demonstrated very restricted spread (Vosloo *et al.,* 2002).

2.2.5 Genetic variation

Changes in the nucleotide composition of the capsid, especially the VP1, are responsible for the genetic or antigenic variability of the virus and can lead to the evolvement of new subtypes (Haydon *et al.,* 2001; Mateu *et al.,* 1989; Strohmaier *et al.,* 1982; Carillo *et al.,* 1984; Beck and Strohmaier 1987; Baxt *et al.,* 1989;

Lews *et al.*, 1991; Meyer *et al.*, 1994). Thus the generation of new variants is considered as one of the major problems in the control of FMD by vaccination. Since there is continual antigenic drift in enzootic situation, this is an important factor to consider when selecting vaccine strains (Grubman and Mason, 2002).

2.3 History of FMD

Foot and mouth disease has been recognized as a significant epidemic disease threatening the cattle industry since the sixteenth century. The earliest description of probable FMD was given by Hieronymi Fracastorii in 1546. He described the disease, which occurred in northern Italy in 1514, as being unusual and affecting only cattle. In 1780 Le Vaillant (1795) from southern Africa, described a disease in cattle which "attacked the feet of oxen causing them to swell prodigiously and after producing suppuration, sometimes the hooves dropped off". Gordon Cumming (1850) and General S.J.P. Kruger (1858) also described a disease in southern Africa which was probably FMD; Hutcheon (1894) recorded an outbreak in South Africa, originating in Mashonaland and the Northern part of the Transvaal in 1893. In 1896, a panzootic outbreak of rinderpest swept through southern Africa and only isolated pockets of wildlife and cattle survived. It is estimated the total 2 ruminant population was probably reduced by 95%.

There are no additional reports of FMD in southern Africa until 1931 when the disease was observed in Rhodesia (modern Zimbabwe), except for outbreaks of disease in Cape Town in 1903 which originated from the importation of live, diseased animals from Argentina and were successfully controlled. In Germany,

the existence of FMD was first reported by Adami in 1754, while in Great Britain it was first recorded in August 1839. FMD was endemic in continental Europe during this period and outbreaks were also recorded for the first time in Canada and the United States in the late 18th century (Knowles, 1990).

Foot and mouth disease may have been present in Africa for long time due to the supporting evidence of SAT types of FMDV which are uniquely adapted to long term survival in free living African buffalo populations in South, Central and Eastern Africa (Bastos *et al.*, 2000). Furthermore these SAT types of FMDV are immunologically and genetically distinguishable from the other four serotypes of FMDV - O, A, C, and Asia 1 which presumably evolved in Asia and Europe. All the FMDV serotypes are endemic in Africa except Asia 1. Foot and Mouth disease is endemic in Kenya with recorded cases dating back to 1915 although the Maasai community was familiar with the disease prior to the records. It is probable that the disease was introduced into Kenya by settlers from Europe and South Africa. It was first characterized in 1932 and typing results have been available in Kenya since 1954 (Wariru, 1994). The disease was spread extensively by movement of trade and military transport cattle.

The logistically difficult and costly efforts required to eradicate the disease resulted in countries which had achieved eradication becoming wary of re-importing it from endemic areas. They consequently instituted measures to prevent this by placing trade embargoes on livestock and livestock products imports from countries where efficient control is not practiced or where the

epidemiological situation with FMD had not been accurately established (James and Rushton, 2002). This is the main reason why Kenya cannot export livestock or frozen meat to countries like the United States of America and the European Union. The primary control strategy was the slaughter of infected and exposed animals using one-kilometre radius from the infected farm as the zone of slaughter and a three-kilometre intensive surveillance zone. In Kenya and most other countries where FMD is endemic, control is by vaccination and quarantine, as slaughter of animals would be too costly for developing countries and have no ability to compensate the farmers.

2.4 FMD Epidemiology

Foot and mouth disease is a highly contagious viral vesicular disease of cloven-hoofed domestic and wild animal species and is characterized by fever, salivation and vesicular eruptions on the feet and mouth (Blood *et al.*, 1983; Thomson 1994). Morbidity can be as high as 100% in susceptible populations but mortality is low in adults. Infected animals show responses to FMD ranging from inapparent infection to severe disease and death.

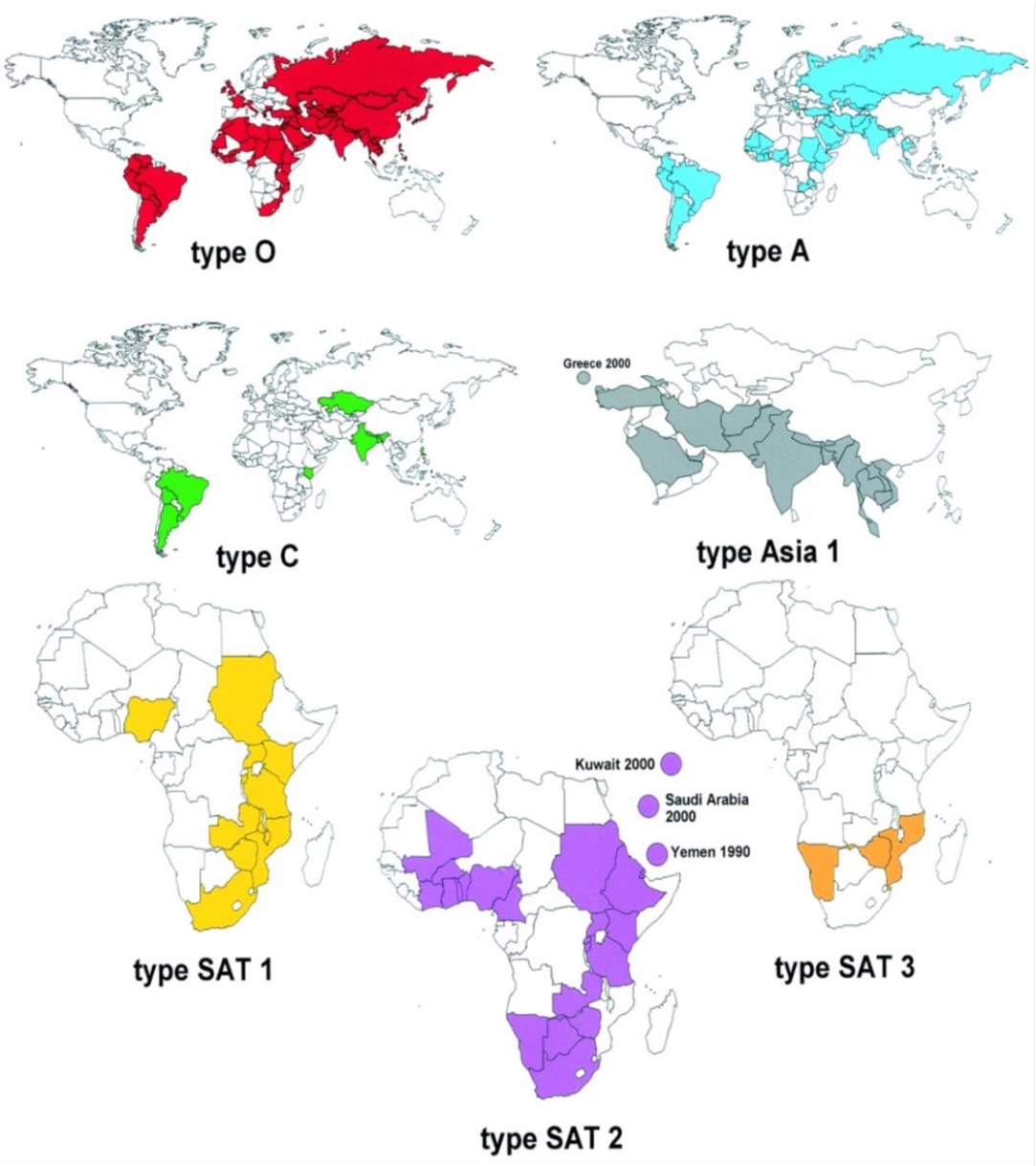
2.4.1 Geographical distribution

FMDV has an essentially global distribution, with the exception of North America, Western Europe (Greenland, New Zealand) and Australia. The FMD status of any particular country or region can be defined as endemic, epidemic (sporadic), or free. The USA, Canada, Mexico, Australia and Scandinavia haven't had the disease for many years (Samuel and Knowles, 2001b). At present, most

developed countries have successfully controlled or eradicated the infection and have implemented strict control measures especially regarding imports of animals and animal products to prevent re-emergence of the disease. According to Office International des Epizooties (OIE, 2012), 36 of the 162 member countries of the OIE have obtained FMD disease free status.

However, FMD is endemic in several areas of the world including Africa, Asia, part of South America, and the Middle and Far East with most outbreaks due to serotypes O and A (Anonymous 1998a; Kitching 1998; Samuel and Knowles 2001b; Knowles and Samuel, 2003). This situation exists despite continued efforts to control the disease and the extensive use of FMD vaccine throughout the affected areas of the world (Asseged, 2005). Western Europe has had recent outbreaks, which have all been successfully controlled (Leforban and Gerbier, 2002).

Fig: 2.1: Map showing the global distribution of FMD by serotype



(Adapted from, www.iah.bbscr.ac.uk/virus/picornaviridae/aphthovirus, 2004)

2.4.2 Host range

FMD is highly contagious and affects over 70 domestic and wild *Artiodactyla* species (Hedger, 1981). Of the domesticated species, it causes disease in cattle, pigs, sheep, goats and water buffalo. In addition, many species of cloven-hoofed wild life such as deer, antelope and wild pigs may become infected though their involvement in the epidemiology of FMD in the domesticated species is not certain (OIE, 2004). The susceptibility of these animals can vary with breed of animal and strain of virus. Indigenous breeds of cattle in Africa do not develop severe lesions as compared to high producing exotic breeds (Thomson, 1994; Kitching, 2002a; Kitching and Hughes, 2002; Kitching and Alexandersen, 2002).

2.4.3 The role of carrier animals

A carrier is defined as an animal from which FMD virus can be isolated from the esophageal pharyngeal (OP) area, more than 28 days after infection (Sutmoller *et al.*, 1968). In effect carriers are apparently healthy animals in which the virus is shed in small quantities from basal epithelial cells of the pharynx and dorsal soft palate. Carrier state of animals depends on the ability of virus to persist in the pharyngeal area of these ruminants which signifies a special virus-host relationship while the duration of persistence differs from species to species (Burrows *et al.*, 1981; *Rossi et al.*, 1988). FMD virus may persist undetected in cattle (up to 3 years), African buffalo (up to 5 years), sheep (up to 9 months), goats (between 3-6 month) and consequently become source of new infection to other susceptible animals (Rina and Martin, 1976). Transmission of FMDV by livestock in carrier status is at most an extremely rare event. Conversely where

African buffalo are concerned such transmission both in cohorts of buffalos and cattle has been clearly demonstrated (Hedger 1972; Hedger, 1976).

2.4.4 The role of wildlife in transmission

FMD has been reported in several species of wildlife. Buffaloes are believed to be the ultimate source of infection for livestock in southern Africa and East Africa due to their ability to both maintain and transmit the disease (Sangare, 2002). The mechanism facilitating SAT-type virus transmission from buffalo appears to occur readily when there is close contact between the two species during acute stage of infection while huge amounts of virus is being shed.

A general observation has been that wherever in the world FMD has been eradicated from livestock, it has also generally disappeared from wildlife in those regions (Thomson *et al*., 2003). Similarly, outbreaks of FMD in zoological gardens have coincided with outbreaks of FMD in domestic animals. In Sub-Saharan Africa, wildlife is clearly involved in the maintenance of FMD. Wildlife in South Africa, particularly the Cape buffalo ((*Syncerus caffer)* has been identified as natural hosts for the SAT serotypes of FMDV, although they may be infected by all serotypes (Hedger, 1976).

In East Africa, however, though little is known about the occurrence and distribution of FMD diversity in wildlife, findings have shown that African buffalo (*Syncerus caffer*) in selected national parks in Uganda have FMD antibodies (Ayebazibwe *et al*., 2010). Cattle in many areas in Africa including

Kenya are grazed on open communal rangelands with potential contact with wildlife populations. This wildlife-livestock interface is critical for disease transmission particularly around common watering points and pastures. Other factors include cattle straying into wildlife conservancies especially during the dry seasons in search of pasture with subsequent contact with wildlife species. In South Africa fences have been erected to separate wildlife from domestic animals (buffalos and cattle) in addition to continued vaccinations within the buffer zone and have resulted to improved disease control. The only locality in which overt FMD has been reported regularly in wildlife over the last 60 years is the Kruger national park in South Africa where there have been 31 reported outbreaks of FMD in impalas since 1938 (Bastos *et al.*, 2000).

The disease has been reported in several species of wildlife such as the African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), kudu (*Tragelaphus strepsiceros*) species, warthog (*Phacochoerus aethiopicus*), and African savanna and forest elephants (*Loxodanta Africana/Loxodanta cyclotis* respectively) with an ability to both maintain and transmit the disease. The virus can persist in an isolated herd of buffalo for up to 24 years, whilst an individual animal can maintain the infection for up to five years. Furthermore, buffalo have unequivocally been shown to be source of infection for cattle under both natural and experimental conditions. The mechanism facilitating SAT-type virus transmission from buffalo appears to occur readily when there is close contact between the two species during acute stage of infection in which large amount of virus is shed (Sangare, 2002).

Impala (*Aepyceros melampus*) is the most frequently infected species and act as an intermediary in disease transmission between livestock and buffalos. Although studies have established that individual impala do not become carrier, it appears that the disease can persist in impala populations for between 6 and 13 months Kudu (*Tragelaphus strepsiceros)* has been shown to gradually get infected with the carrier status of between 106-140 days being demonstrated. Experimental infection of warthog (*Phacochoerus aethiopicus*) with SAT2 type virus resulted in severe clinical signs of infection and transmission to contact animals (Vosloo *et al.*, 2002). Rare case of FMD has also been reported in Indian elephant (*Elephas maximus*) and in the African elephant (*Loxodo africana*) (Thomson, 1994).

2.4.5 Mode of transmission

FMD is highly contagious and transmission of disease can occur by direct contact between infected and susceptible animals during the acute phase of the disease, by animal products (e.g. meat, milk, wool), by airborne route (Cooper *et al.*, 1978; Woodbury, 1995), inoculation with contaminated vaccines, insemination with contaminated semen and by contaminated animal handlers. In addition, vehicles and formites have also been responsible for transmission of the disease (Sellers, 1971). Immediate freezing of carcasses after dressing enhances preservation of live infectious virus with outbreaks across international borders ascribed to this manner (Leforban and Gerbier, 2002).

2.4.6 Pathogenesis of foot-and-mouth disease

In animals infected via the respiratory tract, initial viral replication occurs in the pre-pharyngeal area and the lungs followed by viremic spread to other tissues and organs before the onset of clinical disease (Brown *et al*., 1992). FMD virus is then distributed throughout the body to reach multiplication sites such as the epithelium of oropharynx, oral cavity, feet, udder and heart. Viral excretion commences about 24 hours prior to the onset of clinical disease and continues for several days. The acute phase of the disease lasts about one week and viremia usually declines gradually coinciding with the appearance of strong humoral responses (Murphy *et al.,* 1999).

2.4.7 Immune Response to FMDV infection

The protection of a susceptible host against FMD virus correlates with the neutralizing antibodies level. Infection with one serotype produces complete protection against homologous virus but little or no protection against heterologous viruses (Samina *et al*., 1998). Serotype specific immunity is based on the presence of neutralizing antibodies to the VP1 viral capsid protein which develops 7 to 21 days after exposure to the virus. The immunoglobulin M (IgM) is most prevalent in the early convalescent serum and is less specific to the different serotypes than IgG. Immunoglobulin G is produced in the later stage during the FMD infection and the reaction between the serotype and the homologous antibodies is highly specific. Although serum antibody levels play an important role in host protection against FMD virus infection, the cellular responses mediated by T-helper and T- cytotoxic cells also play a role in the immune

response to FMD virus infection (Sanz-Parra *et al.,* 1998). Recovered cattle produce neutralizing antibodies and can resist re-infection by the same subtype of virus for up to one year (Samina *et al.,* 1998).

2.4.8 Transmission and clinical signs of FMD

When susceptible animals acquire disease through natural infection, the incubation period may range between 2-14 days (Kitching, 2002a). Excretion of the virus from infected animals in all secretions and excretions usually begin before the appearance of visible clinical signs (Kitching 2002a). The severity of clinical signs varies with the strain of the virus, the exposure dose, age, breed of the animal, the host species and the degree of immunity. The signs can range from mild or inapparent in sheep and goats to severe in cattle and pigs (OIE, 2004).

In cattle, the initial signs are fever of between 39.4-40.6^{o}C (103-105^{o}F), dullness, anorexia and fall in milk production. These signs are followed by excessive salivation, smacking of the lips, grinding of the teeth, serous (later turning mucopurulent) nasal discharge; shaking, kicking of the feet or lameness; and vesicle (blister) formation. The predilection sites for vesicles are areas where there is friction such as on the dorsum of the tongue, dental pad, gums, soft palate, nostrils, muzzle, teats, inter-digital space and coronary bands with consequent lameness (Sahle, 2004; Woodbury, 1995). The vesicles usually rupture within 24-48 hours leaving shallow erosions making it susceptible to secondary bacterial infection.

Pregnant cows may abort (Blood *et al.*, 1994) and young calves may die suddenly without developing any vesicle because of inflammation of the heart (myocarditis) (Blood *et al*, 1994). Morbidity can approach 100% but mortality in adult animals is rare, although in young animals, mortality can exceed 50% due myocarditis (Woodbury, 1995).

Most animals recover within two weeks although recovery of mouth, feet and teat lesions may delay. The complications may include hoof deformation, mastitis, low milk production, failure to gain weight and breeding problems (Tesfaye, 2006). In swine other vesicular diseases such as swine vesicular disease (SVD), vesicular stomatitis and vesicular exanthema of swine cause signs so similar to those of FMD thus complicating the clinical diagnosis (Bachrach, 1968).

In swine lesions often occur on the snout. Initial signs include fever of 40-40.6^{o}C (104-105^{o}F) 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 (no drooling), sows may abort and piglets may die without showing any clinical sign (Radostits *et al*., 2000).

In sheep and goats, the clinical signs tend to be very mild and may include dullness, fever and small vesicles or erosions on the dental pad, lips, gums and tongue. Mild lameness may be the only sign. In lame animals, there may be

vesicles or erosion on the coronary band or in the interdigital space. Infected animals may abort and nursing lambs may die without showing any clinical signs (Hughes *et al.*, 2002). Mouth lesions are less common and may proceed directly from epithelial necrosis to erosions without vesicle formation. In sheep and other small ruminants lesions commonly occur on the dental pad where they may be difficult to detect.

Usually the mortality in adult animals is negligible (1-2%) although it may be considerably high in young animals due to myocarditis. The course of an FMD infection is 2 to 3 weeks. The virus is capable of replicating fast and spreading at an alarming rate. This phenomenon was demonstrated in the 1997 Taiwan outbreak in which the first case was reported in March 1997 and within 3 weeks had spread to almost the entire island (Yang *et al.*, 1999).

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*). Sheep often masks the appearance of clinical signs after infection and therefore, during the transportation of apparently healthy animals, the virus is transmitted from one area to another (*maintenance host*). When contact occurs between sheep and cattle, the latter develop severe clinical signs of slobbering and lameness often raising the red flag of infection (and so the term *indicator host*).

Chronic FMD has been encountered historically in cattle both in Europe and S. America and is also recognized by pastoralist in East Africa (Mugera, 1979). The condition is usually seen 3-6 months after acute FMD in adult cattle and is manifested by heat intolerance resulting in an increased respiratory rate, reduced milk production, mastitis, a wooly or hairy coat, general unthriftiness and abortions and subsequent sterility sometimes occur. It mostly affects yearling cattle due to damage to their glandular tissue such as the thyroid. These animals are sometimes referred to as hairy panters' and may affect up to 5% of cattle in a herd. It has been suggested that heat intolerance is a sequel to FMD and is caused by damage to the endocrine system by the virus (Radostits *et al.*, 1994)

2.5 Foot-and-mouth disease pathology

In cattle, the diagnostic lesions are single or multiple vesicles ranging from 2 mm to 10 cm. These can occur at all sites of predilection. Gross lesions on the mouth cavity and tongue may appear as small-blanched whitish area in the epithelium, a vesicle (blister) or an eroded (red) area covered with yellow, brown, green or gray fibrinous coating (Woodbury, 1995). The vesicle in the inter-digital space is usually large because of the stress on the epithelium caused by movement and weight. The lesion at the coronary band at first appears blanched then followed by separation of the skin and horn. When healing occurs and new horn is formed, a line resulting from the coronitis is seen on the wall of the hoof. Animals that die may have grayish or yellowish streaking in the myocardium indicating degeneration and necrosis "tiger heart". Skeletal muscle lesions occur but are rare (Woodbury, 1995)

Lesions due to foot-and-mouth disease

Plate 1: FMD, foot lesion
AVIS Foot and mouth disease (FMD)

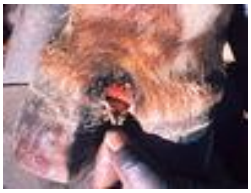


Plate 2: Inter-digital FMD erosions and ulcerations in cattle (Univ. Pretoria: J.A.W. Coetzer; R.C. Tustin; G. R. Thomson)

Plate 3: FMD, foot lesion in pigs (Univ. Pretoria: J.A.W. Coetzer; R.C. Tustin; G. R. Thomson)

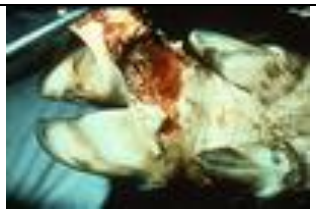


Plate 4: Foot lesion (from Master file - Mexico-US Commission for the Prevention of FMD)

Plate 5: Drooling of saliva in cattle

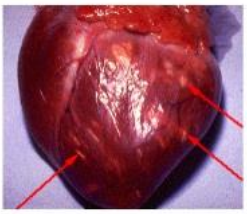


Plate 6: Focal myocarditis

2.6 Economic importance of the foot-and-mouth disease

Foot-and-mouth disease is probably one of the most important livestock diseases in the world in terms of economic impact. The disease ranks highly among the most economically devastating animal diseases in the world (OIE, 2004). Economic losses can be attributed to both direct and indirect costs. Direct effects of the disease include loss of milk production, loss of draught power, growth

retardation, abortion in pregnant animals, death in calves and lambs whereas indirect losses are attributed to the disruption in trade of animals and their products (Blood and Radostits, 1989). Its effects are found to be more important than the acute illness itself (Woodbury, 1995).

For example, Taiwan had been free of FMD for 68 years until the 1997 outbreak which culminated into the slaughter of more than 4 million pigs, almost 38% of the entire pig population at an approximated cost of $6 billion (Yang *et al.*, 1999). Taiwan was thus declared an FMD-infected zone and lost its pork export market. Later in 1999 to 2000 another outbreak occurred in Taiwan affecting cattle and goats but was more limited than the 1997 outbreak. Nucleotide sequencing of the virus isolated from infected animals revealed that the virus was different from the 1997 virus but closely related to viruses circulating in the Middle East and India Losses due to foot and mouth disease outbreak in Taiwan is estimated to have cost approximately $15 billion (Huang *et al.*, 2001).

In March 2000, a large FMD outbreak occurred in South Korea after being free from the disease for 66 years. A much more limited outbreak also occurred in Japan after 92 years of FMD freedom. The Korean outbreak was controlled by the slaughter and vaccination of all cloven-hoofed animals within the affected provinces and resulted in the slaughter of over 500,000 animals mainly cattle (Joo *et al.*, 2002). In 2001 and 2007, this pandemic spread to Great Britain, which had also been free of FMD since 1981. In this outbreak, 2030 cases occurred between February and September 2001 and spread to Ireland, France and the Netherlands

(Scudamore and Harris, 2002). As a result, farmers in the UK were compelled to slaughter approximately 4 million infected and in contact animals whose cost was estimated to be more than $29 billion (Defra, 2005). In Kenya, a dairy farm with 200 head of cattle had estimated losses of up to Ksh.1.2 million in a single outbreak in 2001 (Mulei *et al.*, 2001).

2.7 Diagnosis of foot-and-mouth disease

2.7.1 Field diagnosis of FMD (sample collection and transportation)

Foot-and-mouth disease in cattle should be considered whenever fever, salivation and lameness occur simultaneously and when vesicular lesion is seen. Differential diagnosis for FMD should include vesicular stomatitis, rinderpest, malignant catarrhal fever, the bovine herpes 1 infections, swine vesicular disease, vesicular exanthema of swine and blue-tongue (Blood *et al.,* 1994). An appropriate sample should be collected followed by fast and reliable laboratory diagnosis.

Since both the fluid contained in the vesicles and the epithelium covering the vesicles usually contain high concentration of the virus ($\geq 10^6$ infectious dose/ml or gm) these are the specimen of choice in acute cases. The fluid is easily aspirated from un-ruptured vesicles using a needle and syringe while the epithelial fragments can be simply cut free from the edges of the lesion with scissors. Epithelial specimen contains detectable virus quantities for 4 days after appearance of clinical signs while that of the feet is detectable for 7 days. Once collected, the samples from the large ruminants should then placed in transport fluid either glycerol/phosphate buffered saline composed of (0.08 M phosphate

buffer containing 0.01% bovine serum albumin, 0.002% phenol red, antibiotics [1000 units/ml penicillin, 100 units/ml mycostatin, 100 units/ml neomycin,and 50 units/ml polymyxin], and adjusted to pH 7.2) (Kitching & Donaldson, 1987). The antibiotics is used to prevent epithelial fragments putrefaction. Glycerol should not be added to vesicular fluid since it is toxic to cell cultures. On reaching the laboratory glycerol may be removed from the specimen by washing with PBS or using absorbent paper. The sample is then processed using OIE standard tests (O.I.E Manual, 2009).

2.7.2 Laboratory diagnosis

Due to the highly contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory (OIE, 2004), but this may not be the case in already endemic countries, Otherwise many endemic countries do not have such facilities. The samples include; vesicular fluid usually contains the highest quantity of virus. Epitheliums from early vesicles and from recently ruptured vesicles are tissue of choice for virus isolation (OIE, 2004). Other samples such as blood with anticoagulant, serum, lymph nodes, thyroid gland, adrenal gland, kidney and heart are good sources of specimens from postmortem.

Identification of the agent include; virus isolation and characterization "golden standard" and although it is a very sensitive method, it is on the other hand laborious and expensive and there is the risk of the dissemination of the virus in the environment (Kitching *et al.,* 1989). Immunological methods include; enzyme

linked immunosorbent assay (ELISA) and complement fixation test (an alternative test for international trade) (Roeder *et al.,* 1987). Nucleic acid recognition methods using the polymerase chain reaction (PCR) to amplify the genome fragments of FMD virus are applicable. Reverse Transcription combined with real time has sensitivity comparable to that of virus isolation and automated procedures to enhance sample thoroughput (Reid *et al.,* 2003).

Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and those that detect antibodies to viral nonstructural proteins (NSPs). The SP tests are serotype-specific, highly sensitive and detect antibodies elicited by vaccination and infection. They include; virus neutralization test (VNT) (Golding *et al.,* 1976), the solid-phase competition ELISA (SPCE) (Macckay *et al.,* 2001; Chenard *et al.,* 2003; Paiba *et al.,* 2004) and the liquid phase blocking ELISA (LPBE) (Hamblin *et al.,* 1986a; Hamblin *et al*., 1987).

The detection of antibody to the NSPs of FMDV can be used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated. Therefore the tests can be used to confirm suspected cases of FMD and to detect viral activity or to substantiate freedom from infection on a population basis (Diego, 1997). The 3 ABC Elisa test entails detection of antibodies to the non-structural polyprotein and acts as a useful indicator of FMD virus infection with any of the seven serotypes of FMD virus (Mackay *et al.,* 1998). Antibody to the NSP is only found in virus-infected animals but not in vaccinated animals (Bergmann *et al.,* 2000).

2.8 Control of foot-and-mouth disease

The degree of control of FMD thus varies as follows (Gonzalez *et al.,* 1992**)**: In disease free counties, strict movement controls and slaughter of infected and contact animals when outbreaks occur is applied (OIE, 2004). In endemic areas, the disease is generally controlled by vaccination and movement restriction of animals (Asseged, 2005). It is therefore important to consider that the vaccine contain the same subtype of virus as is present in the area (Gonzalez *et al.,* 1992). Intratypic variation of the field strains of FMD viruses must also be considered in the selection of seed virus for vaccine production (Grubman and Mason, 2002).

Several control measures such as vaccinations and animal movement control are used in Kenya to reduce the impact of FMD (Chema, 1975; Ngulo, 1980; Ngichabe, 1984) but the strategies' have not been applied at an intensity that could curtail the transmission and maintenance of the disease. The antigenic relationships between isolates need to be determined by serological tests to assess the vaccine strains to be used for the control of outbreaks (Nderitu, 1984). The vaccines are produced by growing the virus in suspension cultures of baby hamster kidney (BHK) cell lines and subsequent inactivation using binary ethyleneimine (BEI) as recommended by OIE (2004). This means efficient methods of diagnosis are required as a prelude to production of effective vaccines, disease surveillance, screening and control.

For countries that use vaccination as their control strategy, it is important to differentiate antibodies resulting from vaccination with those resulting from patent

infection. This will serve to detect persistently infected animals (otherwise known as carriers) that may occur. This status is important in clearing animals for export to FMD free countries in accordance to standards set by (OIE 2004). According to international standards set by OIE, vaccine purity is an import/export pre-requisite as the presence of trace amounts of non-structural proteins (NSPs) in vaccines may cause false positive reactions in animals that have been repeatedly vaccinated (Brocchi *et al.,* 2006).

The more affluent FMD free nations, those with an economically significant live animal and animal product export trade, and those whose livestock are highly susceptible to FMD employs the following approaches; stamping out policy with compensation, emergency vaccination within an infected area and/or protective vaccination in animals not already exposed to FMD virus (Sangare, 2002; Woodbury, 1995,;OIE, 2004; Asseged, 2005).

Some of the control practices.

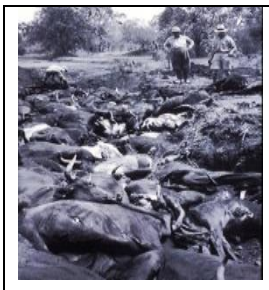


Plate 7: Disposal of carcasses

Plate 8: Burning carcasses

 Plate 9: Strict quarantine	 Plate 10: Disinfecting premises

2.9 Foot-and-mouth disease in Kenya

Foot and mouth disease is endemic in Kenya with recorded cases dating back to 1915 although the Maasai community was familiar with the disease prior to the records (Wariru, 1994). FMD in Kenya and the East Africa has had six of the seven serotypes known namely; O, A, C, SAT1, SAT2 and SAT3 reported. Serotypes SAT3 has been recorded only in Uganda (Vosloo *et al.*, 2002). During the period of 2004-2006, circulating FMD serotypes in Kenya have included types A, C, O, SAT1 and SAT2. In this period and more recently, an upsurge of SAT1 and SAT2 outbreaks were recorded (Sangula, 2006). In the past, the majority of outbreaks in Kenya have been caused by serotypes O and SAT 2. Serotypes A occurs on a lesser frequency while serotype C has been rare with only one outbreak last reported in 2004 (OIE, 2009).

Phylogenetic analysis at WRL Pirbright showed that the rise in SAT 1 cases were as a result of an incursion of a new strain which was first detected in Transmara district in 2009 and spread to many areas of the country causing major outbreaks even in vaccinated herds in central and eastern provinces in 2010 (FMD Laboratory Annual Report, 2009/2010). On phylogeny reconstruction analysis,

this strain was divergent from the vaccine strain by about 10% and may have been antigenically divergent leading to lack of protection. Antigenic matching with Kenyan vaccine strains was however not carried out to ascertain this (Chepkwony *et al,* 2012)

The rift valley province has all the five serotypes and the highest prevalence rate of outbreaks between 2001-2007 followed by Central, Eastern, Nyanza, Nairobi, Coast and lastly Western provinces (FMD laboratory annual report, 2007). Between 1995 -1999 the most prevalent serotype in outbreaks recorded was SAT2. Recently serotype O has dominated but in 2009 SAT 1 incidence rose steadily reaching a peak by mid-2010 (FMD laboratory annual report, 2009/2010.)

Foot-and-mouth disease was first characterized in Kenya in 1932 and sero-typing results are available since 1954 (Wariru, 1994). Five serotypes namely A, O, C, SAT 1 and SAT 2 have been confirmed in Kenya and every district in the country has recorded at least one serotype. According to a study carried out by Wariru, (1994) serotypes A and O were predominant up to 1974 and serotypes O and SAT2 became predominant in 1975. Serotypes O and SAT2 remain endemic while serotype A occurs sporadically in a few districts and so does serotype C which causes outbreaks only sporadically and was common mainly in Koibatek District. The SAT1 outbreaks in the past have been shown to have originated from neighboring countries of Tanzania and Uganda (Wariru, 1994).

The existence of multiple lineages in Kenya is suggestive of introductions from the cross border animal movements (Nderitu, 1984). The recent studies conducted by Ayelet *et al.,* 2009 in Ethiopia on FMD samples collected between 1981 and 2007 throughout the country from different species of animals showed the presence of serotype O, A, C, SAT1 and SAT 2. South Omo (Ethiopia) shares border with Kenya and Sudan with no strict prevention of animal movement among the different pastoral communities in these areas thus making the situation favorable for the transmission of the disease (Molla *et al.,* 2010).

In Kenya, there is a risk of spread of type SAT 1 and SAT 2 to neighboring areas in Great Lakes countries attributable mainly to uncontrolled animal movement. The wild reservoir harboring SAT type viruses in countries situated within the region makes FMD very difficult to control. In Kenya, as in other part of Africa, the use of vaccines is sub-optimal in relation to the size of population and most of the FMD susceptible animal populations are at risk (Molla *et al.,* 2010)

It has been suggested that the pastoralist livestock keeping areas in the East African region form an ecosystem in which FMD is maintained. These ecosystems also play an important wildlife-livestock interface hosting large populations of FMD susceptible wildlife. The ecosystems include the Maasai ecosystem on the Kenya-Tanzania border and the Somali ecosystem on the Kenya-Somali border (FAO/AU-IBAR/PACE FMD workshop, 2006).

The FMD control measures which include vaccinations and animal movement controls have not been applied at an intensity that could curtail the transmission and maintenance of the disease. The multiplicity of serotypes, antigenic variations with evidence of an upsurge of SAT 1 and SAT2 outbreaks and the low levels of vaccination coverage only serve to complicate the control efforts (Sangula, 2006).

The 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 of and relevant strain of vaccines. The goal of these measures is to establish FMD free zones in parts of the country to promote livestock export trade and production earnings for livestock farmers.

2.10 Challenges in attaining effective FMD control

Several challenges exist that act as barrier to attaining effective disease control include: uncontrolled livestock movement either for trade or in search of water and pasture, extensive wildlife-livestock interaction, limited availability of vaccines, occurrence of multiple serotypes and sub-types and the need to match vaccines to field strains. Other constraints include; droughts leading to widespread movement of livestock in search of pasture and water, thereby spreading diseases , high levels of insecurity, inadequate technical personnel willing to work in the generally hostile pastoral environment, inadequate legislation and poor enforcement of the laws; low community participation in livestock disease control; Inadequate disease diagnosis, surveillance and reporting; and lack of

inter-institutional, regional and international collaboration in disease control (Chepkwony *et al*., 2012)

2.11 FMD Diagnostic Assays in Kenya

The country's FMD reference laboratory at Embakasi uses virus isolation in tissue culture as a confirmatory laboratory diagnosis and serotype determination using antigen detection ELISA virus neutralization test (VNT). The VNT is a quantitative test for detecting FMD antibodies. VNT requires specialized laboratory conditions e.g. cell culture and carbon dioxide source facilities, the use of live virus which is a highly risk process because the virus may easily escape from the laboratory into the surrounding causing an outbreak and the process is very laborious and therefore it implies that one can process very few samples at a time coupled with the fact that it takes too long for the same results to come out i.e. it takes about 2–3 days. This has the implication especially where quick diagnosis is required for containment of the disease.

Traditional assays, such as the virus neutralization test, rely on cells as an indicator of viral activity. Cells may not only become contaminated by other viruses or other contaminants to result in faulty results but may also vary in sensitivity towards the agents being tested. In addition, a wide range of antibodies are involved in the immune response, not just those that neutralize. Therefore non sterile assays that can use either live or inactivated antigens, measure all antibodies binding to a virus, and use an enzyme reaction as an indicator, are often preferred (OIE Terrestial Manual, 2012)

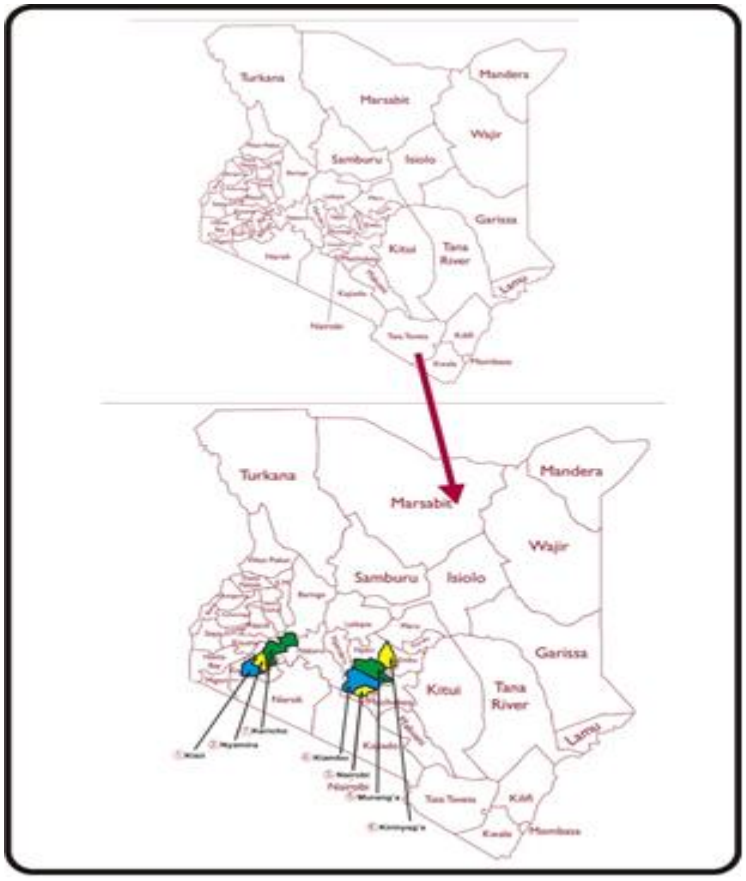
CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study Area

Kenya has 47 counties but only 39 of them were considered for study due to availability of samples from those areas. The counties included; Nyeri, Nyandarua, Mombasa, Kwale, Kilifi, Tana River, Lamu, Taita Taveta, Malindi, Marsabit, Isiolo, Meru, Tharaka Nithi, Embu, Kitui, Machakos, Makueni, Garissa, Wajir, Mandera, Moyale, Kisumu, Siaya, Migori, Turkana, West Pokot, Trans-Nzoia, Kajiado, Narok, Nakuru, Baringo, Laikipia, Samburu, Uasin Gishu, Keiyo Marakwet, Nandi, Bomet, Kakamega and Bungoma. The missing counties included; Kiambu, Murang'a, Nairobi, Nyamira, Busia, Kirinyaga, Kericho and Kisii.

Fig 3.1: Map showing the counties of Kenya and 39 counties covered



Source: http://softkenya.com/county/kenya-counties-map/

3.2 Study sample and sample size determination

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 throughout the country, 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 district. The sera collected were from bovine and porcine species. The sampling unit was considered to be the county. Initially, districts had been considered as units of analysis but due to gazettement challenges for the recently created districts, the county unit was preferred. Some other districts have since then been subdivided.

The minimum sample size for cross-sectional survey was calculated using the formula by Dohoo *et al.,* (2003) as shown below:

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

- 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% (This equation often written with 4 replacing $(1.96)^2$ so that the estimate is at the 95% level of confidence).

$$\frac{(1.96)^2 \times 0.5(1-0.5)}{(0.05)^2} = \frac{3.8416 \times 0.25}{0.0025} = 384$$

The calculated sample size was 384; however, the sample size was increased by four fold to 1383 taking into consideration the number of test kits available and level of precision. 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. The counties included; Elgeyo-Marakwet, Nandi, Trans Nzoia, Bungoma, Kakamega, Bomet, Mombasa and Malindi.

For counties that had slightly above the average number of samples required, simple random selection was done by creating a frame and randomly selecting a paper corresponding to the number of sample. For counties that had a very large number of serum sample pool and considering the geographical size of such counties, a higher number of serum samples selected was considered. This was aimed at offsetting the deficit experienced in other counties. Sample selection was done before running any test. All the 3709 bovine samples and 180 porcine samples were subjected to FMD screening test; NSP-ELISA (AniGen FMD NSP Ab ELISA).

	
Plate 11[a]	Plate 11 [b]

All the 4262 sera samples were individually verified and entered into a new data sheet with the following details; animal laboratory identification, location and coordinates of the source (district and county), species (either bovine or porcine), sex (either male or female), age of the animal (stratified as follows <1 year, 1-2 years and >2 years) and vaccination history (either vaccinated, non-vaccinated or unknown). Of the total 4262 serum samples entered, 372 of them were either missing or empty representing about 8% of the total samples. The individual

verification was necessary to physically ascertain whether the vial had serum or empty. A total of 3889 samples: 3709 bovine and 180 porcine samples were subjected to analysis.

3.3 Reagents and equipments

Equipments such as the photometer, orbital shaker, automatic washer, reagent troughs, antigen coated plates, 96-well NUNC Maxisorp Elisa plates, adhesive plate sealer, glass/plastic ware and incubator in addition to chemicals (such as washing solution, chromogen, hydrogen peroxide, substrate buffer capsules, coating buffer capsules & Tween-20) and biological reagents (such as positive and negative controls, enzyme conjugate, freeze dried anti-FMDV trapping antibody and freeze dried anti-FMDV detecting antibody) required as indicated in the manual of diagnostic tests and vaccines for terrestrial animals (OIE, 2009) were provided by the foot and mouth disease reference laboratory, Embakasi. Some other materials were sourced locally and from South Korea, Netherlands and Pirbright, UK through support from the national council for science and technology (NCST). Both chemical and biological reagents were prepared according to the kit manufacturer instructions.

3.4 Reported vaccination coverage

The reported vaccination coverage was assessed from the information on history of vaccination available for each of the 3709 bovine species and 180 porcine species samples. Each vial of samples was labeled as either vaccinated "yes" or not vaccinated "no". The information was used to determine the national foot and mouth disease vaccination cover as well as on each county.

3.5 Determination of seroprevalence and serotype distribution on various disaggregated units

Kenya borders Ethiopia to the North, Somalia to the East, South Sudan to the North West, Uganda to the West and Tanzania to the South. The seroprevalence and circulating serotypes on various borderlands was determined through analysis of sera samples from the respective border counties.

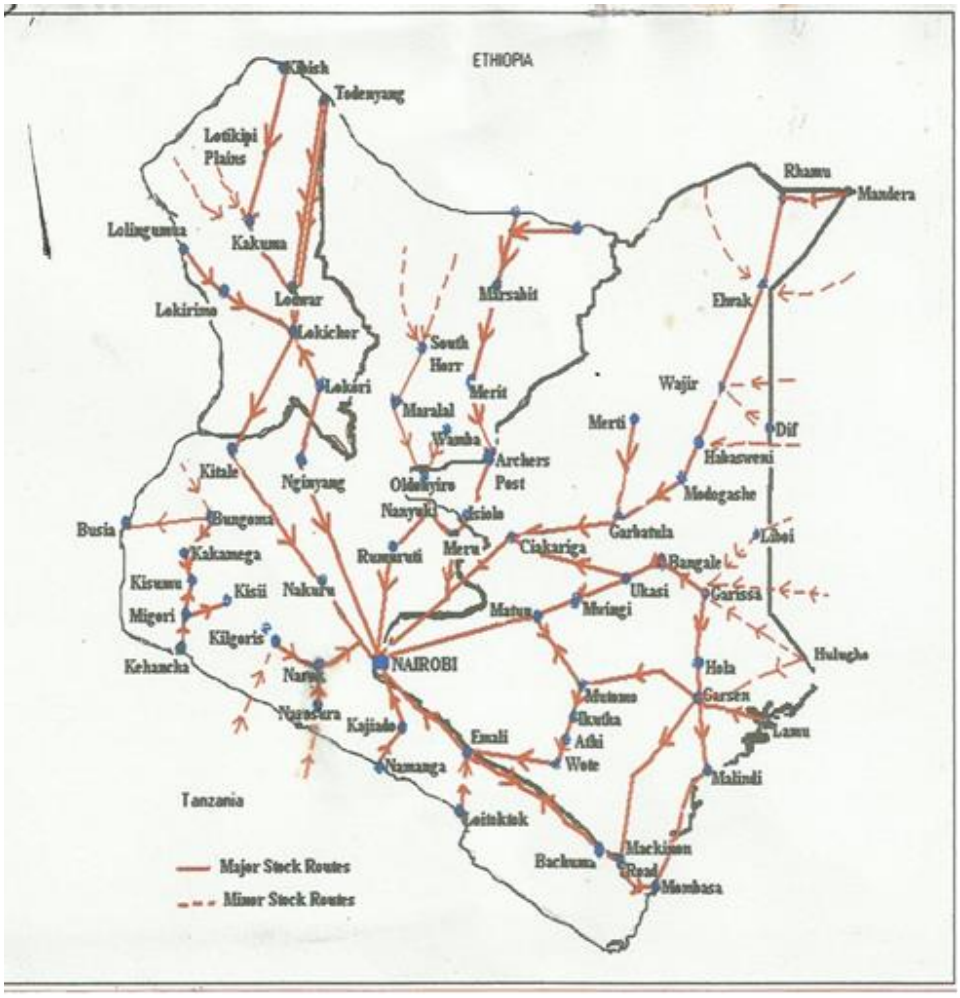
The country is zoned into several agro-ecological areas i.e. from I-VII. Agro-ecological zones V-VII constitute the pastoral areas (also known as ASAL areas) and occupy mainly the northern and the southern part of the country. They comprise the following counties: Turkana, West Pokot, Baringo, Mandera, Wajir, Garissa, Isiolo, Samburu, Moyale and Marsabi to the north and Taita Taveta, Narok, Kajiado, Kitui, Lamu, Laikipia and Tana River to the south. The mean seroprevalence of this agro-ecological zone was determined through analysis of sera samples from all the above counties. In addition, the seroprevalence of non-pastoral areas, occupying agro-ecological zone I-IV and which comprise of the

following counties; Elgeyo Marakwet, Uasin Gishu, Nandi, Trans Nzoia, Bungoma, Kakamega, Bomet, Migori, Nakuru, Siaya, Machakos, Makueni, Nyeri, Embu, Meru, Nyandarua, Tharaka Nithi, Kilifi, Malindi, Mombasa, Kwale and Kisumu, was determined by subjecting all sera from the counties above to the nonstructural protein Elisa test.

The Ministry of Agriculture, Livestock and Fisheries development is in the process of setting up several disease free zones in line with vision 2030. This will act as buffer zones in addition to enabling the country achieve effective disease control and to access lucrative international markets for livestock and livestock products. The proposed DFZ's include; Coast, Laikipia/Isiolo complex, Machakos/Kajiado complex, Central and North Rift. The sera samples from counties within the disease free zones above were identified and all screened using the NSP Elisa with the randomly selected positive samples subjected to serotype titration to determine the circulating serotypes within each DFZ.

Foot and mouth disease seroprevalence around major national parks and game reserves was determined through identification of sera samples from the surrounding counties and subjecting them to screening (NSP Elisa) and titration (LPBE) tests to determine their seropositivity and circulating serotypes respectively. FMD seroprevalence was analyzed in the following national parks and reserves: Sibiloi, Meru, Mt. Elgon, Mt. Kenya, Abardares, Lake Nakuru, Hells Gate, Amboseli and Tsavo national parks in addition to Maasai Mara, Samburu, Losai, South Turkana and Marsabit game reserves.

Fig 3.2: Map showing several stock routes in Kenya



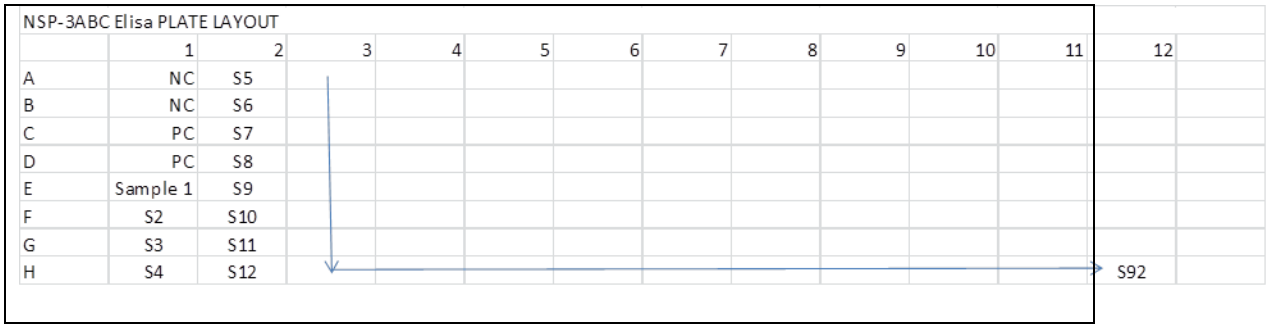
Source: AU-IBAR & NEPDP (Kenya livestock sector study, 2006)

Several stock routes that supply animals to either Nairobi or Mombasa exist. The stock routes act as dissemination route for foot and mouth disease virus. The stock routes include those from north eastern corridor, northern corridor and the southern corridor. In order to determine the seroprevalence along several stock routes, sera samples along these areas were identified and screened for the presence of foot and mouth disease virus using the nonstructural protein Elisa.

3.6 laboratory analysis methods

3.6.1 Non-structural protein enzyme-linked immunosorbent assay

Table 3.1: Table showing NSP Elisa Layout



NSP-3ABC Elisa PLATE LAYOUT												
	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S5										
B	NC	S6										
C	PC	S7										
D	PC	S8										
E	Sample 1	S9										
F	S2	S10										
G	S3	S11										
H	S4	S12										S92

The laboratory analysis was carried out as described by Bionote® AniGen FMD NSP Ab ELISA, South Korea. Wells A1 and B1 were dispensed with 50μl undiluted negative control serum (normal bovine serum with protein stabilizer preserved in phosphate buffer) while C1 and D1 were equally filled with 50μl of undiluted positive control serum (rabbit polyclonal antibodies to FMDV with protein stabilizer preserved in phosphate buffer). All other remaining wells were filled with 50μl of corresponding serum samples.

50μl of diluted enzyme conjugate were added to all wells (the dilution was done at 1:100 i.e. 10μl of enzyme conjugate stock in 1ml of conjugate diluent). The plates were then covered with an adhesive plate sealer, placed on an orbital shaker (to ensure reproducible results) and incubated at 37^{o}C for 90 minutes.

The plates were then washed 6 times with 350ul of diluted washing solution (prepared using dilution 1:9 i.e. 10ml of washing stock solution in 90ml of distilled/deionized water, enough for the plates). After the last washing, the plates

were tapped and 100ul of ready to use substrate were dispensed to all wells and incubated at room temperature for 15 minutes for color development. After incubation, 100ul of stopping solution was dispensed to each well therefore stopping any further reaction. The absorbance of the antigen-antibody complex (if any) was read using bichromatic spectrophotometer at 450nm with reference wavelength of 620nm.

The specially selected NSP antigens were used as capture material in the test. This enabled the FMD NSP Ab ELISA to identify FMDV outbreak antibodies in sera, with high degree of accuracy. For validation of the assay, the Optical Density of the positive control ought not to exceed 2.0 and the negative 0.5 respectively. The difference between the negative and positive control ought to be ≥ 0.4.

Calculation of the results: the OD of the positive control (OD_{pos}) as well as OD of the samples (OD_{sample}) was then corrected by subtracting the OD of the negative control (OD_{neg}).

$$\text{Positive control} = OD_{pos} - OD_{neg}$$

$$\text{Sample} = OD_{sample} - OD_{neg}$$

The samples in relation to the negative and positive controls were then analyzed using the formula

$$\text{Value (\%)} = \frac{(OD_{sample} - OD_{neg})}{(OD_{pos} - OD_{neg})} \times 100\%$$

Interpretation of the results was based on the following criteria;

OD Value	<20%	20-30%	>30%
Interpretation	Negative	Ambiguous	Positive

3.6.2 Liquid phase blocking ELISA (LPBE)

The chemical and biological reagents were prepared according to the manual (Kenya, FMDV Elisa Kit, Bench protocol, 2009). All the sera samples that had been selected and turned seropositive on NSP screening were subjected to liquid phase blocking Elisa (LPBE). LPBE acted as a titration test; where it was used to determine the serotype responsible for the seropositivity on NSP. A total of 738 samples from 39 counties were subjected to the test. Each of these sera was screened against the five serotypes of FMD virus known to be or have been in circulation in Kenya, i.e., O, A, C, SAT I and SAT 2 using the strains for vaccine production namely, 'A'K5/80, 'O'K77/78, 'C'K267/67, SAT1T155/71, SAT2K52/84 for serotype A, O, C, SAT1 and SAT 2 respectively.

3.6.2.1 Coating of the microplates

The contents of vials of rabbit anti-FMDV trapping antibody stock (FMDV serotypes O, A, C, SAT1 and SAT2) were gently agitated. A 1:1000 working dilution of trapping antibody stock was then prepared in coating buffer in a volume sufficient for the number of plates required (6µl of rabbit antibody stock in 6ml of coating buffer per plate) and then agitated.

For the serotypes that were being tested, 50µl volumes of the working dilution of the respective trapping antibody were immediately dispensed to all the 96 wells of appropriately labeled and correctly aligned polystyrene microplates (NUNC Maxisorp cat. No. 442404). The sides of the microplates were then tapped to ensure that the trapping antibody was evenly distributed over the bottom of each well. The microplates were then covered and incubated at +1 to 8^{o}C overnight.

3.6.2.2 Test and control serum incubation (Liquid phase)

The test and control sera were gently agitated to ensure homogeneity. A 1/16 dilution of each control and test sera was then prepared in suitable dilution tubes as follows: for each *control serum* (C++, C+ and C-, 15 µl of undiluted control bovine serum were added to 225 µl of Diluent Buffer A and for each *test serum*, 10µl of undiluted serum were added to 150µl of Diluent Buffer A and gently agitated.

The test and control sera were then added to the wells of polypropylene U bottom microplate. 50µl of pre-diluted test and control sera (strong positive C++, weak positive C+ and negative C-), were then added into wells of polypropylene U-bottomed multiwell microplate and 50µl of Diluent Buffer A added to the antigen control (Ca) wells (each serotype with its own plate).

Table 3.2: Liquid phase blocking Elisa plate layout

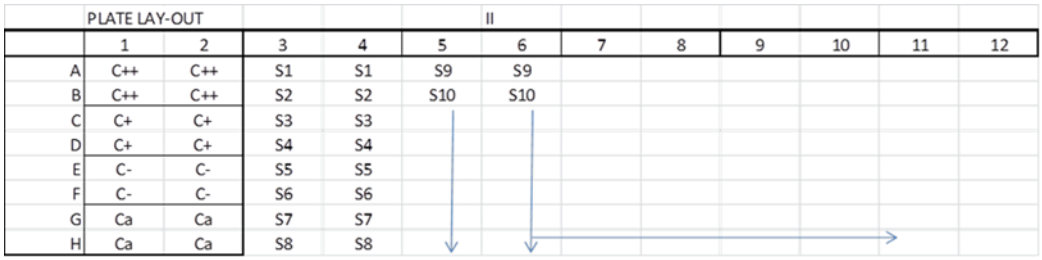


PLATE LAY-OUT						II						
	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	C++	S1	S1	S9	S9						
B	C++	C++	S2	S2	S10	S10						
C	C+	C+	S3	S3								
D	C+	C+	S4	S4								
E	C-	C-	S5	S5								
F	C-	C-	S6	S6								
G	Ca	Ca	S7	S7								
H	Ca	Ca	S8	S8								

50µl of FMDV antigen (serotypes O, A, C, SAT1, and SAT2) homologous to the rabbit antisera used to coat the plates and diluted at suggested working dilutions were added into all 96 wells of the perspective polypropylene U-bottomed microplates. A working dilution of the FMDV antigens (FMDV serotype O, A, C, SAT1 and/or SAT2) was prepared in Diluent Buffer A in a volume sufficient for the microplates used (5ml per plate plus an additional 1ml for two-fold dilution range plates).

Table 3.3: Working dilutions of several serotypes

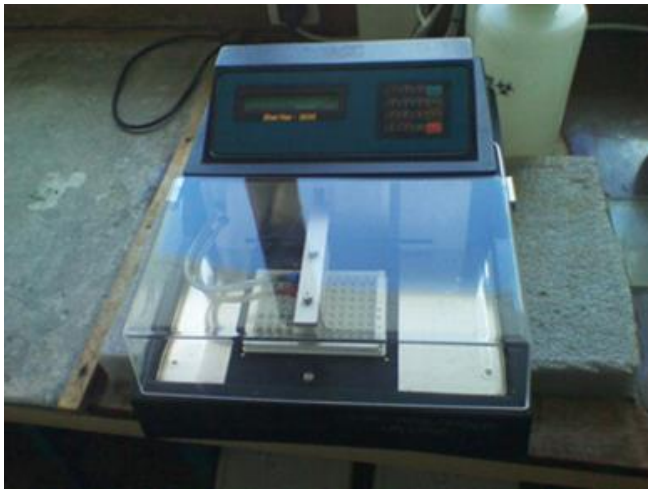
		Suggested	Used dilution
O_1 Manisa	16/2/07	1:100	1:100
A_{22} Mahmatli	16/2/07	1:100	1:140
C PHI 7/84	16/11/04	1:90	1:80
SAT1 (105)	16/11/04	1:120	1:50
SAT2 Eritrea	11/8/04	1:100	1:100

All wells finally contained 100µl total of a serum dilution and antigen. The serum antigen mixture in the U bottom microplate were then placed on an orbital shaker or tapped at the sides of the microplates to ensure thorough mixing. The polypropylene microplates were then covered and incubated at +1 to 8^{o}C overnight. Step1 and step 2 were done simultaneously.

3.6.2.3 Transfer of Serum/Antigen Mixture to the ELISA plate

The microplate was then inverted and using an abrupt downward hand motion, the contents of all antibody coated microplates (NUNC Maxisorp) was discharged into the sink. The inverted microplates were then slapped onto a lint-free absorbent towel to remove all residual contents. Then all the 96 wells of all microplates are filled with wash buffer. After filling, the contents of the microplates were again discharged and the inverted microplates were slapped onto a lint-free absorbent towel to remove all residual contents. This process of filling and emptying was repeated three times.

Plate 12: Washing of microplate using automatic plate washer



After three complete wash cycles and after ensuring that no residual contents were left in the microplates, 50µl of serum-antigen mixture were immediately transferred from the polypropylene U-bottom carrier microplates to the rabbit serum coated NUNC Maxisorp ELISA plate according to the same plate layout as being used for the ``liquid phase''. The microplates were then covered and incubated at 35 to 39^{o}C for 1 hour with continuous shaking.

3.6.2.4 Addition of detecting antibodies

Immediately before the end of the serum/antigen mixture incubation for the first plate, a 1:1000 working dilution from the homologous detecting antibody stock (anti-FMDV serotype O, A, C, SAT1 and/or SAT2) was prepared in Diluent Buffer B with the volume sufficient for the plates being used (60µl of guinea pig antiserum stock in 6ml Diluent Buffer B).

After one hour of incubation, the microplates were then removed from the incubator and the plates washed with buffer as described above. For each serotype and immediately after washing, a 50µl volume of the working dilution of the detecting antibody (FMDV serotype O, A, C, SAT1 and/or SAT2) was added into all the 96 wells of the respective microplates. Then the sides of microplates were then tapped to ensure that the working dilution is evenly distributed over the bottom of each well. The microplates were then covered and placed on an orbital plate shaker at 35 to 39^{o}C for 1hour with continuous shaking.

3.6.2.5 Addition of conjugate

Immediately before the end of the detecting antibody incubation, a 1:200 working dilution of the conjugate was then prepared in Diluent Buffer B in a volume sufficient for all microplates (30µl of conjugate stock in 6ml of Diluent Buffer B). After 1 hour of incubation, microplates then removed from the incubator and washed with buffer as earlier described. Immediately after the washing, a 50µl volume of the working dilution of conjugate was then added into all the 96 wells of each microplate. The sides of the microplates were again tapped to ensure the evenly distribution of the conjugate over the bottom of each well. The microplates were then covered and incubated for 1 hour at +35 to 39^{o}C with continuous shaking.

3.6.2.6 Addition of the substrate/chromogen and stopping solutions

Immediately before the end of the conjugate incubation, the substrate/chromogen solution in a volume sufficient for the number of plates being run was then prepared (e.g. for 1 plate, 30µl of substrate stock (H_2O_2) was diluted in 6ml of chromogen stock solution (OPD). This represented 3.3mM OPD and 4.4mM H_2O_2. This final substrate/chromogen solution appeared colorless as required).

After 1 hour of conjugate incubation, the plates were then washed as described above with focus to ensure that all 96 wells of each microplate had been flooded with wash buffer to eliminate unreacted conjugate. A clean microplate (not coated with trapping antibody) was used as the ``blanking plate'' for the photometric reading.

Immediately after washing, 50µl volumes of the substrate/chromogen solution were then added to the wells of the microplates, starting with the first column of the ``blanking plate'' followed by all 96 wells of the microplates in the test run. Timing was then done immediately after filling the first wells. The plates were then incubated at ambient temperature for 15 minutes without plate shaking.

After 15 minutes of substrate/chromogen incubation, 50µl volumes of the stopping solution (1.25M sulphuric acid) were immediately added starting with the first column of the ``blanking plate'' followed by all the 96 wells of the microplates in the test run. The microplates were then briefly tapped at the sides to ensure even mixing. The end result was that, all wells were to lastly contain 50µl of substrate/Chromogen solution plus 50µl of stopping solution.

3.6.2.7 Measurement of substrate development

The ``blanking plates'' were then placed in the carriage of the photometer and the blanking sequence was then initiated. This was followed by placing the first microplate of the test run in the carriage of the photometer in order to initiate the reading sequence. The run was then repeated for each microplate.

The plates were read at 492nm on a spectrophotometer ELISA reader connected to the computer loaded with ELISA Data Information (EDI) software, which was used to automate the reading of optical density (OD) values and calculate the percentage inhibition (PI), control and plate acceptance. The percentage inhibition (PI) for control and test samples was calculated according to the manual (Ferris, 2004).

The formula for calculation of PI on the control and quality assurance (QA) acceptance

$$PI = 100 - \frac{\text{(Replicate OD of control x 100)}}{\text{Median OD of antigen control (Ca)}}$$

Formula for calculation of PI of test sera and Diagnostic interpretation

$$PI = 100 - \frac{\text{(Replicate OD of test serum x 100)}}{\text{Median OD of Ca}}$$

The data expressed in OD values and PI values for the antigen control (Ca) and data expressed in PI values for the three other controls (C++, C+ and C-) was then used to determine whether or not the test had (been) performed within acceptable limits of variability and therefore, whether or not the test sera data would be accepted for any given microplate.

The replicate OD values of the Ca control were first compared to the UCL and LCL provided for in the Quality Assurance (QA) Information on the test kit before calculating the PI values. Both intermediate OD values (i.e. the two values that remained after discarding the lowest and highest values) had to fall within the limits. If not, the plate was then rejected. Only the two intermediate OD values were used for the calculation of the median Ca OD value and in subsequent PI calculations.

The diagnostic threshold for this assay was set at 50% inhibition (50% PI). If both replicate PI values of a test serum fell below 50 PI then that test serum was be considered to be negative. If either or both replicate PI values of a test serum fell above 50 PI, then that serum was tentatively considered positive and it was retested according to titration assay for confirmation and estimation of antibody titer. The ELISA Data Information (EDI) software was able to calculate the Percentage Inhibition (PI) of each sample and to determine the control and plate acceptance.

Table 3.4: Validity criteria for LPBE

Control	Upper Control Limit (UCL)	Lower Control Limit (LCL)
Ca (OD)	1.9	0.8
Ca (PI)	25	-25
C++ (PI)	100	85
C+ (PI)	85	50
C- (PI)	49	0

3.7 Measurement of agreement between AniGen and Priocheck NSP Elisa

The validation of AniGen and Priocheck FMD NSP Elisa (from Bionote® South Korea and Netherlands respectively) was done by subjecting a total of 500 fresh serum samples collected from coastal disease free zone to both test kits. The absorbance and percentage inhibition was read using ELISA Data Information (EDI) software. This was followed by counting the number of samples which were detected positive and those which turned negative for each test.

3.8 Data analysis

The results of both the NSP ELISA and Liquid phase blocking ELISA were then entered in an Excel spreadsheet (Microsoft Corp) with the following information; sampling location, age, sex, species, vaccination history, AniGen results and LPBE results. The data was imported to SPSS 20 version for analysis. Descriptive statistical analysis was then done to determine the proportion of positive samples and serotype distribution across the country and at county level. Graphs were drawn using Microsoft excel. The association between seropositivity versus age, sex and vaccination cover was determined using the Chi-square test. Measurement of agreement between the AniGen and Priocheck tests was done by calculating the Kappa statistic.

CHAPTER FOUR

4.0 RESULTS

4.1 Nonstructural protein results (AniGen)

Of the 4262 samples earlier collected, a total of 3709 were bovine and 180 were porcine samples. A total of 373 samples of both species were missing (Appendix 1)

4.1.1 Bovine results

4.1.1.1 Sex

A total of 3709 bovine serum samples were all subjected to NSP screening Elisa. There were more female bovine samples 70.4% (2611/3709) compared to males 29.6% (1098/3709) (Figure 4.1).

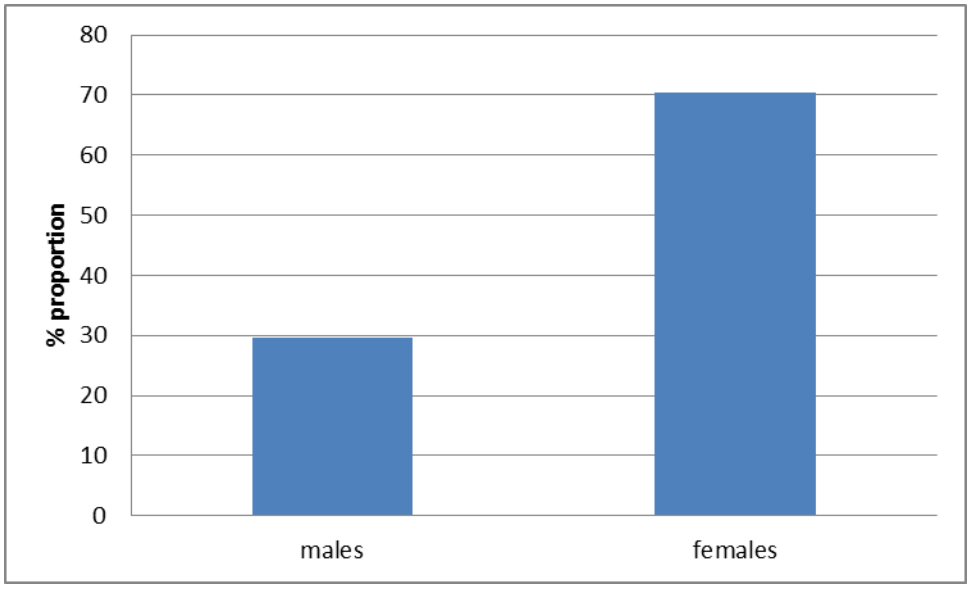


Figure 4.1: proportion of males and females

4.1.1.2 Age

The animal sera had been categorized into three (<1 years, 1-2 years and > 2 years). Samples from adult animals (>2 years) were the majority and accounted for 44.0% (1635/3709). Those of 1-2 years accounted for 28.4% (1052/3709) while serum samples belonging to age group less (<1 year) were 1022 representing 27.6% of the total samples (Figure 4.2).

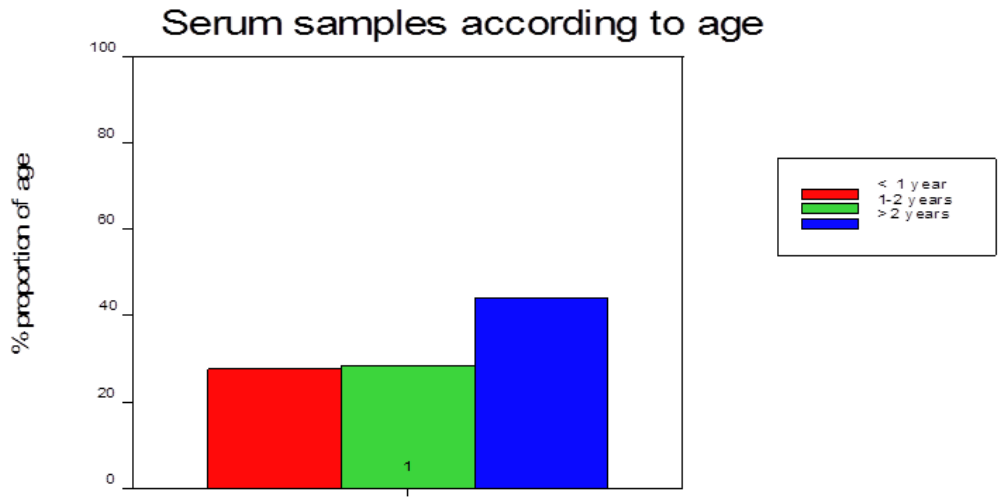


Figure 4.2: proportion of serum samples according to age

4.1.1.3 Vaccination status

Out of the 3709 serum samples, 'yes' were 522 representing 14.0%, 'no' were 2053 representing 55.4% and 'unknown' group were 1134 representing 30.6% [Figure 4.3 (a)]. A large number of animals were unvaccinated with the 'no' and the 'unknown' groups representing 86% [Figure 4.3 (b)]

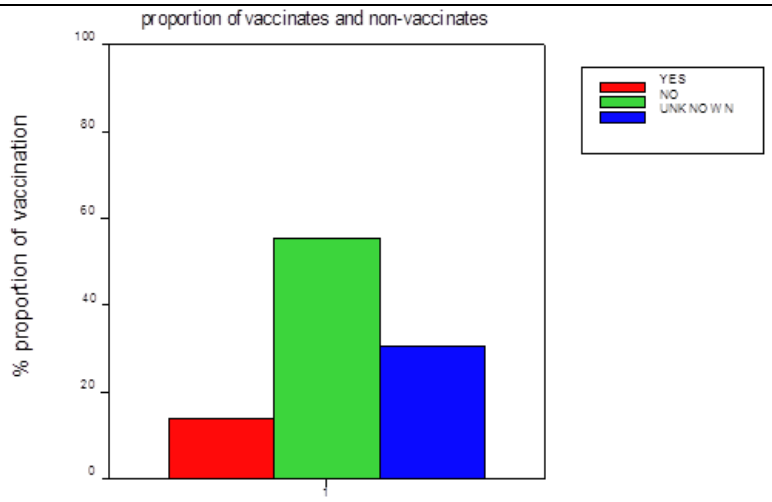


Figure 4.3 (a): vaccination status of serum samples

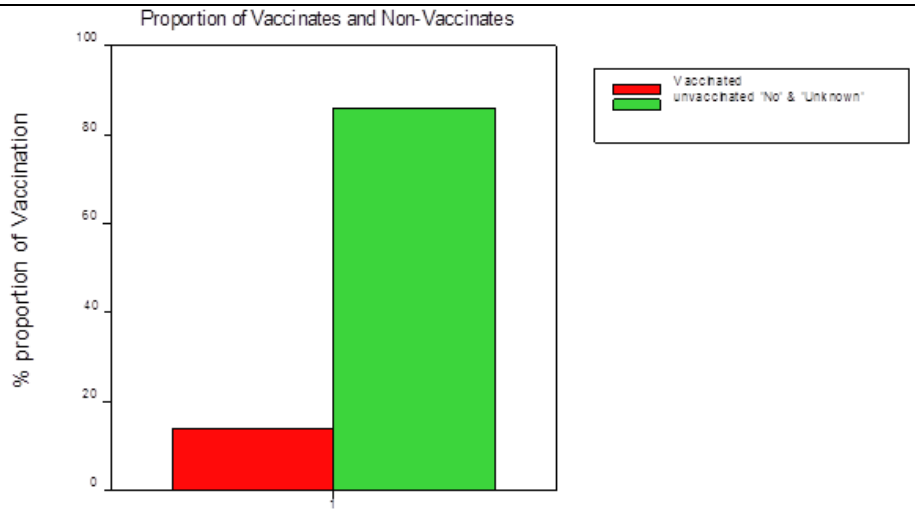


Figure 4.3 (b): proportion of animals vaccinated & those unvaccinated

4.1.1.4 Bovine FMD prevalence

Of the 3709 subjected to NSP screening test, 1947 were positive representing 52.5% while the other 1762 were negative representing 47.5% (Figure 4.4).

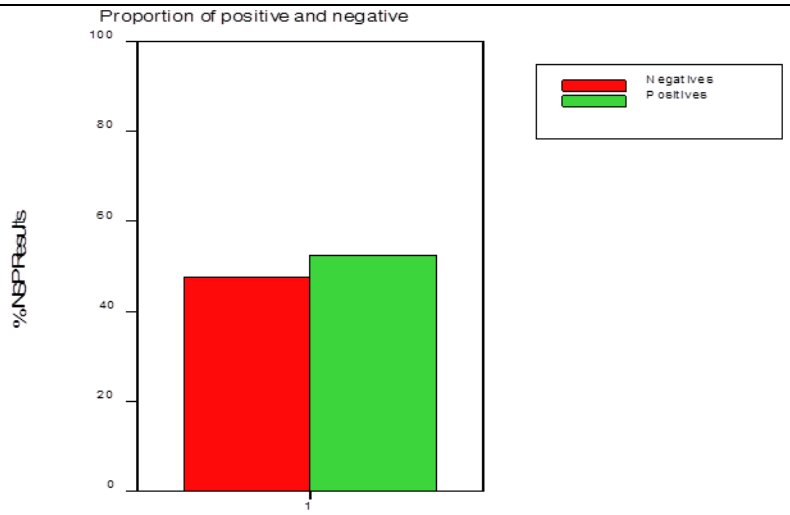


Figure 4.4: FMD NSP seroprevalence in bovines

Total Positives	**Total Negative**	**Mean Positive**	Mean Negative	Variance Positive	Variance Negative	S.D Positive	S.D negati
1947	**1762**	**49.923**	45.179	3252.862	3461.625	57.034	58.836
% total 52.5	**% total 47.5**						

The national bovine FMD seroprevalence was 52.5%. Among the counties that had the highest disease seroprevalence were those in western part of the country which included: Baringo, Elgeyo Marakwet, Uasin Gishu, Nandi, Trans Nzoia, Bungoma, Kakamega and West Pokot counties, all of which had 100% seropositivity. Bovine sera from Narok, Embu, Turkana, Migori, Garissa and Bomet counties showed high seropositivity of >70% (i.e. 90.4%, 82.9%, 80%, 75.6%, 72.9% and 70% respectively) (Figure 4.5)

Serum samples from Kajiado, Lamu, Tana River, Siaya, and Kisumu counties had average seropositivity of between 50 and 70% (i.e. 67.6%, 65.7%, 65.7%, 62.1% and 51.1% respectively). Counties which had seropositivity of between 30% and 50% included Laikipia 49.2%, Machakos 43.2%, Kwale 42.2%, Taita Taveta

40.2%, Samburu 40%, Tharaka Nithi 40%, Nyandarua 37.5%, Meru 35%, Isiolo 33.9% and Wajir 33% (Figure 4.5).

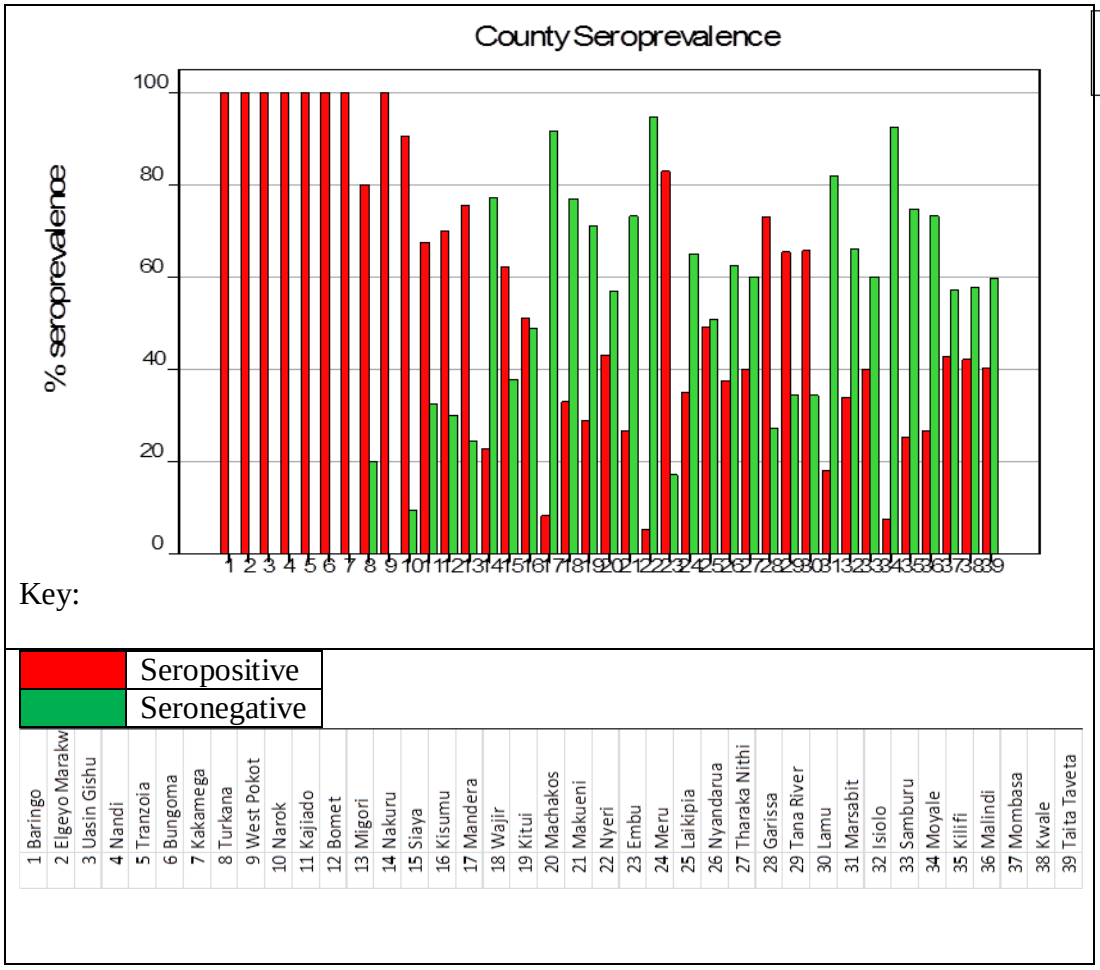


Figure 4.5: FMD prevalence across the 39 counties

The counties that had low seropositivity of <30% included Kitui with 28.8%, Makueni 26.8%, Malindi 26.7%, Kilifi 25.3% Marsabit 18% and Nakuru at 22.7%. The counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3%, 7.5% and 5.3% respectively. 30 counties had

seropositivity of more than 30% (>30%). This accounted of 76.9% of the total serum samples.

4.1.2 Porcine NSP results

A total of 180 porcine samples were analyzed using AniGen FMD NSP Ab screening Elisa. The samples were collected from 15 counties namely; Uasin Gishu, Nandi, Tranzoia, Bungoma, Kakamega, Bomet, Nakuru, Siaya, Kisumu, Nyeri, Embu, Meru, Kilifi, Mombasa and Taita Taveta. The proportion of females was higher than that of males. Females accounted for 72.8% (132/180) while males formed the other 27.2% (49/180) as shown in the figure below (figure 4.6)

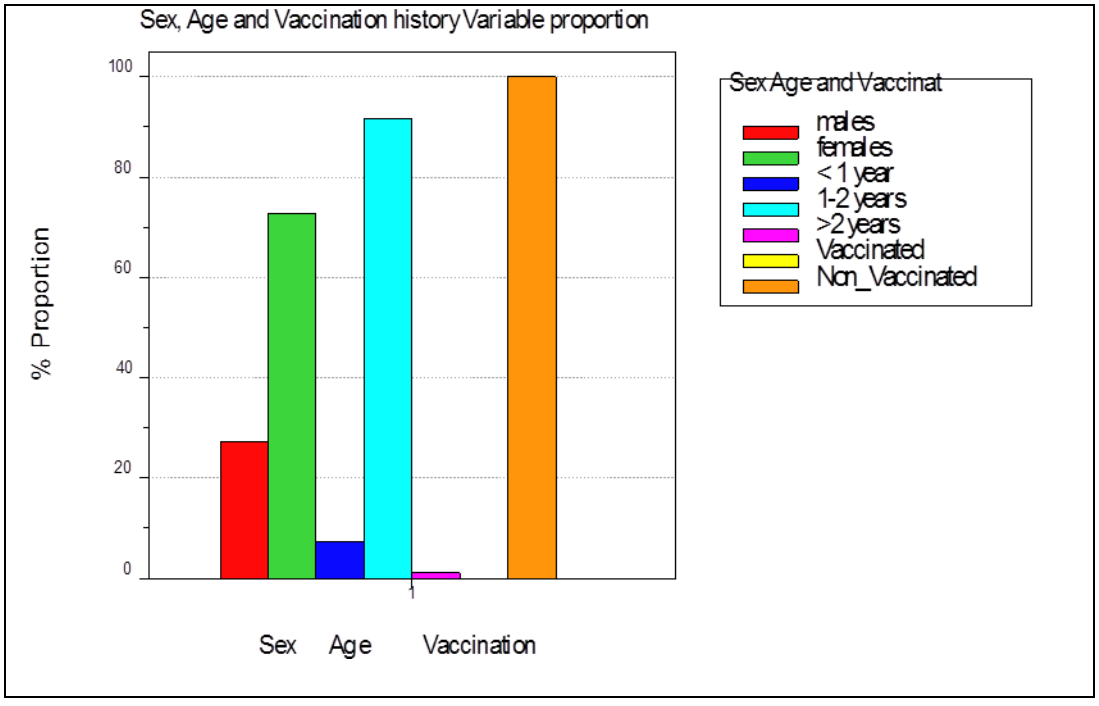


Figure 4.6: Status of serum samples of porcines

The proportion of 1-2 year age group accounted for 91.7% (165/180) and was the majority (Figure 4.6). Young animals of age group <1 years followed with 7.2% (13/180) while adult porcines of age group >2 years were few in number and accounted for only 1.1% of the total (2/180). All porcines sampled had no history of vaccination. This indicates that no FMD vaccination was carried out in porcines (figure 4.6)

4.1.3 Foot-and-mouth disease prevalence

The interpretation was done similar to bovine sera. Samples with Percentage Inhibition (PI) value of above 50 (i.e. ≥ 50.0) on Anigen FMD NSP Ab Elisa were regarded as positive while samples with PI value of less than 49 (i.e. <50.0) were interpreted as negative result.

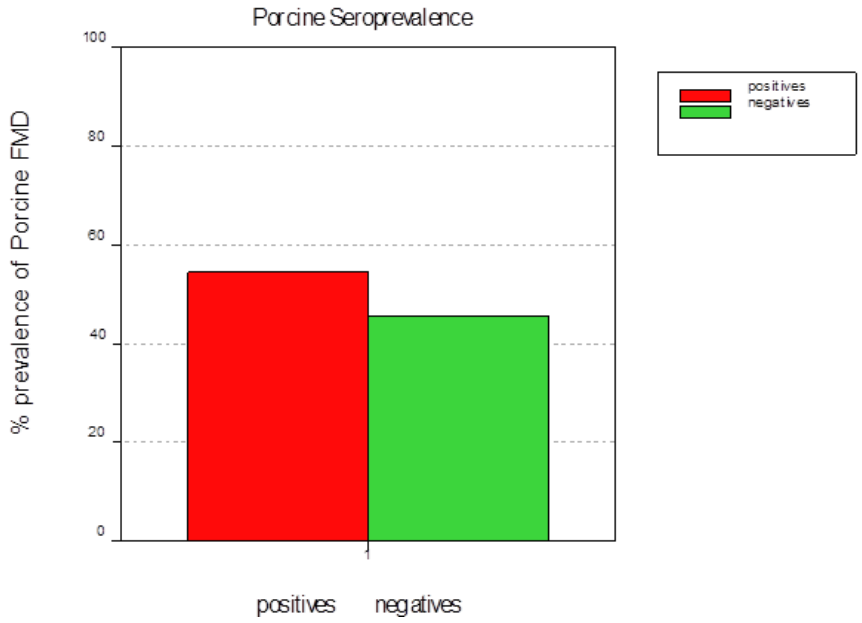


Figure 4.7: Foot and mouth disease seroprevalence in porcines

Of the 180 samples subjected to NSP screening test, 98 were interpreted as positive representing 54.4% while the other 82 samples turned negative representing 45.6% (Figure 4.7). FMD prevalence was higher than that of bovines.

The following counties; Uasin Gishu, Nandi, Trans Nzoia, Bungoma and Kakamega had 100% seropositivity. Porcine sera from Siaya, Kisumu and Taita Taveta counties had prevalence of more than 50% (i.e. 75%, 75% and 62.5% respectively). Porcine sera from Nakuru, Nyeri, Meru, and Mombasa counties had low seropositivity (<50% i.e. 36.8%, 21.2%, 12.5% and 37.5% respectively) while porcine serum from Embu County showed 50% FMD prevalence. FMD antibodies were not detected in samples from Bomet and Kilifi (Figure 4.8).

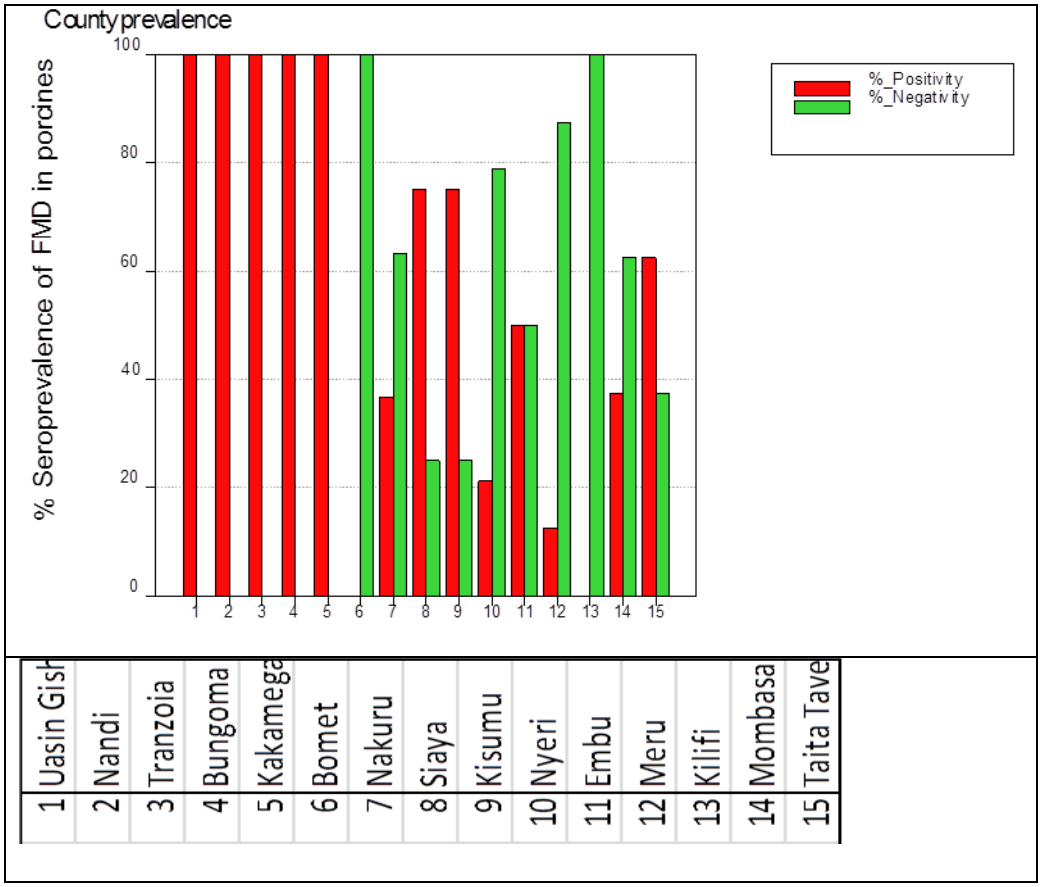


Figure 4.8: Porcine foot and mouth disease prevalence in 15 counties

4.2 Liquid Phase Blocking Elisa results

Serotype SAT 1 was the most prevalent serotype nationally with 50.9% prevalence rate (376/738). It was closely followed by serotype type C with 48.9% (361/738). Serotype A had a national seroprevalence of 37.3% (276/738). SAT 2 had a seroprevalence of 36% (266/738) while serotype O had a national prevalence of 30.9% (228/738) (Figure 4.9).

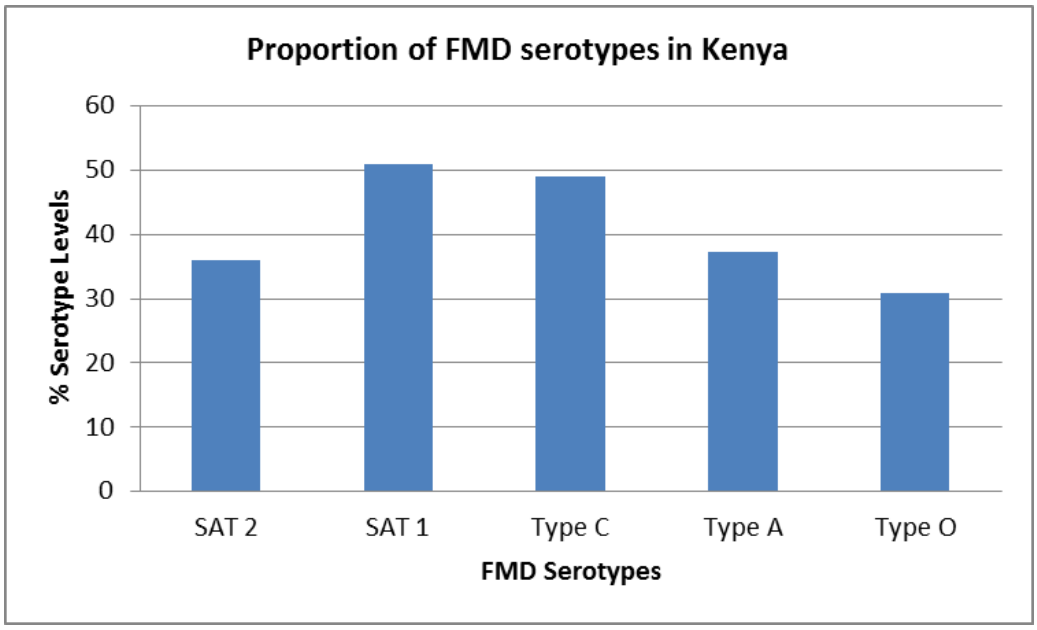


Figure 4.9: National serotype prevalence

Of all the animals that tested positive, (165/1383, 11.9%) showed two serotypes circulating while those with three serotypes were (152/1383, 11.0%). Those with one serotype and four serotypes circulating were (10%, 138/1383) and (97/1383, 7%) respectively. The rest 2.8% of the animals showed 5 serotypes (Table 4.2).

Table 4.2: Number of foot and mouth disease positive cases in Kenya

	Number of serotypes	Frequency	Percentage
	5	39	2.8
	4	97	7
	3	152	11
	2	165	11.9
	1	138	10
	0	147	10.6
Total positive		738	53.3
Total Negative		645	46.7
	Total	1383	100

Of the 39 counties, 35.9% (14/39) had animals with 5 serotypes, 71.8% (28/39) of the animals analyzed had 4 serotypes while 87.2% (34/39) counties had animals with 3 serotypes. 34/39 counties also had animals with 2 serotypes detected representing 87.2% while 33/39 (84.6%) counties had animals with 1 serotype detected. 33 of the counties had at least one or more samples not indicating the presence of any serotype (figure 4.10).

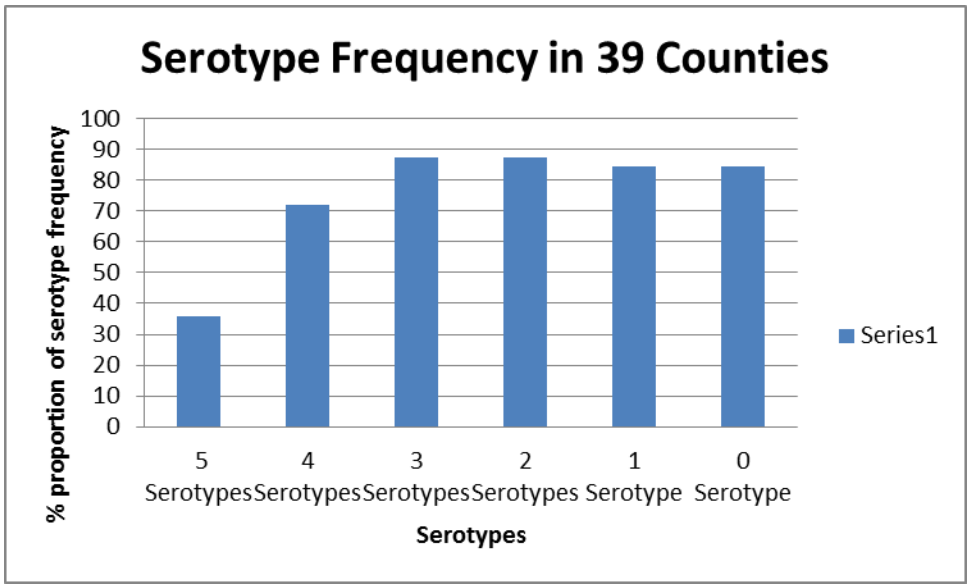


Figure 4.10: Foot-and-mouth disease serotype frequency in Kenya

All sera from the 39 counties subjected to serotype titration indicated the presence of SAT 1 (100%). This was closely followed by Type C with 92.3% (36/39 counties), Type A was present in 34/39 counties representing 87.2%. Serotype O and serotype SAT 2 were detected in 33 and 32 counties respectively representing 84.6% and 82% coverage (Figure 4.11 below).

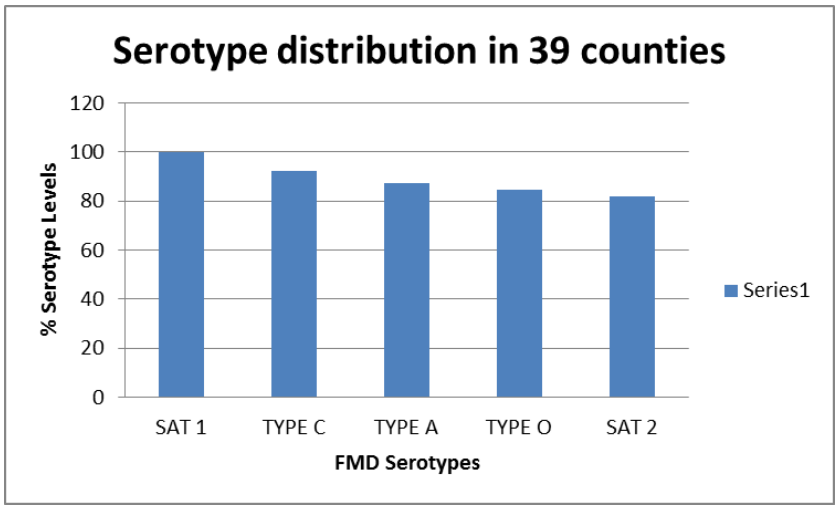


Figure 4.11: Serotype distribution across the country

4.3 Testing for association

4.3.1 Seropositivity versus Sex

The total number of animals sampled and subjected to NSP was 3709 with the majority being females (2611/3709, 70.4%) (Table 4.3). Males were 1098 representing 29.6%. Of the 1383 samples selected, 30% (415/1383) were males while females accounted for the rest 70% (967/1383). 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 whereas 22% (49/220) tested negative.

Table 4.3: Percentage positive cases per serotype per sex

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

Eighty one percent (420/518) of all female animals tested positive for at least one serotype whereas 19% (98/518) tested negative. On chi-square test, there was no significant association between sex and level of seropositivity (p=0.063) [Table 4.4]. Majority of animals tested had two serotypes detected (22.8%, 168/738) with a higher number of these serotypes found in females (16.7%, 123/738) against males 6.1%. Animals with 3 serotypes in each serum sample followed with 20.6%. Those with single serotype came in third with 18.2% while samples with 4 serotypes were 13.4%. Of significance was the 5.3% of animals that had all the five serotypes (Table 4.3). The females accounted the highest in intersex frequency ratio.

Table 4.4 Chi-square test: seropositivity versus sex

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1058.850[a]	990	.063
Likelihood Ratio	247.483	990	1.000
Linear-by-Linear Association	25.263	1	.000
N of Valid Cases	39		

4.3.2 Seropositivity versus Age

Of the total 738 sera samples titrated for FMD serotypes, 30.5% were those of age <1 years, 27.5% (203/738) between the age 1-2 years while the rest 42% (310/738) were adults of age >2 years. 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 (Table 4.5). On chi-square test, there was significant association between age and seropositivity (p=0.002) (Table 4.6). Adult animals had a higher positive reaction to at least one serotype compared to younger animals.

Table 4.5: Percentage positive cases per serotype per age group

	Age-group			
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%

Table 4.6 Chi-square test: seropositivity versus age-group

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	770.120[a]	660	.002
Likelihood Ratio	214.247	660	1.000
Linear-by-Linear Association	22.074	1	.000
N of Valid Cases	39		

4.3.3 Seropositivity versus vaccination history

Of the total 738 serum samples subjected to serotype titration, only 14.9% (110/738) had history of vaccination (vaccinated), 43% (317/738) did have any history of vaccination (non-vaccinated) while 42.1% (311/738) of the animal samples had unclear vaccination history (unknown). 96/591 animals representing 16.2% of the animals vaccinated 'vaccinated group' showed at least one or more serotype, 41.6% of animals not vaccinated (246/591) had at least one or more serotype detected while 42% (249/591) of animals without a known history of vaccination had at least or more detectable serotype (Table 4.7). On chi-square test, there was significant association between serotype level and vaccination history (p=0.048) indicating that the serotype level increases with the absence of vaccination (Table 4.8).

Table 4.7: Percentage positive cases per age group

Vaccination history

SEROTYPES LEVEL	YES	NO	UNKNOWN	Total
0	14	71	62	147
1	19	49	67	135
2	27	79	62	168
3	22	60	70	152
4	17	49	31	97
5	11	9	19	39
≥ 1 serotype	96	246	249	591
% level	16.20%	41.60%	42.00%	80.00%
TOTAL	110	317	311	738
% Total	14.90%	43.00%	42.10%	100%

Table 4.8 Chi-square test: seropositivity versus vaccination status

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	658.970[a]	600	.048
Likelihood Ratio	192.067	600	1.000
Linear-by-Linear Association	5.034	1	.025
N of Valid Cases	39		

4.4 FMDV seroprevalence and serotype distribution in each county

4.4.1 Baringo County

A total of 63 bovine samples from Koibatek and Baringo Districts in Baringo county were analyzed, of those 28.6% (18/63) were males and the rest 71.4%

(45/63) were females. A higher percentage of animals were those below the age of 1 year comprising 36% (23/63) while those between 1-2 years and as well as those >2 years comprised 32% (20/63) each. Only 4.8% (3/63) of all animals sampled indicated that they were vaccinated, 12.7% (8/63) were non-vaccinated while a higher number of them 82.5% (52/63) had unclear history of vaccination. The vaccination coverage was very low (Figure 4.12).

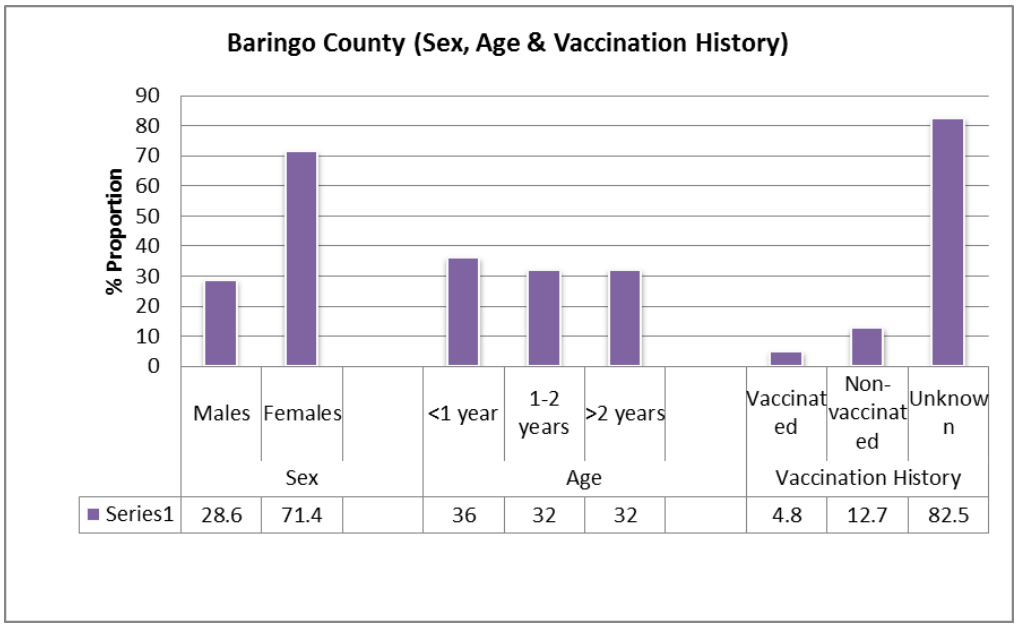


Figure 4.12: Sample status from Baringo

All the 63 sera samples subjected to NSP indicated seropositivity. Of the 41 samples randomly selected for serotype titration using LPBE, 3 (7.3%) of them did not indicate any circulating serotype while 38 (92.7%) indicated at least one or more serotype. 7/41 (17.1%) animals had one circulating serotype, 13/41 (31.7%) had 2 circulating serotypes, 17/41 (41.5%) had 3 serotypes while only one animal

had 4 serotypes circulating. A high percentage of animals had 3 serotypes circulating (Figure 4.13).

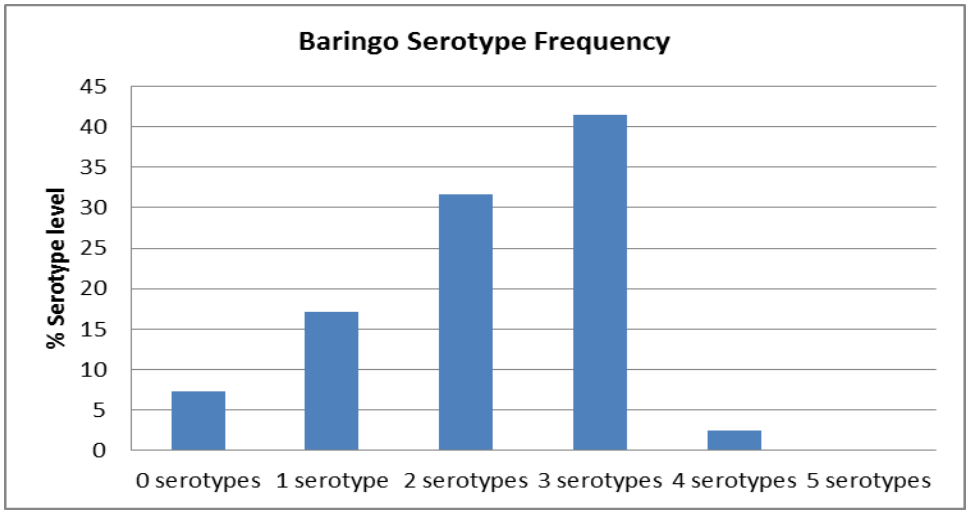


Figure 4.13: Serotype frequency Baringo

Of those with at least one or more serotypes, 28.9% (11/38) were males while 71.1% (27/38) were females. 42.1% (16/38) were of age <1 year, 26.3% (10/38) between 1-2 years while the rest 12/38 (31.6%) were adults >2 years of age. 7.9% of them (3/38) were vaccinated, 13.2% (5/38) were not vaccinated while 78.9% (30/38) indicated unknown vaccination status (Figure 4.14).

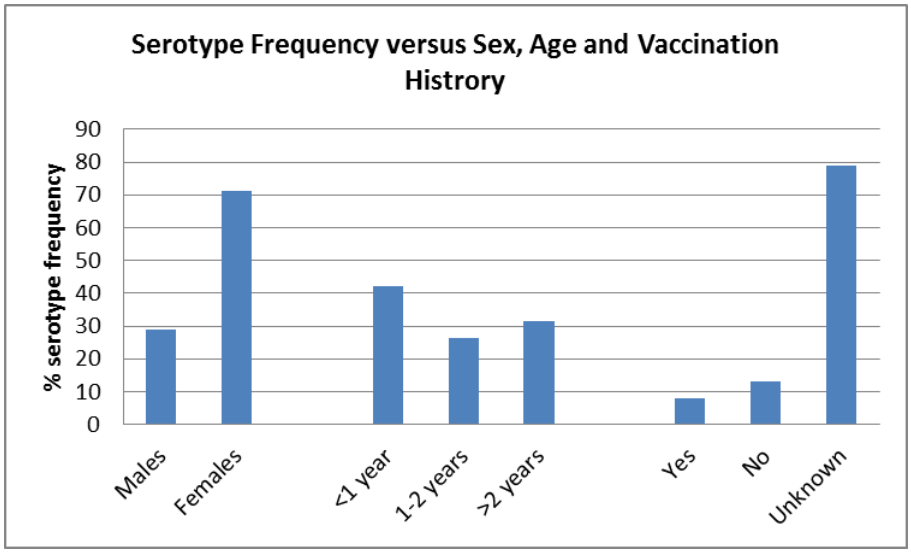


Figure 4.14: proportion of animals with at least one or more serotype

Serotype C was the most prevalent serotype with 85% (35/41) and was followed closely by serotype A with 78% (32/41). 16 animals of the total 41 representing 39% indicated the presence of SAT 1 while only 12.2% (5/41) indicated the presence of serotype O. Serotype SAT 2 was not detected in Baringo county (Figure 4.15).

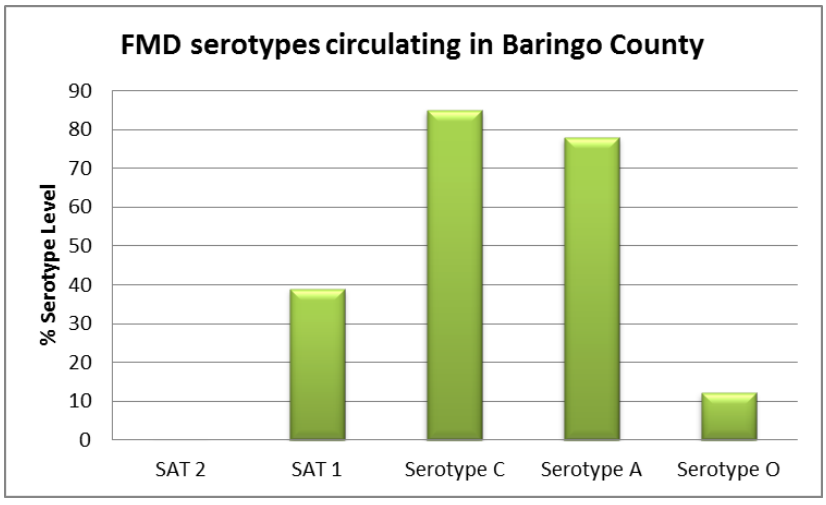


Figure 4.15: Serotype levels in Baringo

4.4.2 Elgeyo-Marakwet County

Of the 14 animals sampled, females were 57% (8/14) while the rest 43% (6/14) were males. Animals >2 years and those between 1-2 years of age were the majority comprising 35.7% (5/14) per age group. Only 4/14 animals (28.6%) were aged <1 year (Figure 4.16) and the samples indicated unknown history of vaccination.

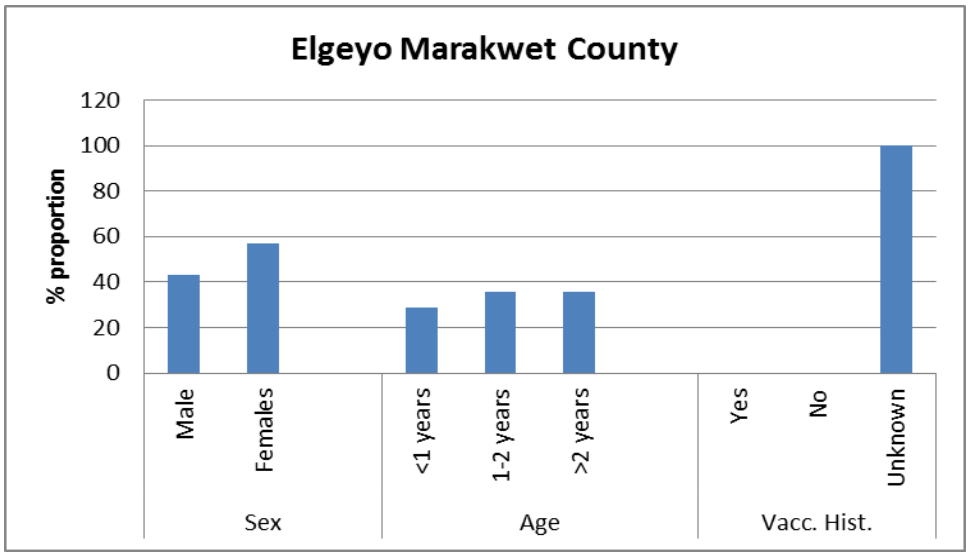


Figure 4.16: Serum sample status of Elgeyo Marakwet

FMD seroprevalence was 100%. All the samples subjected to LPBE titration indicated at least one or more circulating serotypes. 42.9% (6/14) had a single serotype circulating, 14.2% (2/14) of animals had two circulating serotypes while those with three serotypes were 42.9% (6/14) (Figure 4.17).

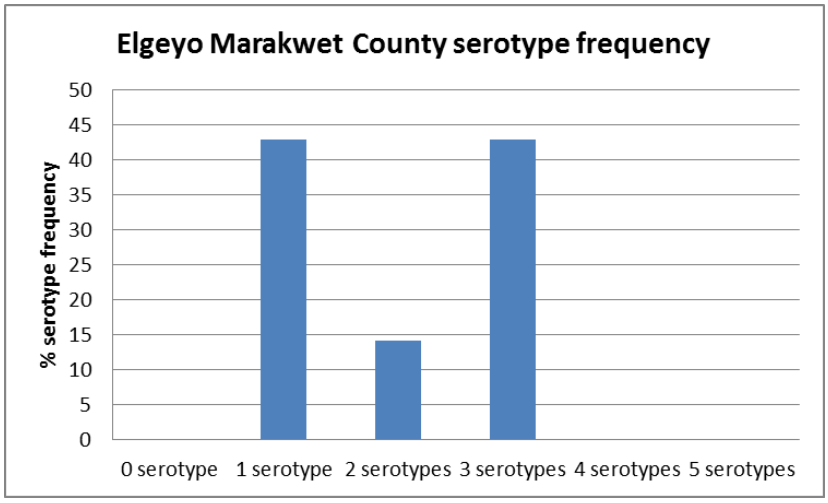


Figure 4.17: Serotype frequency in Elgeyo Marakwet

The serotype frequency was higher in females than in males. 57.1% (8/14) of the females were positive to at least one or more serotype compared to males 42.9% (6/14). Adult animals >2 years had a higher number of serotypes detected at 42.9% (6/14) compared to those of 1-2 years that had 35.7% (5/14). Animals of <1 year had the least serotype frequency at 21.4% (3/14) (Figure 4.18). All the animals had unknown history of vaccination.

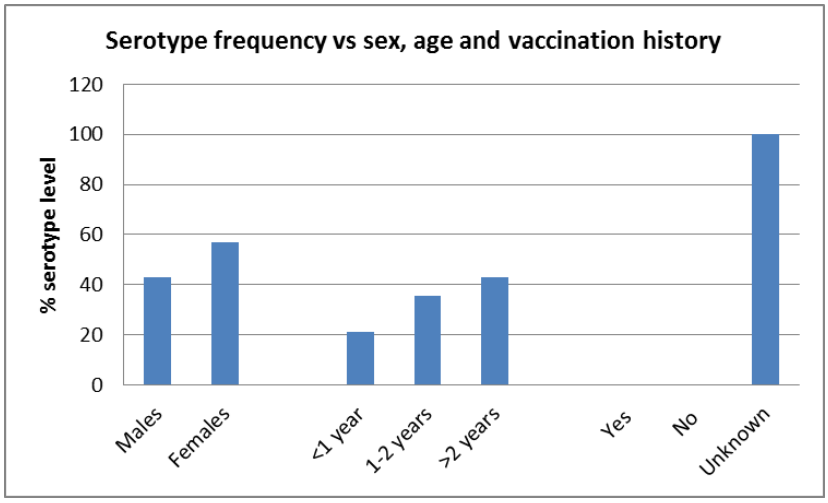


Figure 4.18: Proportion of animals with at least one or more serotype

The most prevalent FMD serotype was serotype C with 78.6% (11/14). Of the 14 serum samples, 10 of them were positive to serotype A, the second most prevalent serotype with 71.4%. Serotype SAT 1 was detected in 50% (7/14) of the samples. SAT 2 and serotype O was not detected in any of the samples analyzed (Figure 4.19).

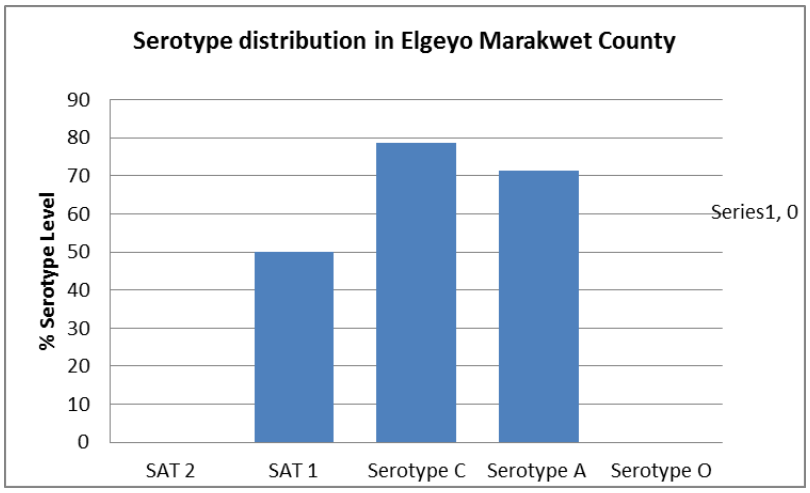


Figure 4.19: Serotype levels in Elgeyo Marakwet

4.4.3 Uasin Gishu County

A total of 60 animals were sampled in Uasin Gishu county for FMD virus analysis. 41.7% (25/60) of those animals were males while 58.3% (35/60) were females. A slightly higher number of samples were those of age <1 year with 35% (21/60) followed by adults > 2 years with 33.3% (20/60) while animals of between 1-2 years were 31.7% (19/60). The reported vaccination coverage was 33.3% (20/60). 11.7% (7/60) were unvaccinated while a higher number 55% (33/60) had unknown vaccination status (Figure 4.20).

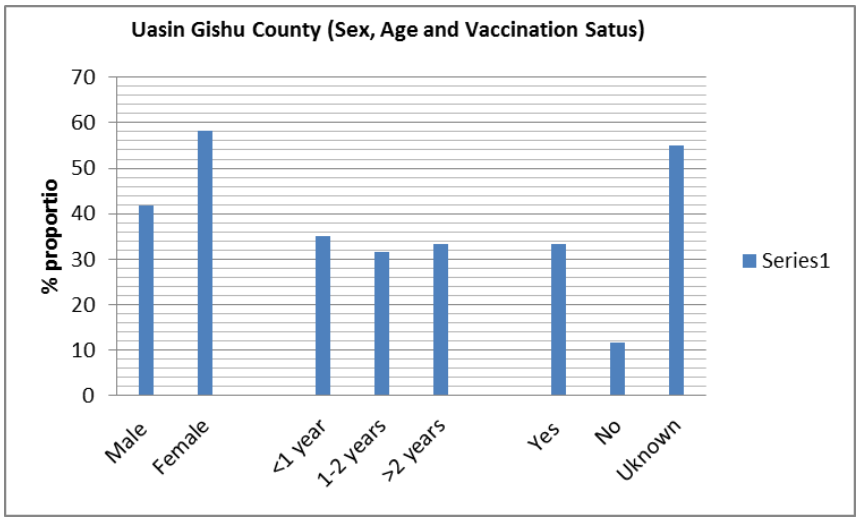


Figure 4.20: Serum sample status of Uasin Gishu

FMD seroprevalence was 100%. Animals with one or more serotypes accounted for 97.5% (39/40). 17.9% (7/39) had single serotype circulating while 18/39 (46.1%) had two serotypes. Animals with three serotypes were 6 and accounted for 15.4% while those with four circulating serotypes made up the rest 20.5% (8/39). Animals with two circulating serotypes were the majority (Figure 4.121).

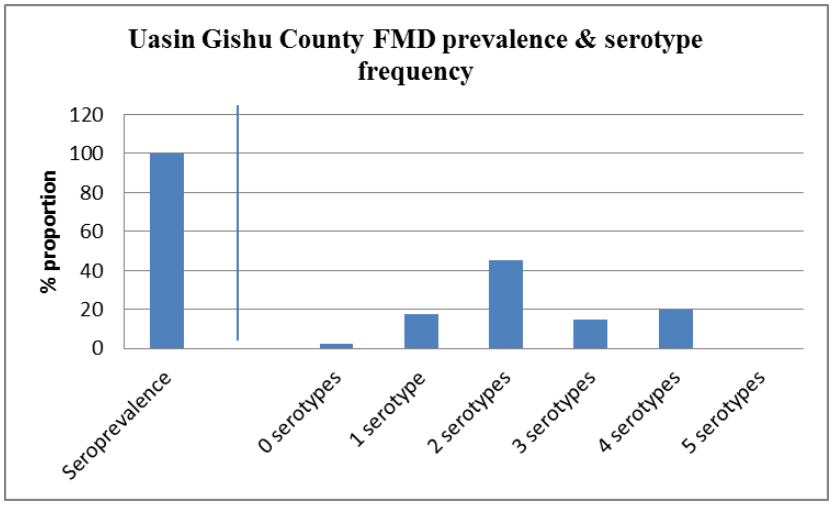


Figure 4.21: Prevalence and serotype frequency levels

According to serotype frequency level results, females accounted for 62.5% (25/40) compared to males 37.5% (15/40). Adult animals (>2 years) had high serotype levels at 37.5% (15/40) compared to those of <1 year which had 35% (14/40) and those between 1-2 years comprised of 27.5% (11/40). Animals with unknown vaccination status comprised 45% (18/40) serotype level while the vaccinated ones comprised 32.5% (15/40) with the least being animals with 17.5% (7/40) Figure 4.22).

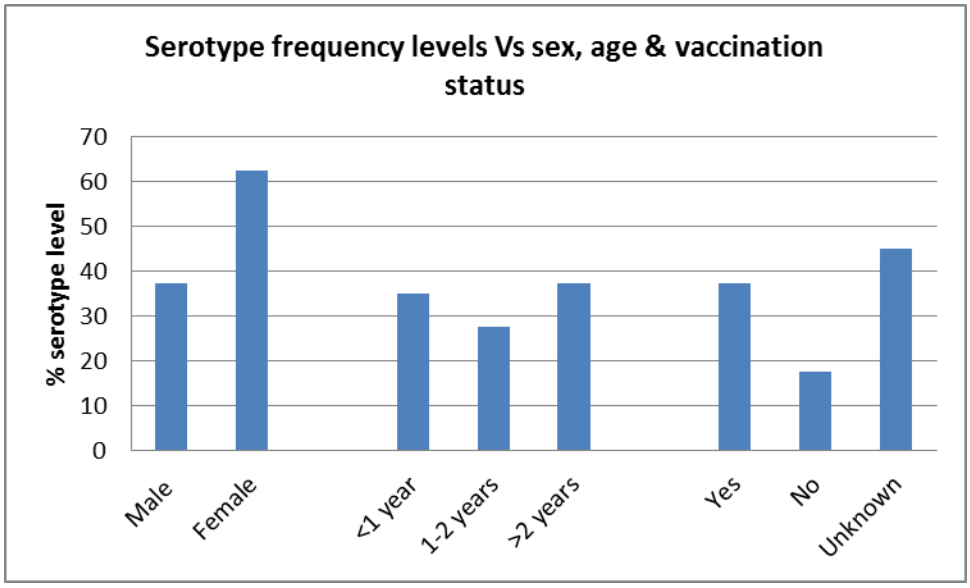


Figure 4.22: proportion of animals with at least one or more serotype

Serotype C was the most prevalent serotype with 84% seroprevalence (34/40). SAT 1 was the second highest prevalent serotype with 50% (20/40) while serotype O was third with 45% (18/40). Serotype A was fourth with detection rate of 35% (14/40). SAT 2 had the least prevalence with 17.5% (7/40) (Figure 4.23).

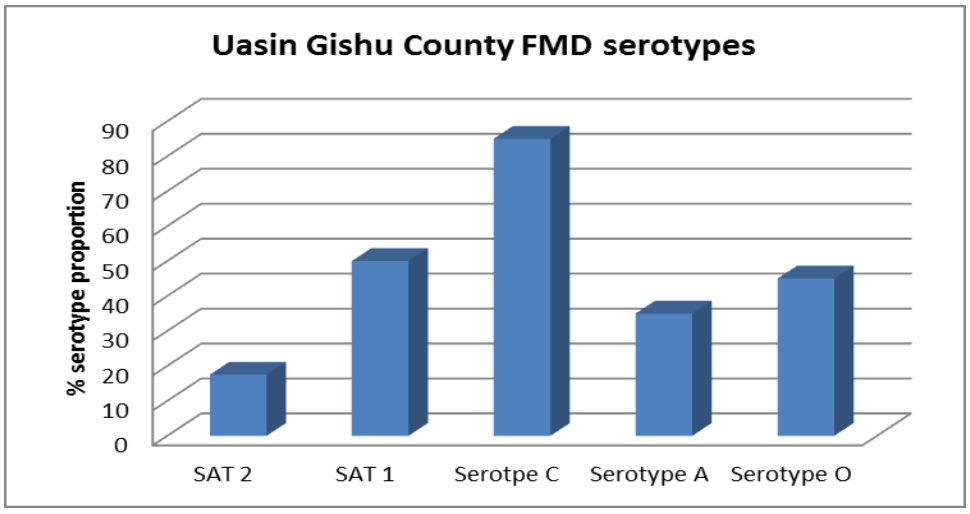


Figure 4.23: Serotype prevalence in Uasin Gishu

4.4.3.1 Foot-and-mouth disease in porcines

19 pigs were sampled in the districts of Uasin Gishu County. None of them had history of FMD vaccination. All the pigs sampled were of 1-2 years of age. All the 19 pigs turned seropositive when subjected to FMD NSP screening test (similar to bovines) (Figure 4.24).

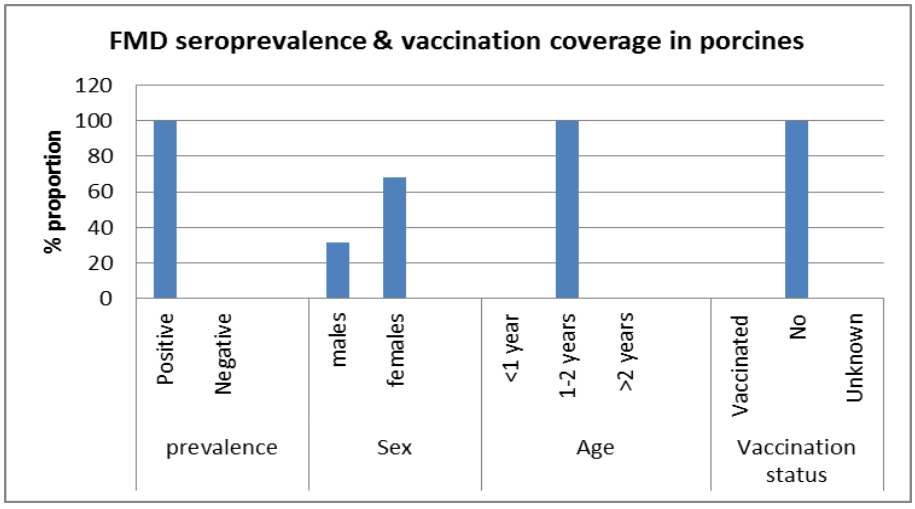


Figure 4.24: Porcine foot and mouth disease prevalence in Uasin Gishu

4.4.4 Nandi County

The males were 13.3% while the rest 86.7% (13/15) were females. All the age groups (<1 year, 1-2 years & >2 years) were equally sampled with each group having 5 animals. A higher number of animals 66.7% (10/15) had unknown vaccination status, 20% (3/20) of them were vaccinated while the rest 13.3% (2/15) were unvaccinated. The reported vaccination coverage was 20% (Figure 4.25).

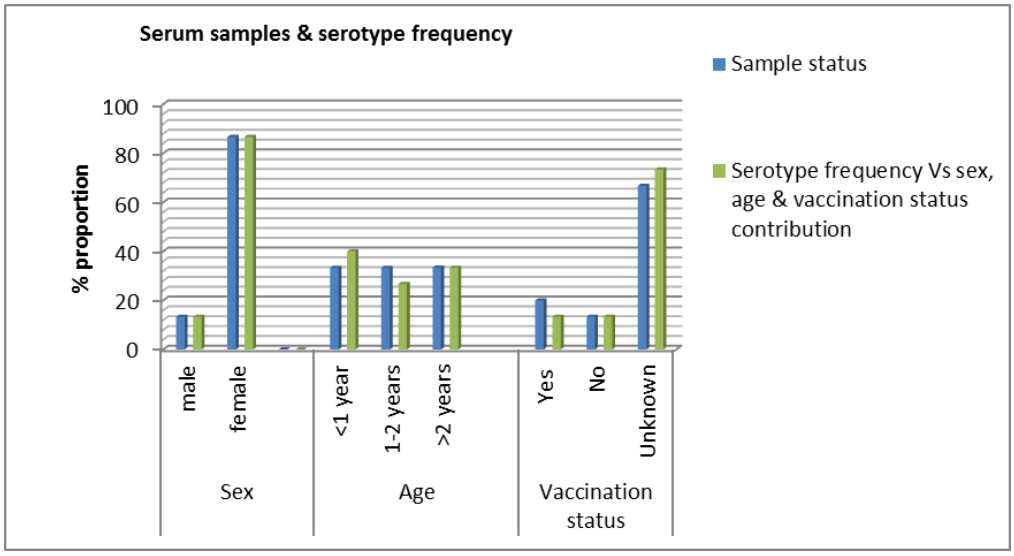


Figure 4.25: Sample status and proportion of animals with at least one or more serotypes

FMD seroprevalence was 100%. 93.3% of the total serum samples subjected to serotype titration had at least one or more serotypes. 50% (7/14) had two serotypes circulating while 5 animals (35.7%) had single serotype (Figure 4.26). A higher proportion of serotype levels were those that belonged to females with 86.7% (13/15) compared to males 13.3% (2/15). Despite equal number of animals

sampled within each age group, young animals of <1 year had a higher proportion of serotype levels with 40% (6/15) compared to adults (>2 years) with 33.3% (5/15) and middle aged group (1-2 years) with 26.7% (4/15). Animals of unknown vaccination status had higher serotype frequency level at 73.4% (11/15) compared to the vaccinated and unvaccinated group which had 13.3% (2/15) (Figure 4.25)

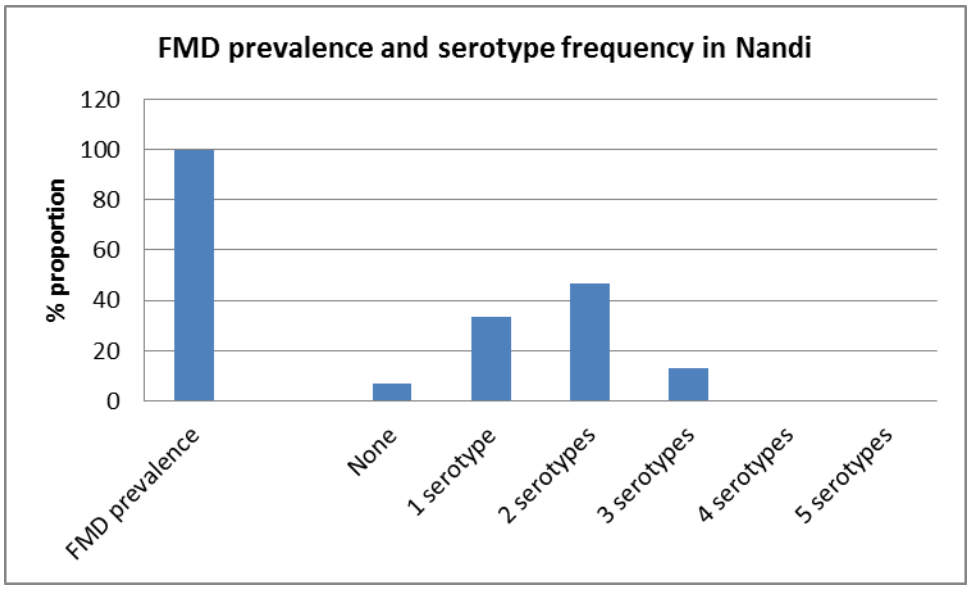


Figure 4.26: FMD prevalence and serotype frequency

Serotype C was the most prevalent serotype with a seroprevalence of 80% (12/15). It was followed by serotype SAT 1 with 40% prevalence (6/15), serotype O with 33.3% (5/15) while serotype A had the least prevalence with 13.3% (2/15). Serotype SAT 2 was not detected (Figure 4.27).

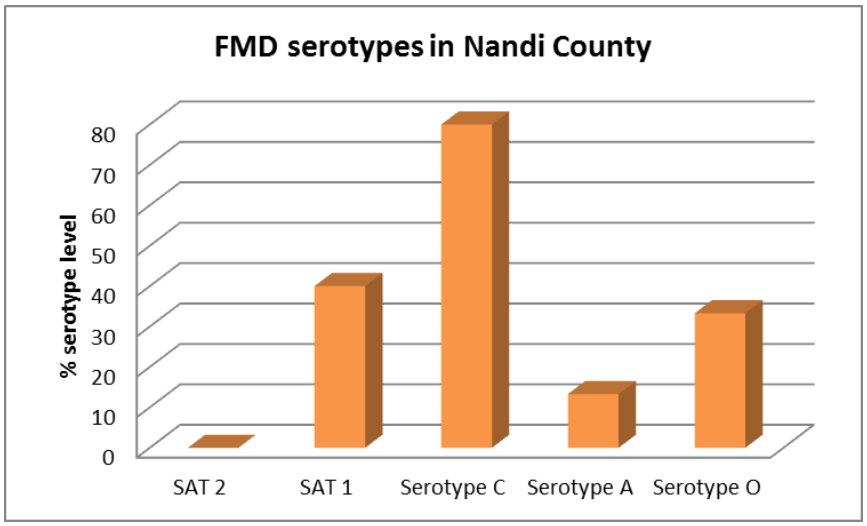


Figure 4.27: FMD serotype levels

4.4.4.1 Foot-and-mouth disease in porcines

All the 10 porcine samples were reportedly unvaccinated and were all seropositive. The seroprevalence in porcines was comparable to that of bovines from the same county. 90% of the porcines sampled (9/10) were of the age group 1-2 years (Figure 4.28).

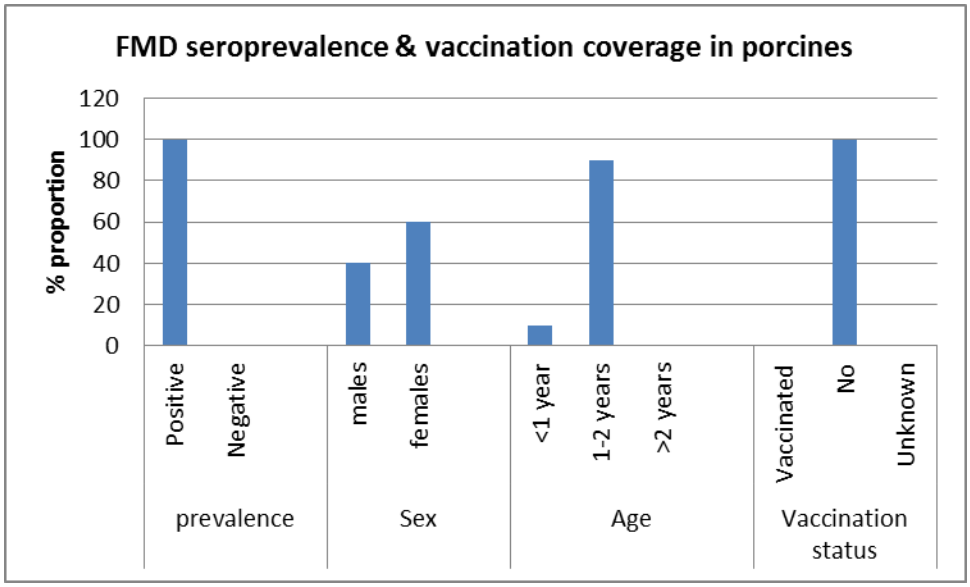


Figure 4.28: porcine FMD prevalence in Nandi

4.4.5 Trans-Nzoia County

The reported vaccination coverage in Trans-Nzoia county was high at 73.3% (11/15) compared to other counties of the North Rift. Females samples were more at 66.7% (10/15) compared to males 33.3% (5/15) (Figure 4.29).

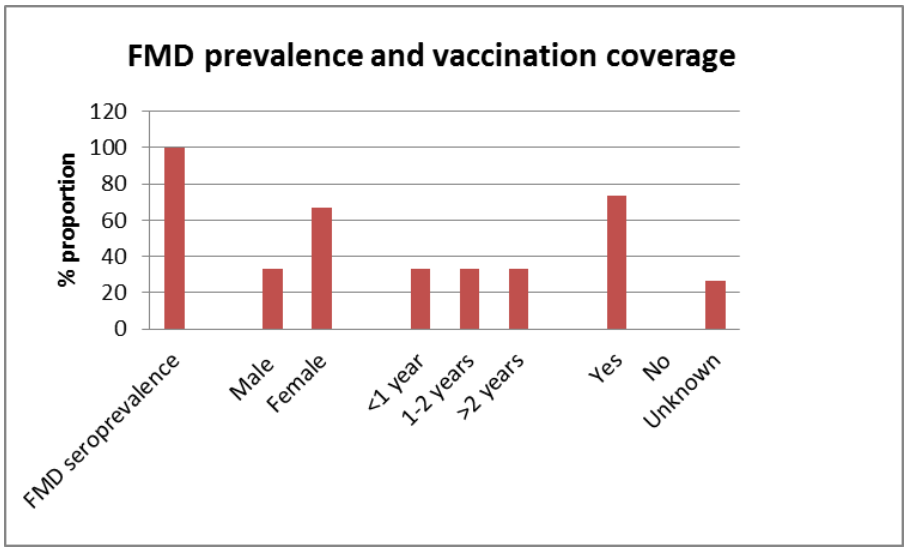


Figure 4.29: FMD seroprevalence and vaccination cover

All animals sampled showed a very high seropositivity (100%) (Figure 4.29). 80% (12/15) of the animals had at least one or more serotype. Animals with single serotypes circulating were 40% of the total samples (6/15) while those with two serotypes were 33.3% (5/15) (Figure 4.30).

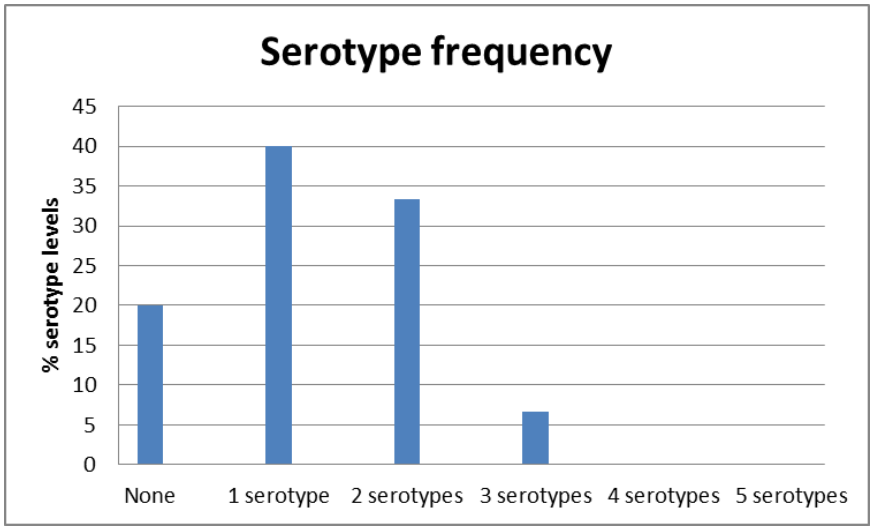


Figure 4.30: Foot and mouth disease serotype frequency

Serotype C had significantly higher prevalence at 66.7% (10/15) compared to SAT 1 and serotype A which had percentage prevalence of 20% each. Serotype O was the least prevalent strain with 13.3% (2/15). Serotype SAT 2 was not detected (Figure 4.31).

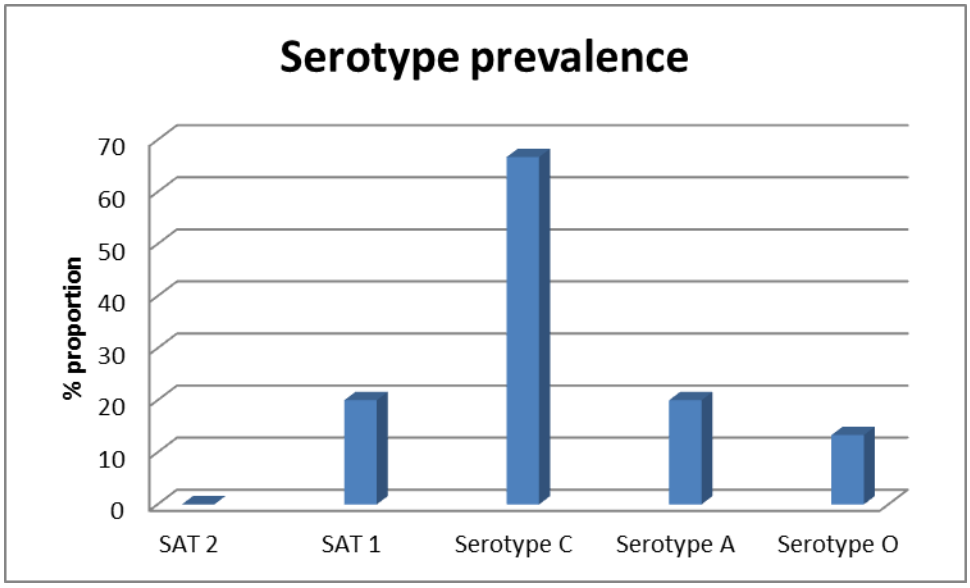


Figure 4.31: serotype prevalence

4.4.5.1 Foot-and-mouth disease in porcines

All porcines sampled were unvaccinated. 87.5% (7/8) were of age 1-2 years. All the samples were seropositive to FMD NSP screening test (Figure 4.32). The FMD prevalence rate was the same as that of the bovines.

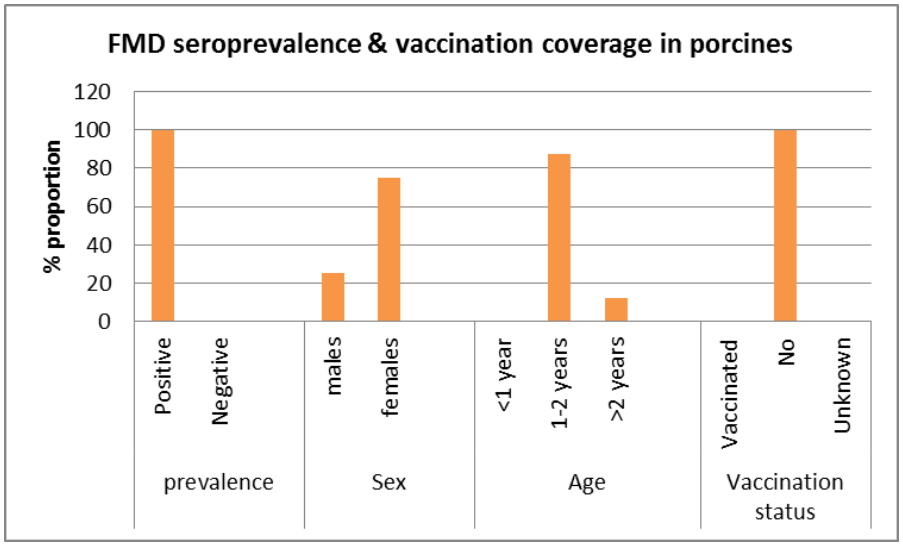


Figure 4.32: Porcine FMD prevalence

4.4.6 Bungoma County

All animals sampled had no history of FMD vaccination. The FMD seroprevalence was very high at 100% (Figure 4.33 below).

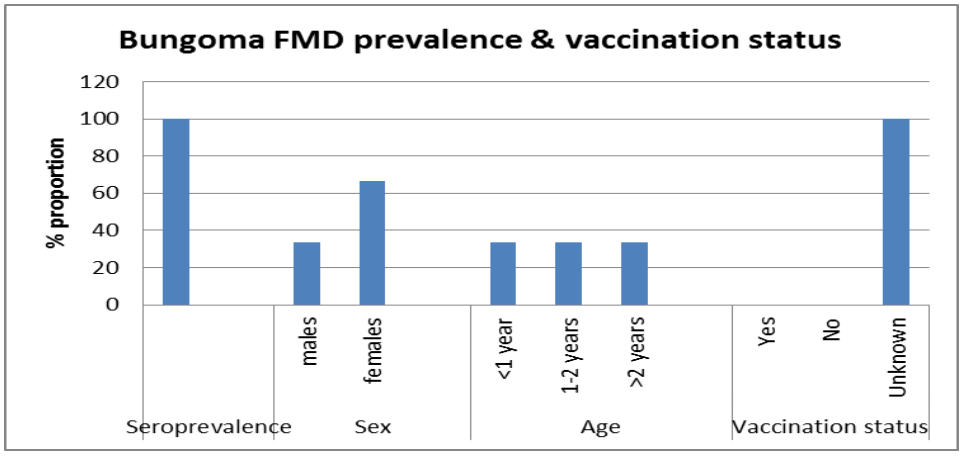


Figure 4.33: FMD prevalence and vaccination cover in Bungoma

50% (7/15) of the animals had at least one or more serotype. Animals with two serotypes were 33.3% (5/15) while those with single circulating serotype made up 13.3% (2/15). Serotype C and serotype A were the most prevalent serotypes with 46.7% (7/15) and 40% (6/15) respectively. Serotype SAT 2 was not detected in all the samples while SAT 1 and O had the least prevalence at 6.7% each (Figure 4.34).

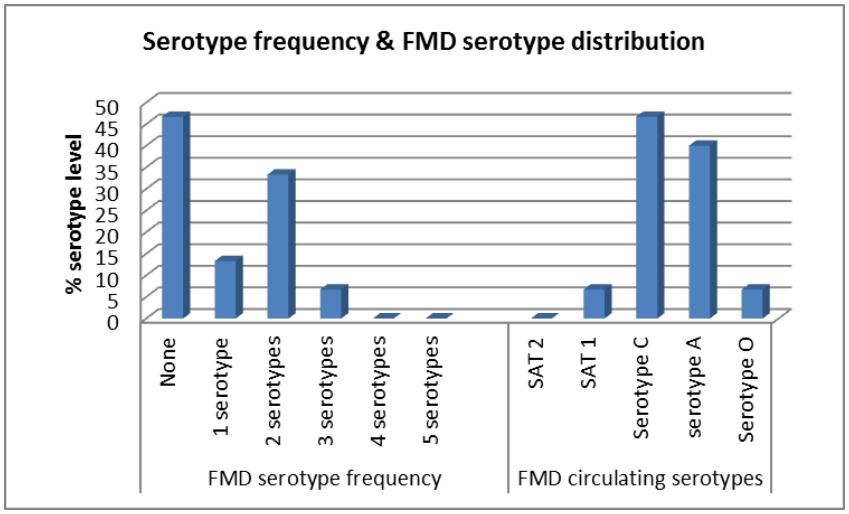


Figure 4.34: Serotype frequency and serotype distribution

4.4.6.1 Foot-and-mouth disease in porcines

All the porcines sampled were of age 1-2 years. None had been reported vaccinated against FMD virus. The prevalence of FMD was 100% (Figure 4.35).

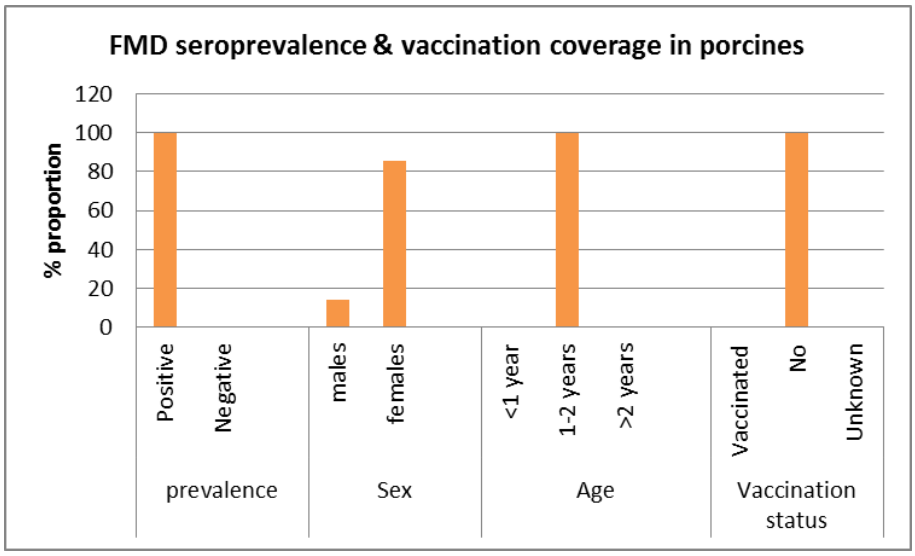


Figure 4.35: FMD seroprevalence in porcines

4.4.7 Kakamega County

According to the farmers interviewed in Kakamega county, no vaccination against FMD had been carried out. There was a very high FMD prevalence of 100% (Figure 4.36).

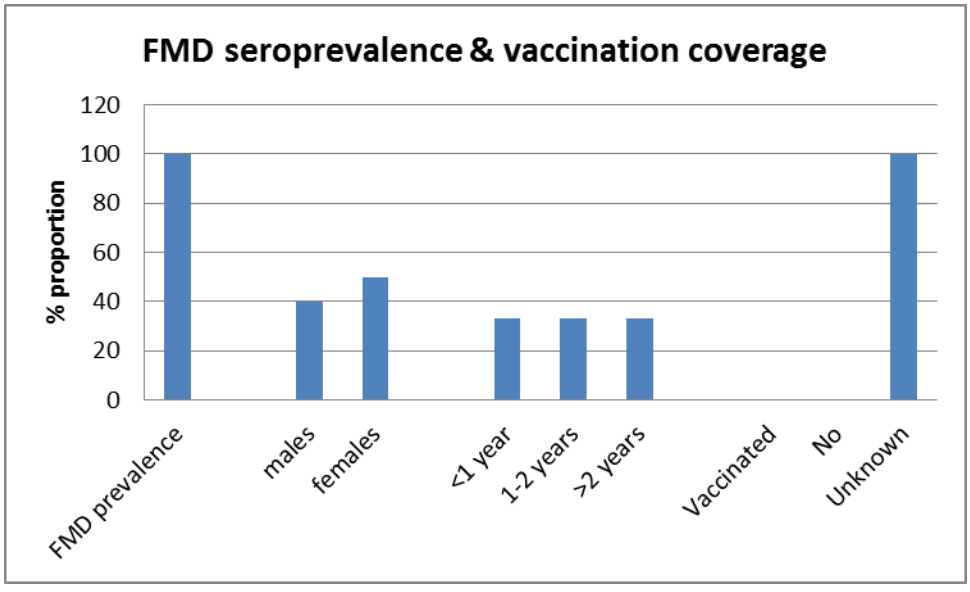


Figure 4.36: FMD seroprevalence and statues of samples

Animals with at least one or more serotypes accounted for 66.7% (10/15). Those with single serotype were significantly higher 40% (6/15). The most prevalent serotype was serotype A with 60% (9/15) prevalence. It was followed by serotype SAT 1 and serotype C with 26.7% and 20% prevalence respectively. Only one animal was positive to serotype SAT 2 (6.7%) with all animals being sero-negative to serotype O (Figure 4.37).

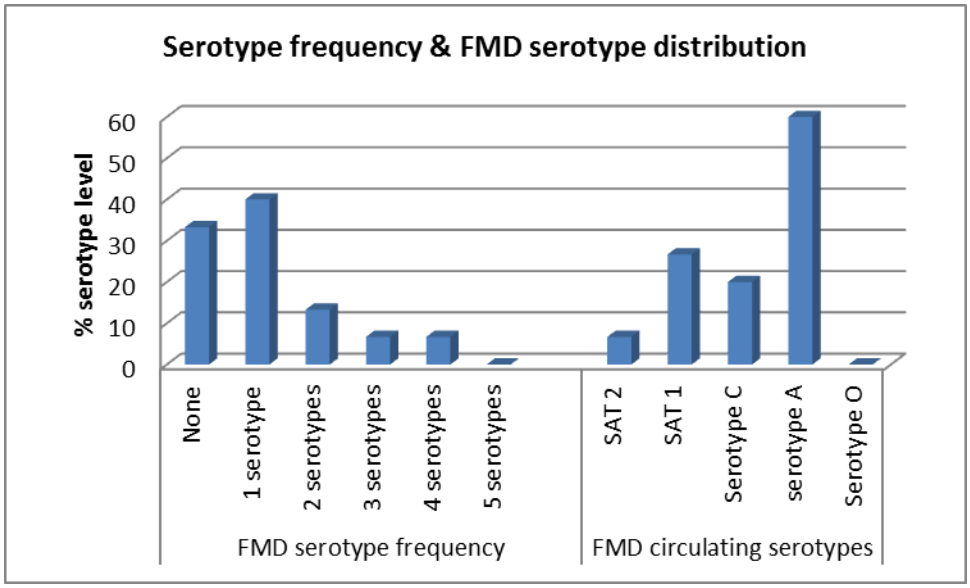


Figure 4.37: FMD serotype frequency and distribution

4.4.7.1 Foot-and-mouth disease in porcines

A total of 9 porcine samples were subjected to FMD NSP screening test. All the serum samples were of 1-2 years group and unvaccinated. The FMD prevalence was very high with all animals showing 100%) seropositivity (Figure 4.38).

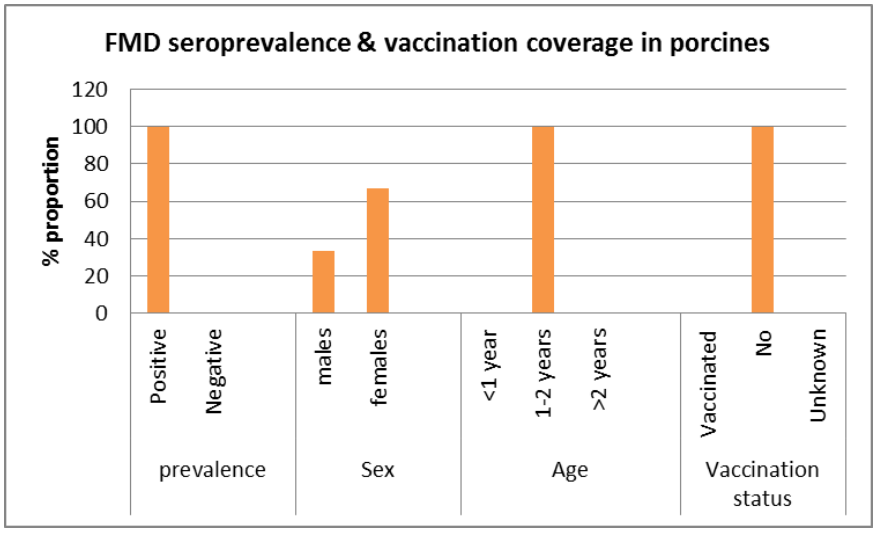


Figure 4.38: Porcine FMD seroprevalence in Kakamega

4.4.8 Turkana County

Out of the 220 animals' sampled and subjected to FMD NSP screening, 176 of the animals were seropositive (80% seroprevalence). All the animals sampled had no history of FMD vaccination (Figure 4.39).

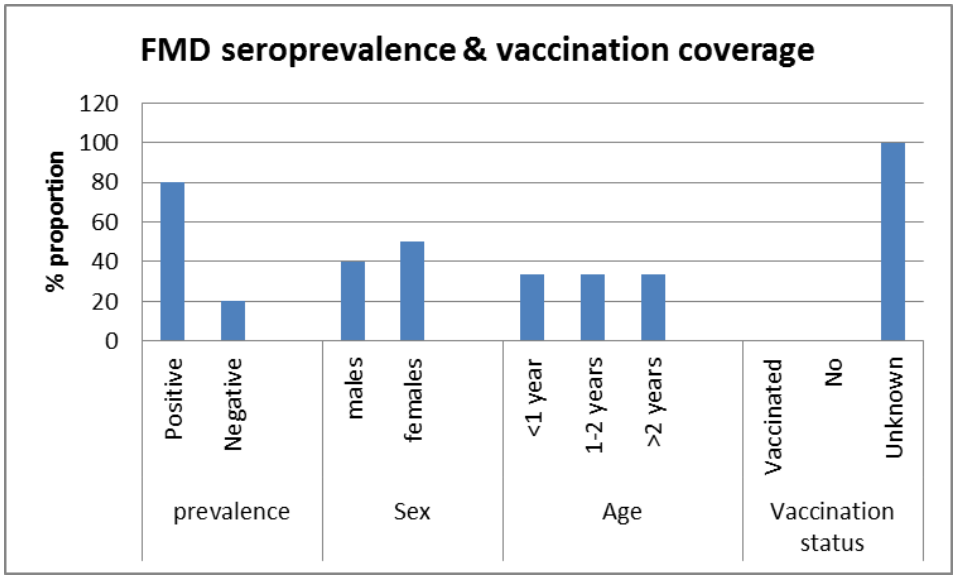


Figure 4.39: FMD seroprevalence and vaccination cover in Turkana

93.2% of the samples subjected to serotype titration (41/44) had at least one or more serotype detected with those containing three serotypes were 31.8% (14/44) while 6.8% (3/44) of them had all the five serotypes circulating. Among the five serotypes circulating in Turkana County, serotype A was the most prevalent with 81.8% (36/44) while serotype SAT 1 and serotype C followed with 56.8% (25/44) and 59% (26/44) prevalence respectively. Serotype O had a prevalence of 38.6% (17/44) with SAT 2 being the least prevalent serotype at 6.8% (6/44) (Figure 4.40).

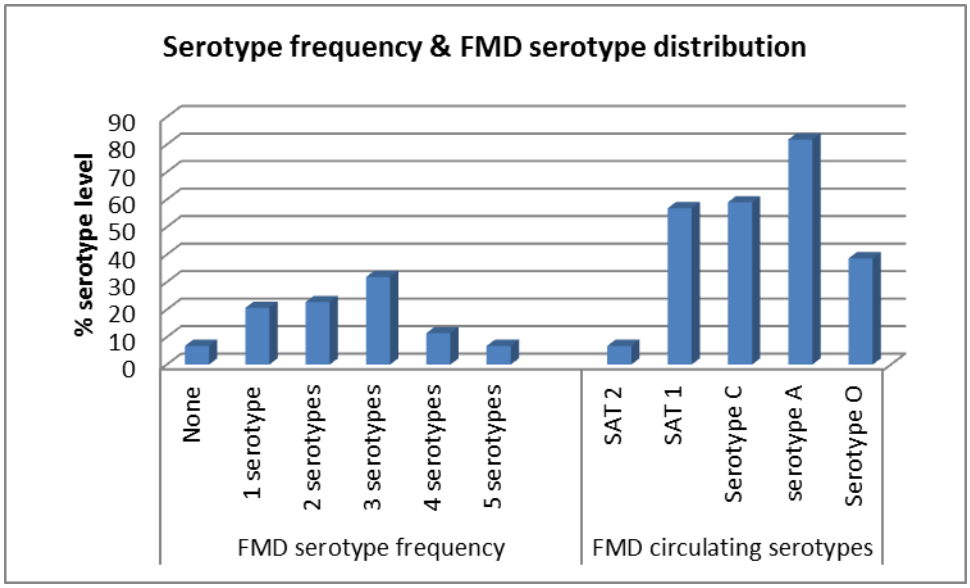


Figure 4.40: Serotype frequency and distribution

4.4.9 Pokot County

100 samples were screened for FMD. Just like the neighboring county of Trans-Nzoia, it had very high seroprevalence of 100%. According to the farmers interviewed, FMD vaccination had not been carried out in the county (Figure 4.41).

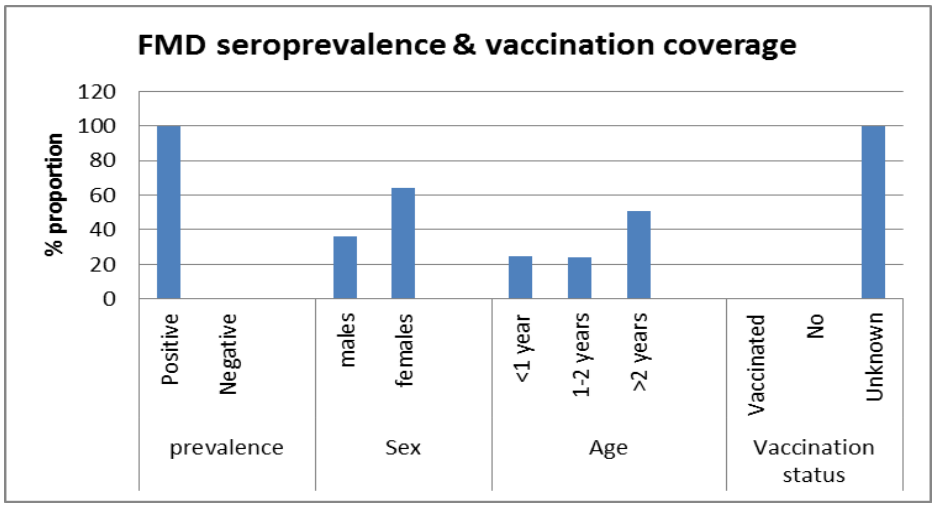


Figure 4.41: FMD seroprevalence and vaccination cover in Pokot

Of the 33 serum samples subjected to serotype titration, 63.6% had at least one or more serotype circulating. Animals with two serotypes were the majority with 27.3% (9/33). A number of them did not show any serotype (36.4%). All the five serotypes were found circulating with serotype C being the most prevalent serotype with 51.5% (17/33). It was closely followed by serotype A with 45.5% prevalence (15/33) (Figure 4.42).

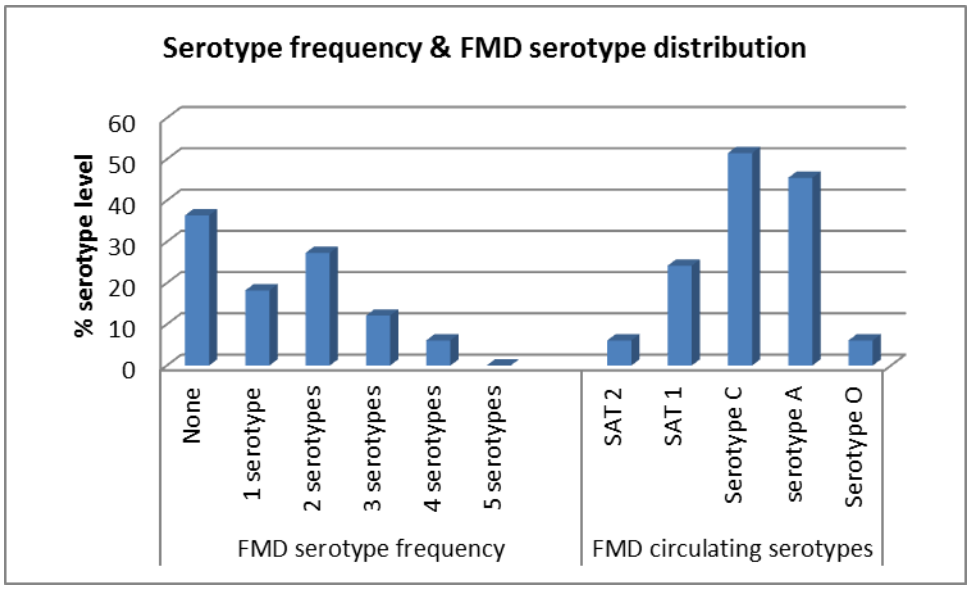


Figure 4.42: Serotype frequency and distribution in Trans Nzoia

4.5.0 Narok County

There was no vaccination carried out in Narok County. The FMD prevalence was high at 90.4% (206/228). Female samples were more at 84.2% (192/228) (Figure 4.43).

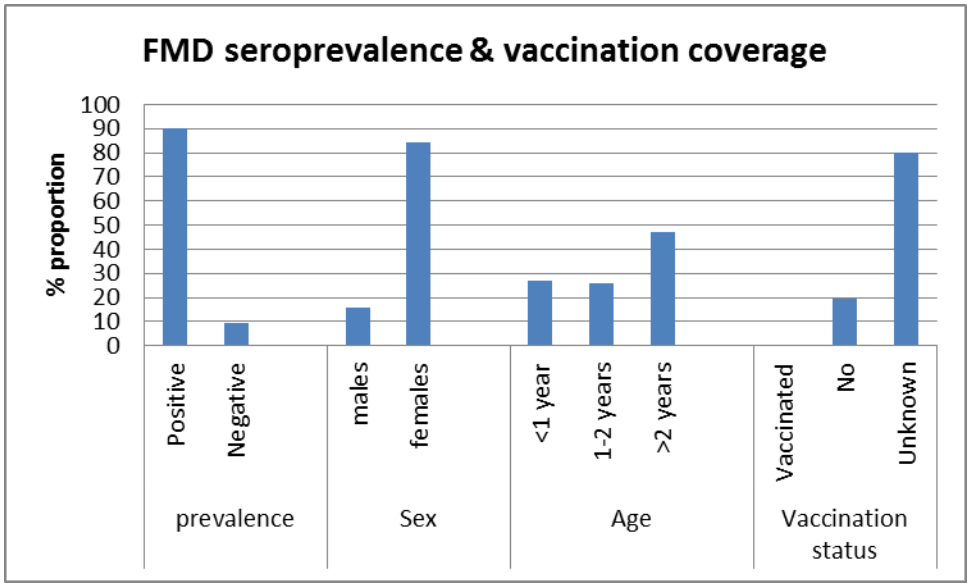


Figure 4.43: FMD seroprevalence and vaccination cover in Narok

Animals with at least one or more serotype were 80% (32/40). 30% (12/40) had three serotypes while 17.5% (7/40) had single serotype circulating. Five animals (12.5%) had all the serotypes circulating within their bodies. Serotype SAT 1 was the most prevalent serotype with 60% (24/40) and was closely followed by serotype C with 55% (22/40) prevalence (Figure 4.44).

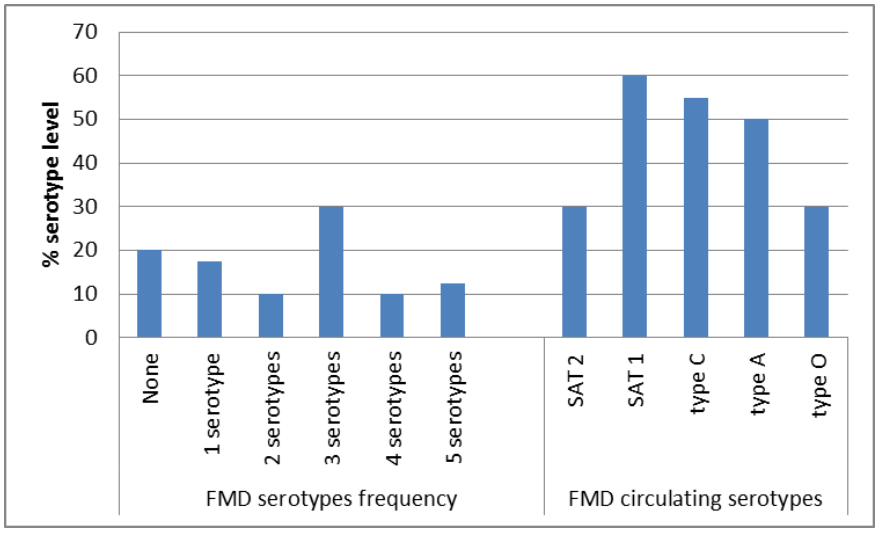


Figure 4.44: serotype frequency and distribution in Narok

4.5.1 Kajiado County

Kajiado and Narok counties form part of the Maasai ecosystem. Reported FMD vaccination coverage was 6.8% (19/281). FMD prevalence was 67.6% (190/281) (Figure 4.45).

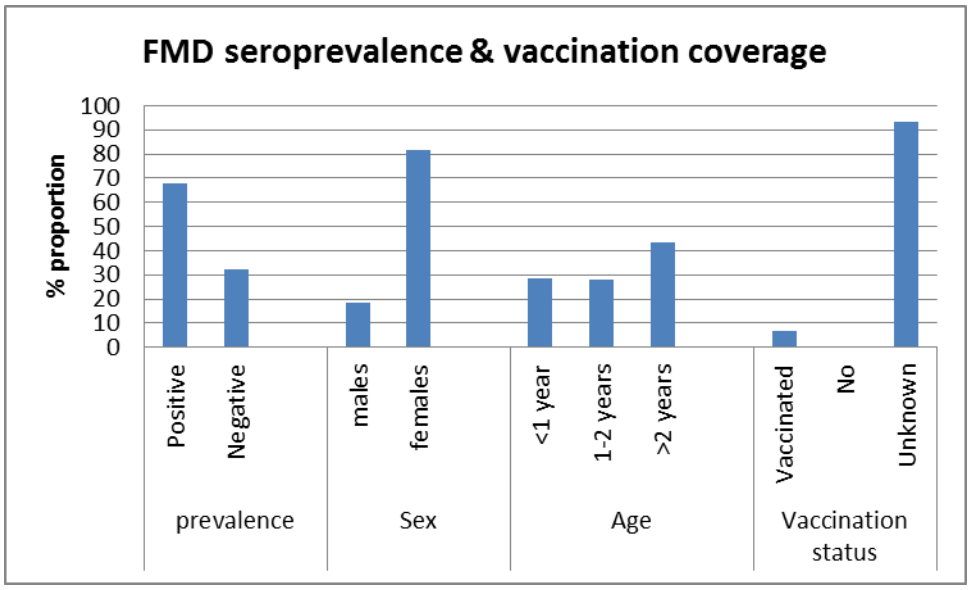


Figure 4.45: FMD prevalence and vaccination coverage in Kajiado

All the five serotypes were detected. 85.7% of the animals (24/28) had at least one or more serotype circulating. 28.6% (8/28) had four serotypes while 21.4% (5/28) had five serotypes. All the five serotypes had significant level of prevalence with serotype C leading with 71.4% (20/28) and were followed by serotype SAT 1 with prevalence of 64.3%, serotypes SAT 2 and serotype A with prevalence of 53.6% and 60.7% respectively (Figure 4.46).

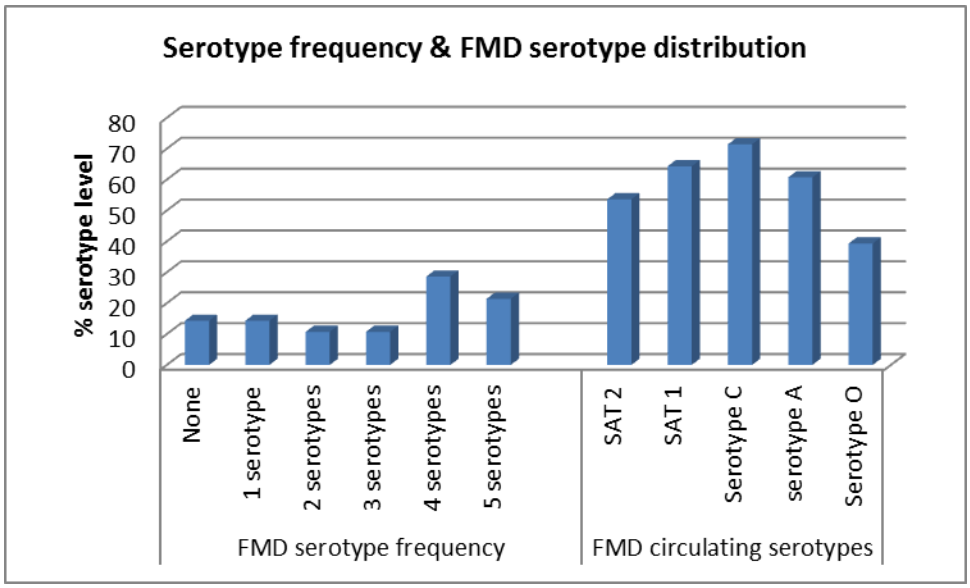


Figure 4.46: FMD serotype frequency and distribution in Kajiado

4.5.2 Bomet County

FMD seroprevalence was 70% (21/30). None of the animals had been vaccinated. (Figure 4.47).

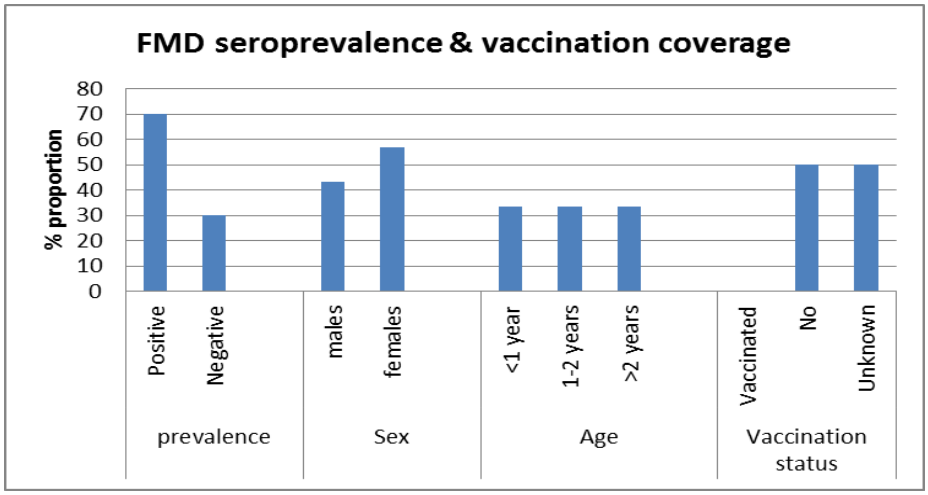


Figure 4.47: FMD seroprevalence and vaccination cover in Bomet

Animals with single serotype accounted the highest at 33.3% (7/21) compared to animals with three serotypes with 23.8% (5/21). Animals with at least one or more serotype accounted for 76.2% (17/21). All the five serotypes were detected. Serotype C was the most prevalent serotype with 57.1% (12/21) and was followed in prevalence by serotype A with 52.4% (11/21). Serotype O was the least prevalent (3.3%) (Figure 4.48).

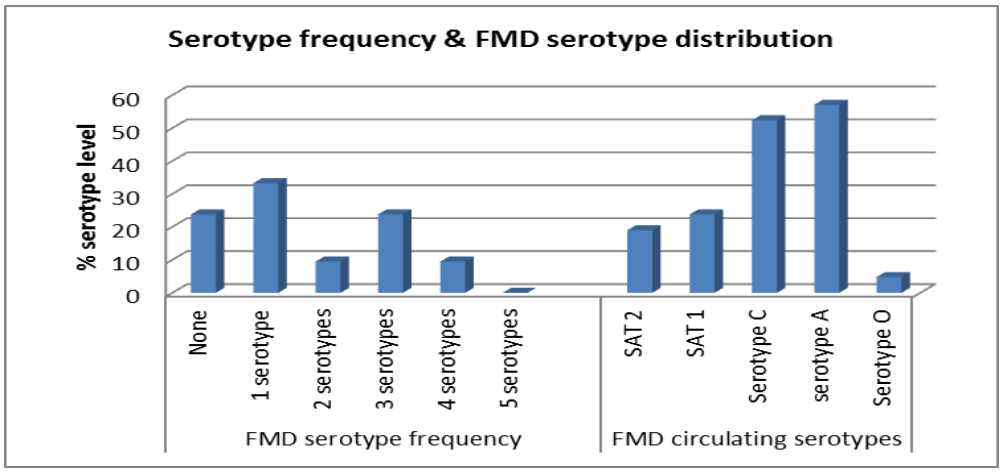


Figure 4.48: serotype frequency and distribution in Bomet

4.5.2.1 Foot-and-mouth disease in porcines

All the three porcine samples were of <1 year of age and had not been vaccinated against FMD. From the results of FMD screening, all the porcines were naïve to FMD infection i.e. all were seronegative (100%) contrary to high seroprevalence of FMD in bovines which had 70% Figure 4.47 and Figure 4.49.

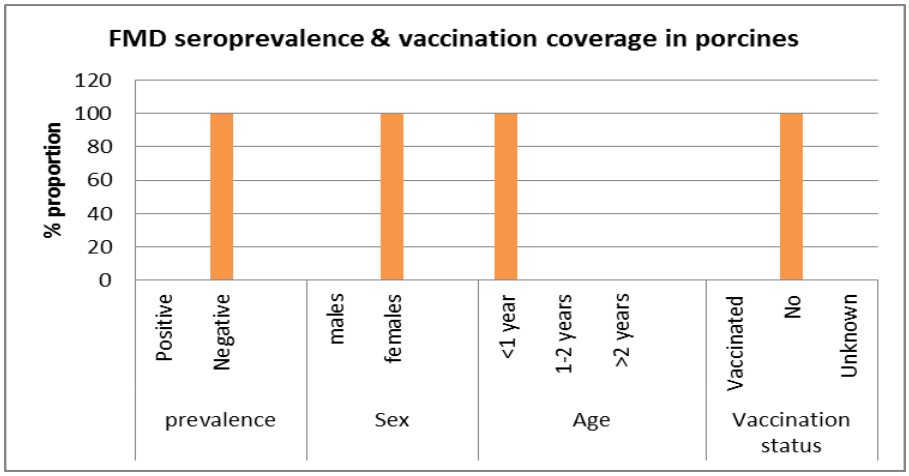


Figure 4.49: FMD prevalence in porcines from Bomet

4.5.3 Migori County

34/45 animals screened for FMD virus were seropositive representing 75.6% seroprevalence. All animals sampled were unvaccinated (Figure 4.50)

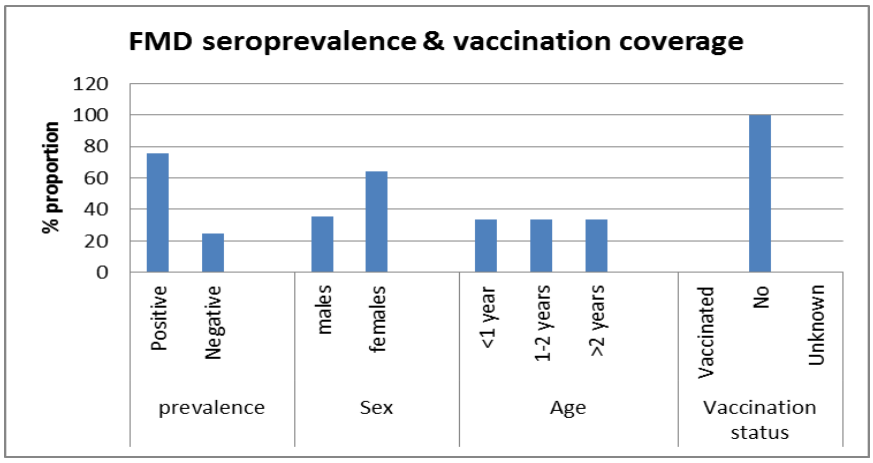


Figure 4.50: FMD seroprevalence and vaccination cover in Migori

56.7% (17/30) had at least one or more serotype detected. Animals with one and two serotypes comprised of 23.3% each (7/30). All the five serotypes were detected with serotype SAT 1 being the most prevalent serotype (43.3%, 13/30). It was closely followed in prevalence with serotype C which had a detection rate of 30% (9 animals positive) (Figure 4.51).

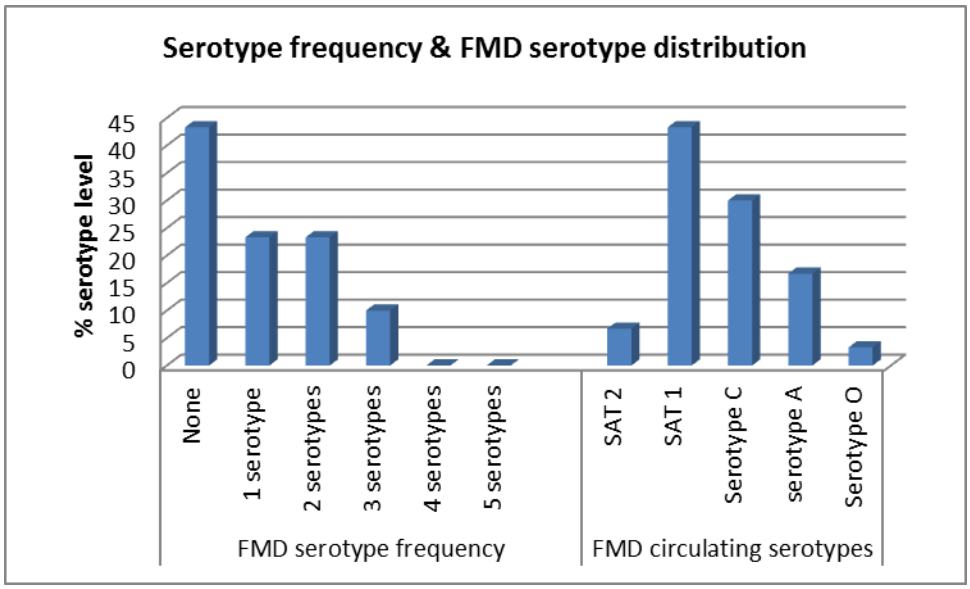


Figure 4.51: serotype frequency and distribution in Migori

4.5.4 Nakuru County

A total of 44 animals had been sampled. The reported vaccination coverage was 31.8% (14/44). FMD seroprevalence was 22.7% (10/44) (Figure 4.52).

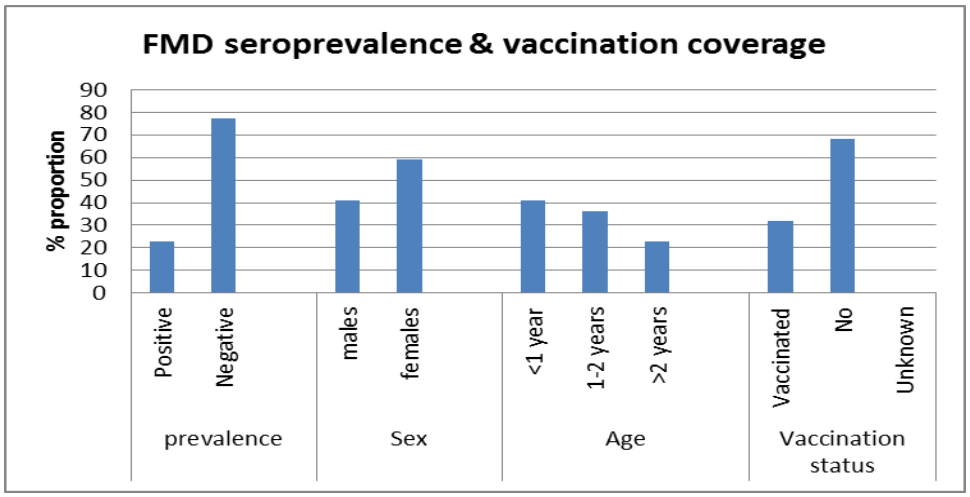


Figure 4.52: FMD seroprevalence in Nakuru

A higher number of samples did not indicate presence of any serotype (70%). Two animals had one serotype while the other had all the four serotypes. All the five serotypes were detected with serotype A being the most prevalent. All the other four serotypes (SAT 1, C, A & O) had equal prevalence of 10%. Adult animals >2 years had high serotype frequency at 40% (4/10) compared to those aged <1 year 30% (3/10) (Figure 4.53).

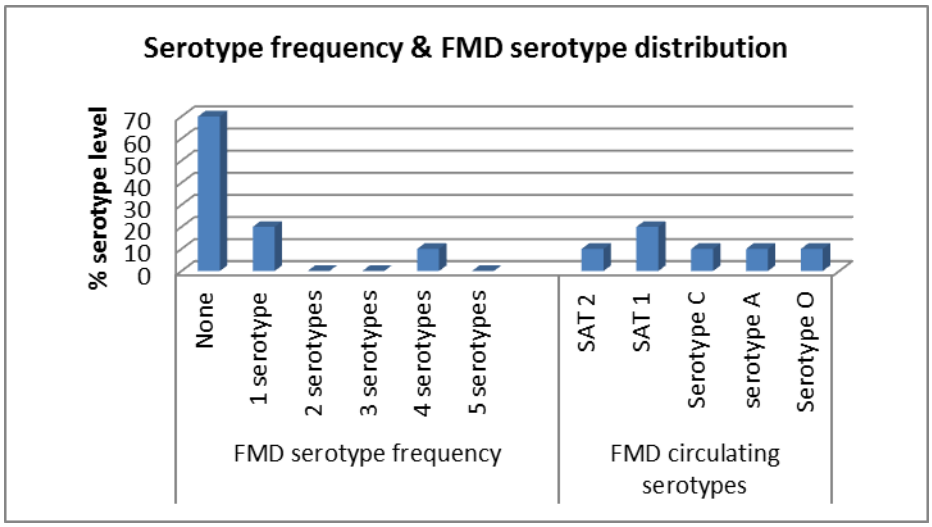


Figure 4.53: serotype frequency and distribution in Nakuru

4.5.4.1 Foot-and-mouth disease in porcines

All the 19 porcines sampled had not been vaccinated against FMDV. The prevalence of FMD in porcines within the districts of Nakuru County was fairly low at 36.8% (7/12). The porcine species seroprevalence was however higher compared to bovine species which had 22.7% (Figure 4.54 and Figure 4.52).

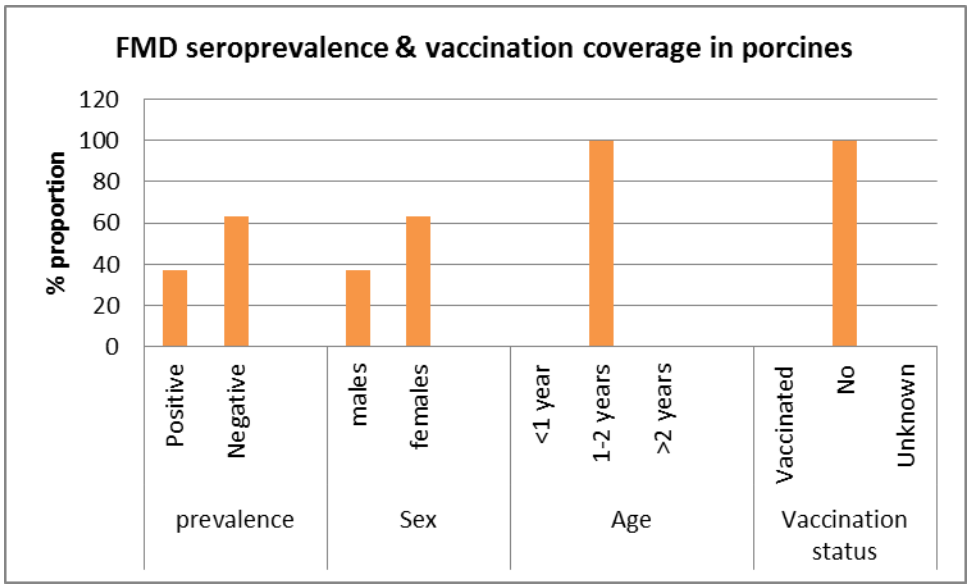


Figure 4.54: porcine FMD prevalence in Nakuru

4.5.5 Siaya County

All animals sampled were unvaccinated against FMDV and FMD seroprevalence was 62.2% (28/45). Females and middle-aged group were the majority with 73.3% (33/45) and 35.6% (16/45) respectively (Figure 4.55).

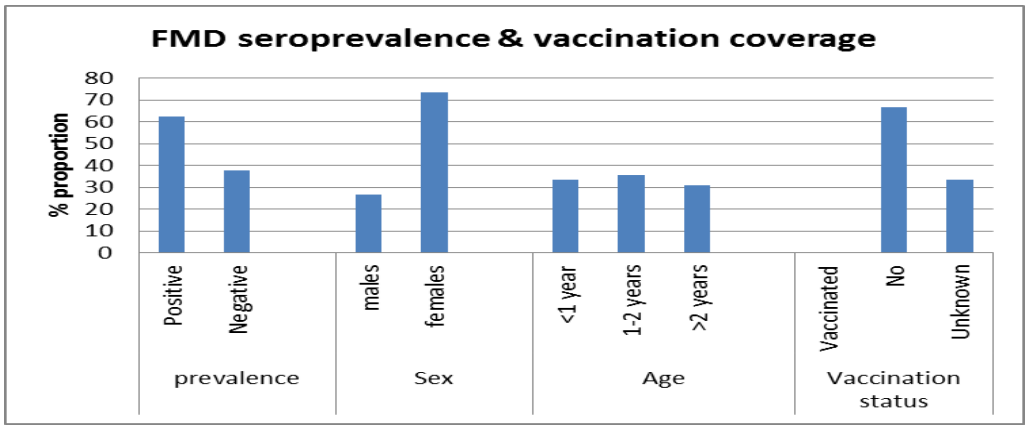


Figure 4.55: FMD prevalence and vaccination cover in Siaya

54.2% (13/24) of the samples had no single serotype detected. Animals with three serotypes were 20.8% while those with two serotypes making up 16.7%. Young animals of <1 year were the highest contributors of serotype frequency at 41.7% (10/24) compared to those of age 1-2 years (29.2%, 7/24). Only three serotypes were detected. Serotype C was the most prevalent serotype with 45.8% (11/24) and was followed by Serotype SAT 1 and SAT 2 with prevalence of 33.3% (8/24) and 25% (6/24) respectively. Serotypes O and A were not detected (Figure 4.56).

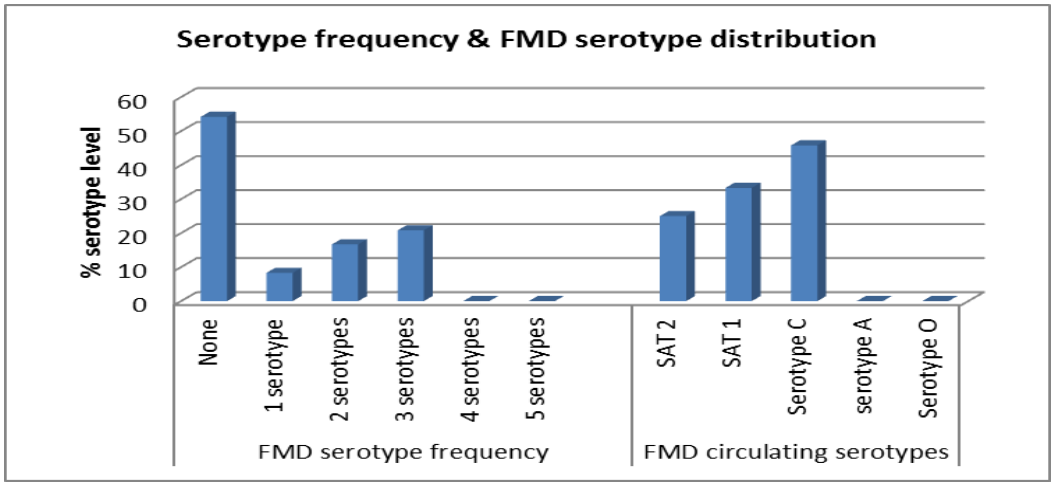


Figure 4.56: serotype frequency and distribution in Siaya

4.5.5.1 Foot-and-mouth disease in porcines

All the 16 porcine samples were unvaccinated with majority of them being of <1 year at 56.3% (9/16). FMD prevalence was 75% (12/16). The porcine FMD prevalence was however higher than that of bovines which was at 62.2% (Figure 4.55 and Figure 4.57).

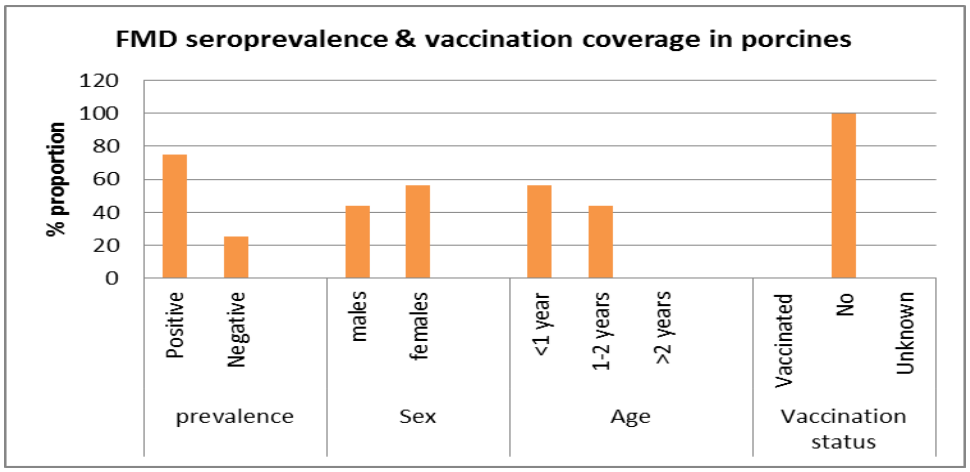


Figure 4.57: Porcine FMD prevalence in Siaya

4.5.6 Kisumu County

A high number of animals sampled comprised of females at 82.2% (37/45) and adults >2 years 35.6% (16/45). All animals were unvaccinated. FMD seroprevalence was 51.1% (23/45) (Figure 4.58).

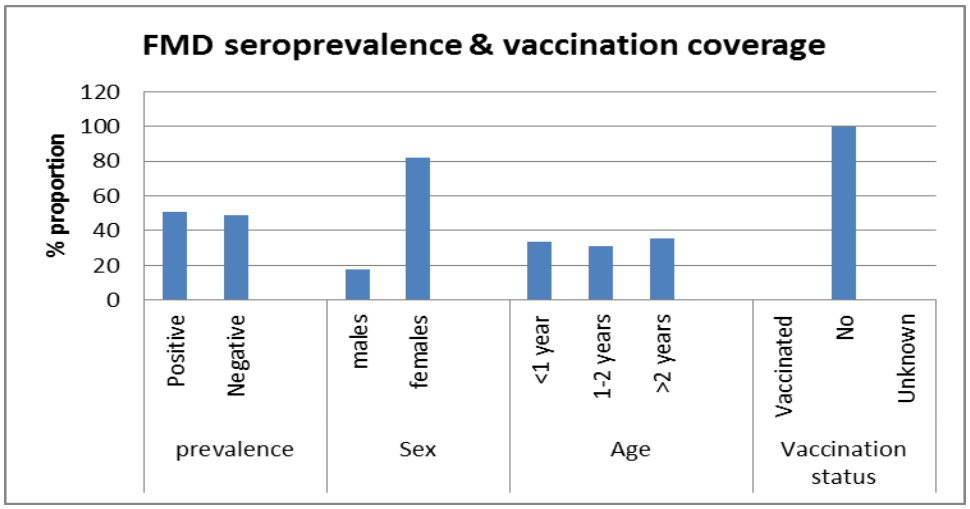


Figure 4.58: FMD seroprevalence and vaccination cover

Only 33.3% (7/21) had at least one or more serotype detected, 66.7% (14/21) did not show any serotype. Animals with single serotype were 14.3% (3/21) same as those with two circulating serotypes as shown in figure 4.159. All the five serotypes were detected with serotype SAT 1 being the most prevalent serotype with 23.8% (5/21). Serotype C trailed SAT 1 with 19.0% (4/21). 42.9% (9/21) of serotypes were detected adult animals (Figure 4.59)

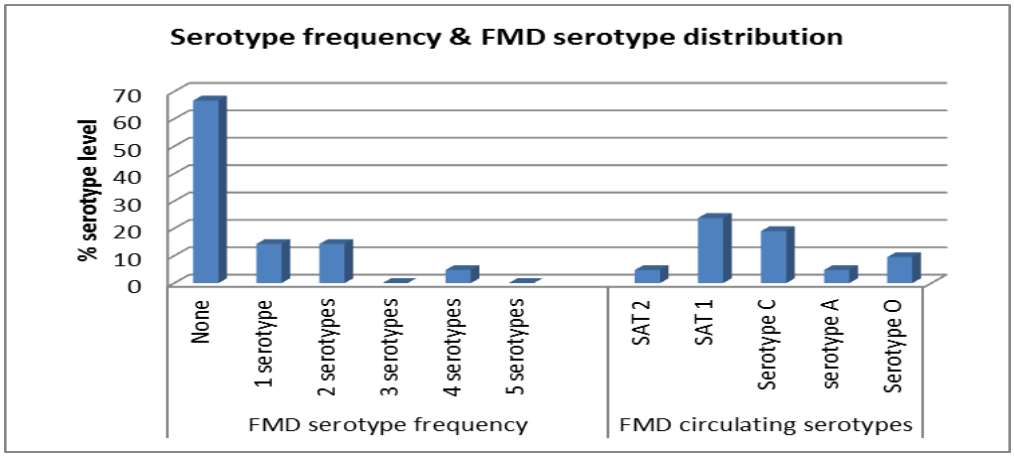


Figure 4.59: Serotype frequency and distribution in Kisumu

4.5.6.1 Foot-and-mouth disease in porcines

All the 8 porcine sera subjected to FMD screening test were of age 1-2 years. None had been vaccinated. FMD seroprevalence was 75% (6/8). The seroprevalence in porcines was higher than that of bovines in the same county (Figure 4.60 and Figure 4.58).

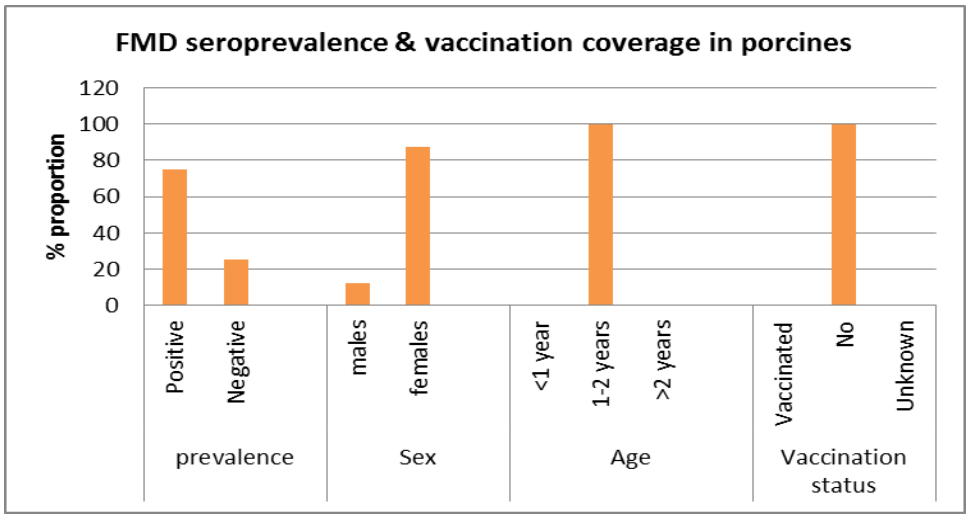


Figure 4.60: Porcine FMD seroprevalence in Kisumu

4.5.7 Mandera County

A significant number animals sampled were females 96.7% (68/70) and adults >2 years 46.7% (28/60). The vaccination cover was nil. FMD seroprevalence was very low with only five animals having detectable FMD antibodies (8.3%). Significant numbers of animals were seronegative on NSP (91.7%) (Figure 4.61).

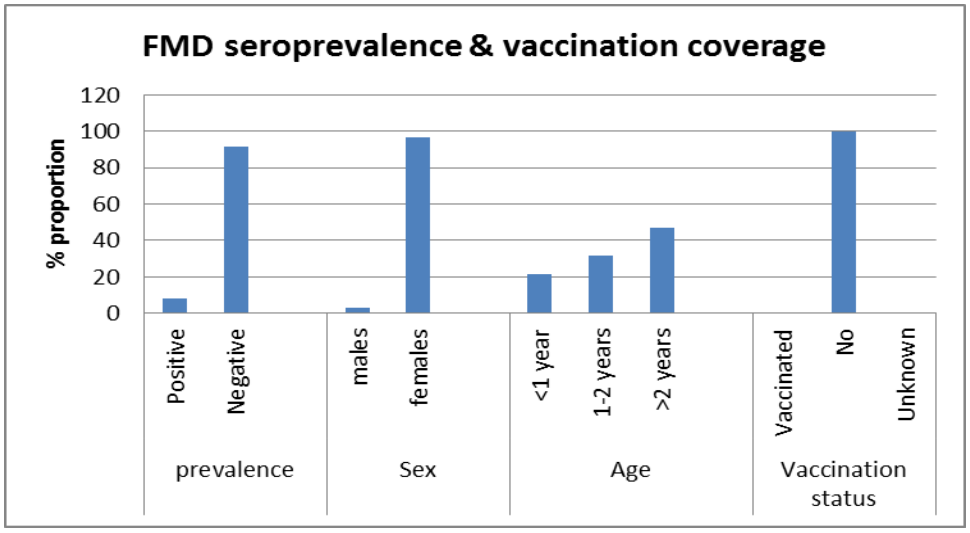


Figure 4.61: FMD prevalence and vaccination cover in Mandera

Only 3 samples were subjected to serotype titration, with two of the samples showing no detectable serotype. Only serotype SAT 1 was detected (Figure 4.62).

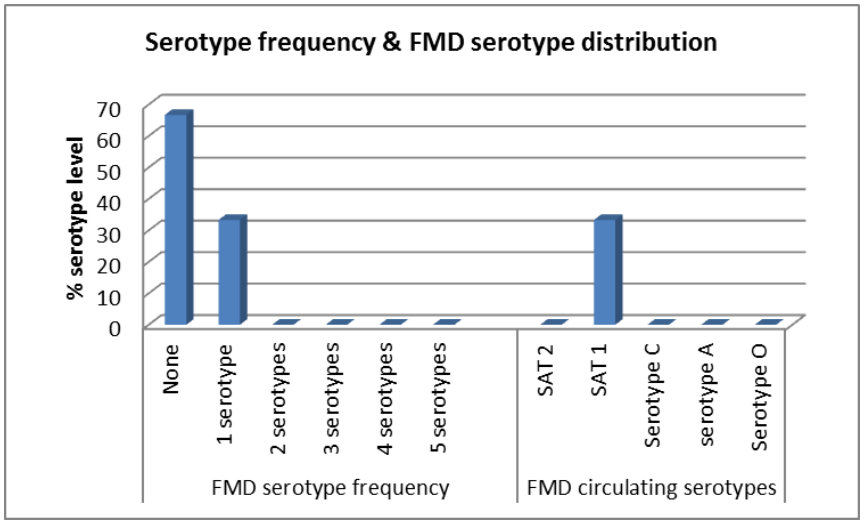


Figure 4.62: serotype frequency and distribution in Mandera

4.5.8 Wajir County

75 animals from Wajir County had detectable levels of FMD antibodies (33% FMD seroprevalence). All animals sampled were unvaccinated against FMD virus. Female samples were significantly higher at 93.4% (212/227) compared to males (Figure 4.63).

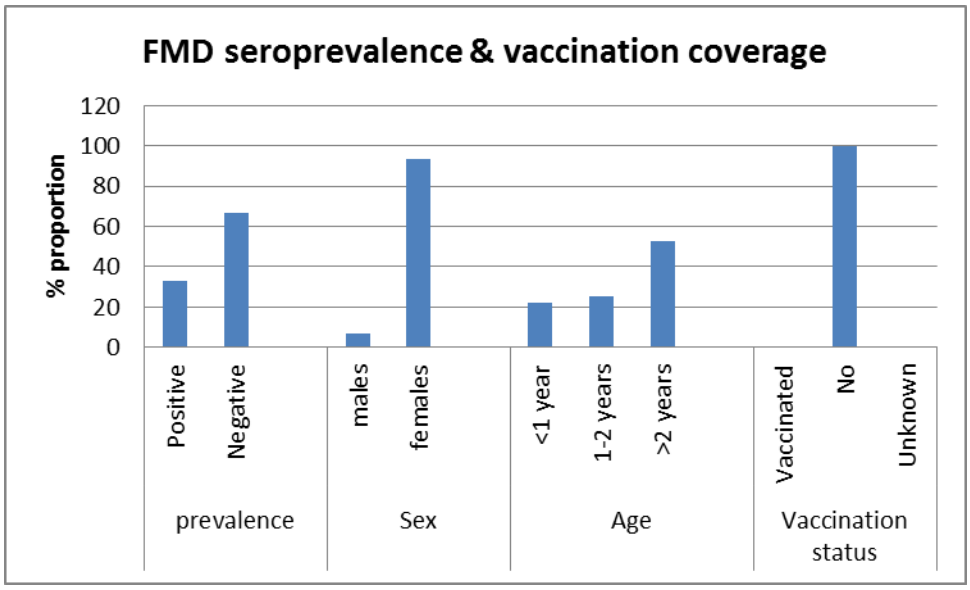


Figure 4.63: FMD prevalence and vaccination cover in Wajir

16 samples that were seropositive were subjected to serotype titration. 87.5% (14/16) had at least one or more serotype circulating. 31.2% (5/16) had four serotypes while only one animal had all the five serotypes. Serotype SAT 1 was the most prevalent serotype at 81.3% (13/16) followed by serotype C which had 75% (12/16) prevalence. All the five serotypes were detected in females (Figure 4.64).

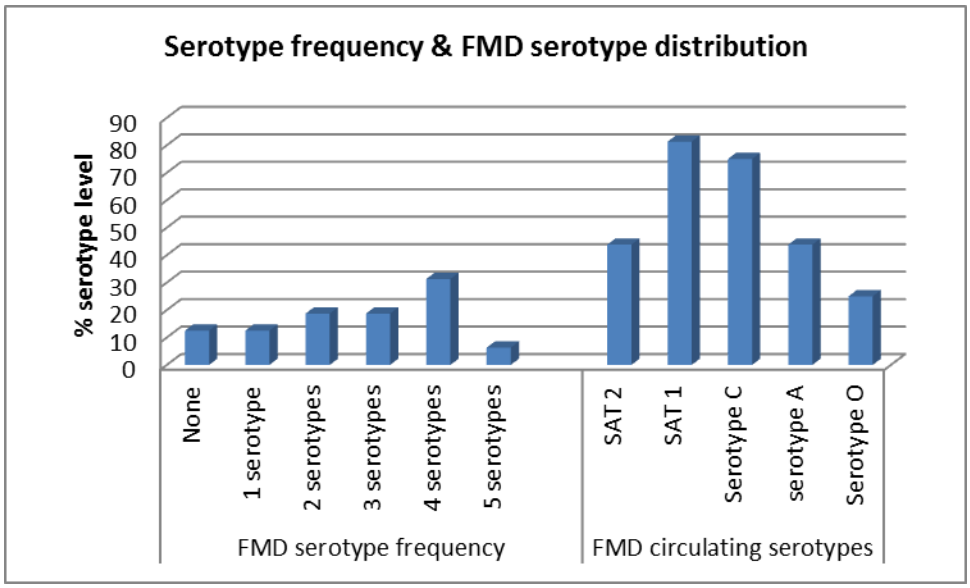


Figure 4.64: FMD serotype frequency and distribution in Wajir

4.5.9 Kitui County

Equal number of males and females were sampled. The vaccination coverage was low with 26 out of the total 264 animals reported vaccinated (9.8%). FMD seroprevalence was 28.8% (76/264) and was lower than the national seroprevalence (Figure 4.65 and Figure 4.14).

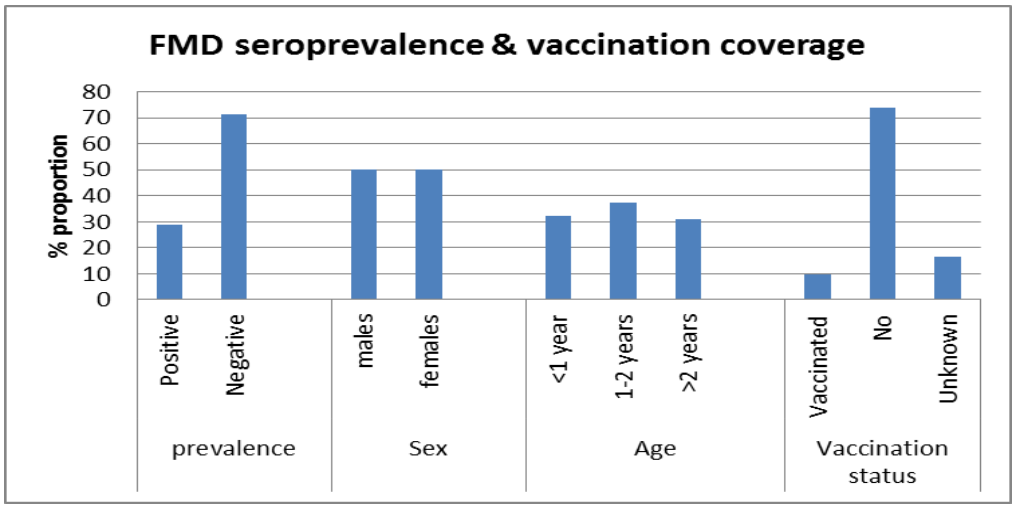


Figure 4.65: FMD seroprevalence and vaccination coverage in Kitui

78.6% of the animals (11/14) had at least one or more serotype. 90.9% (10/11) with at least one or more serotype were not vaccinated. Young animals <1 year had the highest number of serotypes with 50% (7/14) having at least one or more serotype. This was also true for the males where 57.1% (8/14) had detectable serotypes. A higher number of animals had between one and two serotypes i.e. 35.7% and 28.6% respectively. Only four serotypes were found circulating with SAT 1 being the most prevalent serotype with 50% (7/14). Serotype SAT 2 and serotype C had equal level of prevalence at 42.9% (6/14). Serotype A was not detected (Figure 4.66).

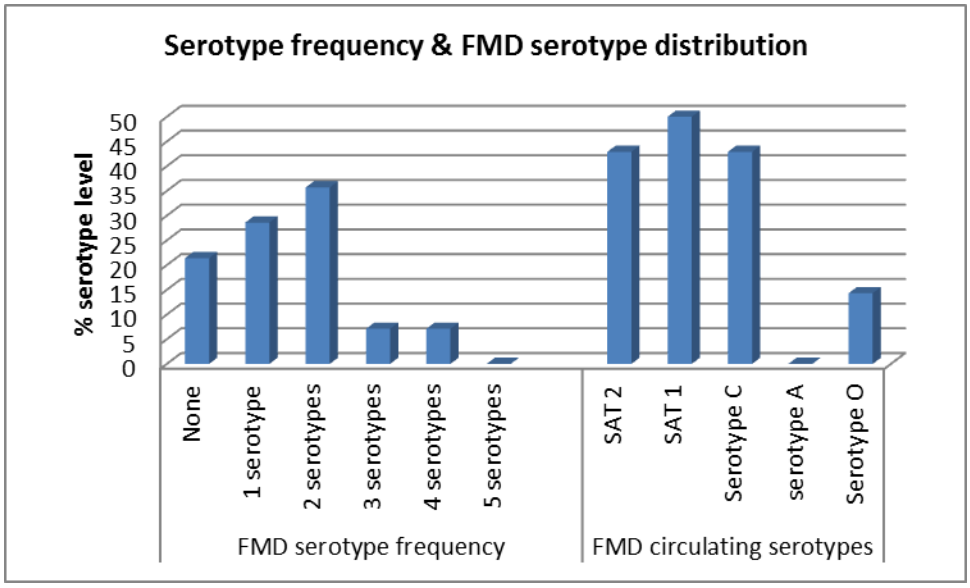


Figure 4.66: serotype frequency and distribution in Kitui

4.6.0 Machakos County

A total of 65 animals were sampled with a higher proportion being males at 63% (41/65). According to the history of the animals sampled, none of them had been vaccinated. FMD seroprevalence was at 43.1% (28/65) (Figure 4.67)

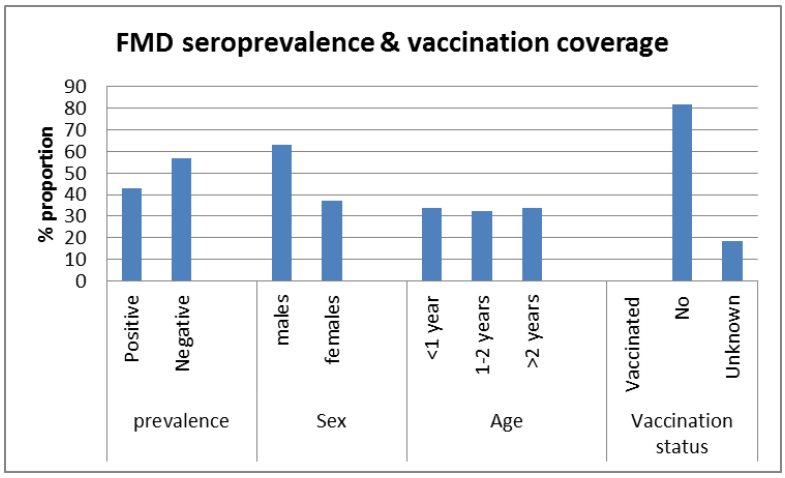


Figure 4.67: FMD seroprevalence and vaccination cover in Machakos

7/15 animals showed no detectable serotype (46.7%). The rest 53.3% of animals (8/15) had at least one or more serotype detectable. Significant number of them (26.7%, 4/15) had four circulating serotypes. All the five serotypes were detected with serotype SAT 2 being the most prevalent serotype at 53.3% (8/15) and was followed by serotype SAT 1 with 46.7% (7/15). Serotype O was the least prevalent with only one animal positive to it (Figure 4.68).

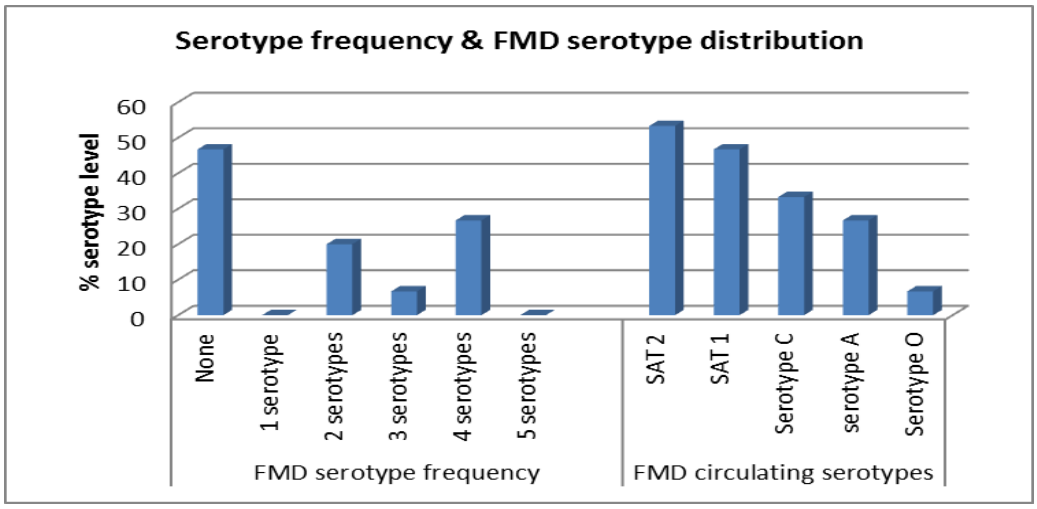


Figure 4.68: Serotype frequency and distribution in Machakos

4.6.1 Makueni County

The vaccination cover in Makueni County was significantly low at 1.6%. FMD seroprevalence was 26.8% (34 animals out of 127 had detectable levels of antibodies to FMD virus) (Figure 4.69).

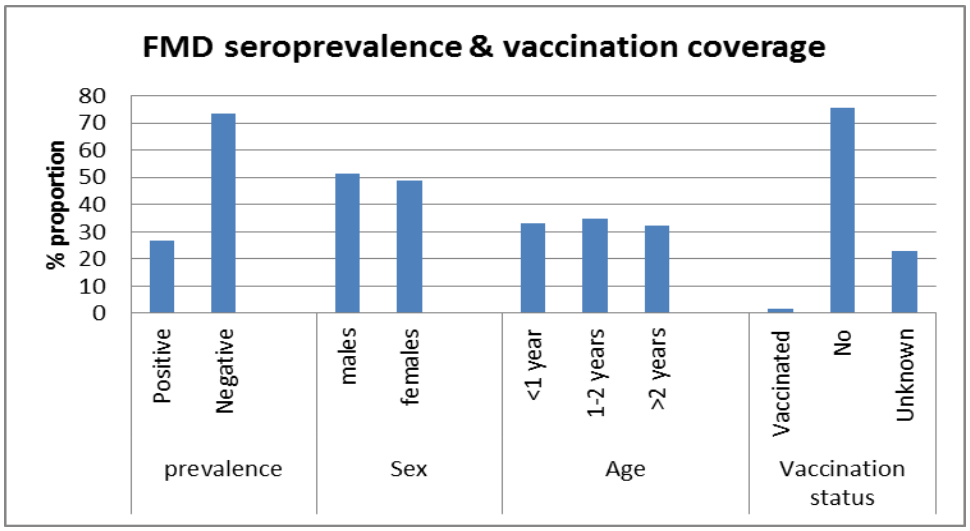


Figure 4.69: FMD seroprevalence and vaccination cover in Makueni

Six animals had detectable antibodies to one or more serotypes (45.5%, 5/11). The rest 55.5% did not have any serotype detected. Animals with two and three serotypes were the majority with 27.3% and 18.2% respectively. A high number of serotypes were detected in females 81.8% (9/11) and adults >2 years. Only four serotypes were detected with SAT 1 being the most prevalent serotype at 54.5% (6/11). Serotype O was the second most prevalent serotype with 36.4% (4/11) and was followed by serotype SAT 2 and type C with each having 27.3%. Just like in Kitui, serotype A was not detected (Figure 4.70).

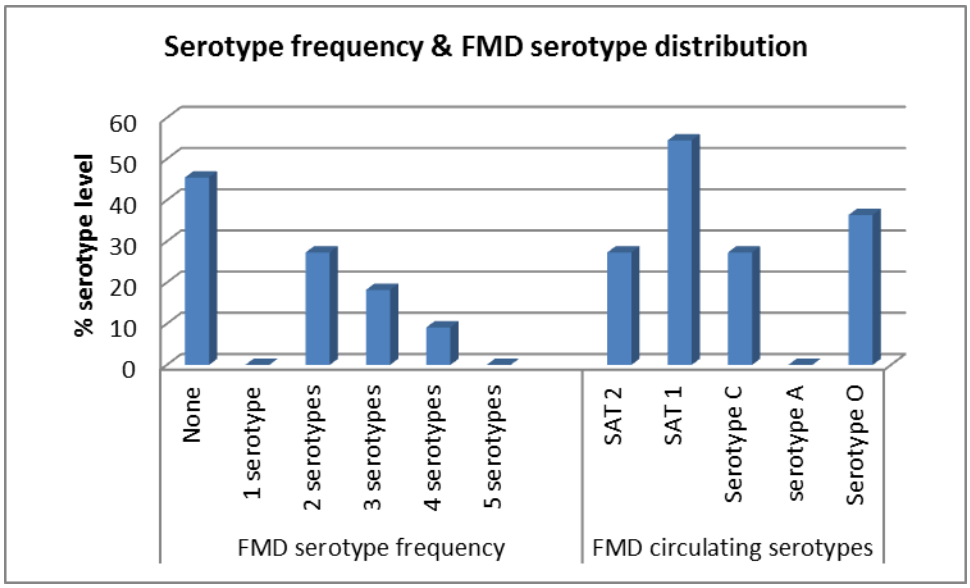


Figure 4.70: serotype frequency and distribution in Makueni

4.6.2 Nyeri County

A total of 76 animals were sampled from different parts of Nyeri County of which 72% (55/76) were females. The reported vaccination cover was 60.5% (46/76 animals vaccinated). FMD seroprevalence was very low at 5.3% (Figure 4.71).

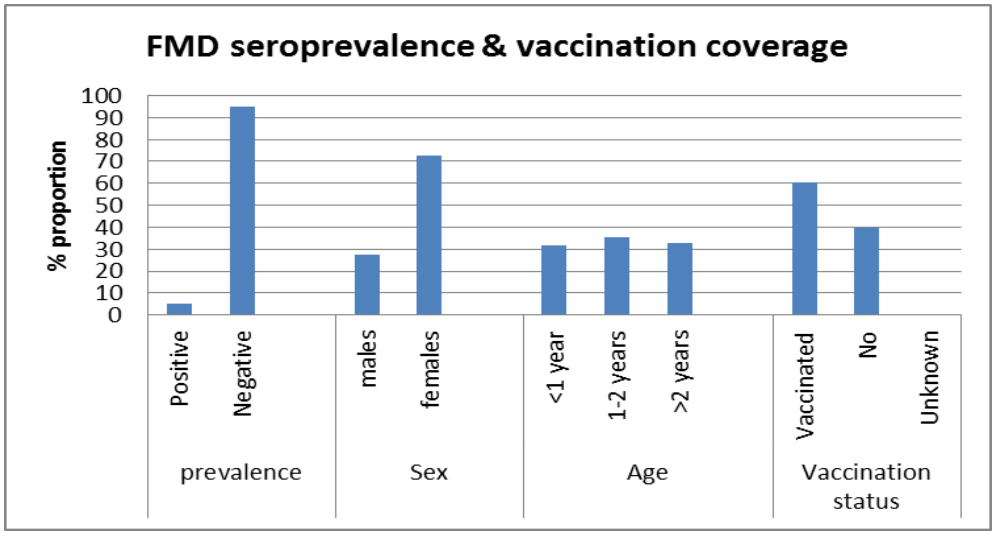


Figure 4.71: FMD prevalence and vaccination cover in Nyeri

Only one sample positive on NSP was subjected to serotype titration. The animal had three circulating serotypes namely SAT 2, SAT 1 and type C (Figure 4.72).

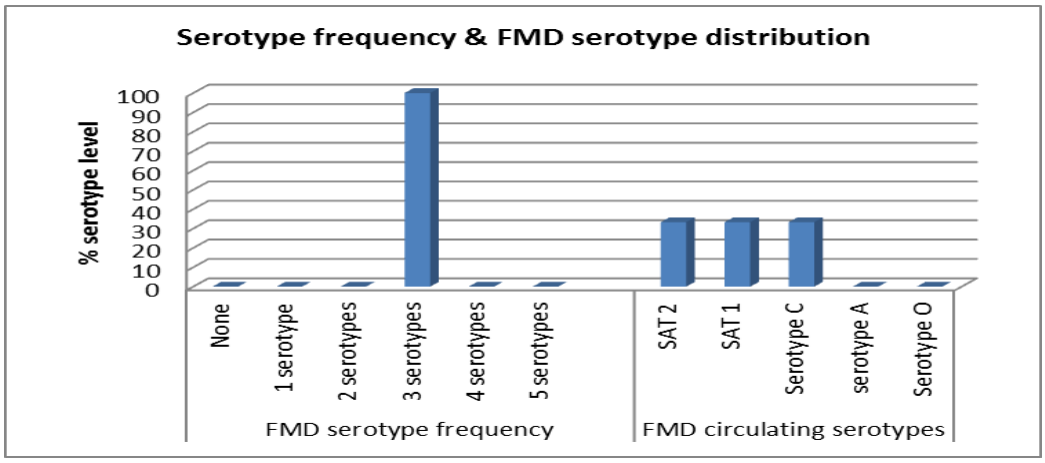


Figure 4.72: serotype frequency and distribution in Nyeri

4.6.2.1 Foot-and-mouth disease in porcines

All the 33 porcine samples subjected to NSP were unvaccinated. Only 7 porcines were seropositive 21.2% (7/33). FMD prevalence in porcines was higher than in bovines (Figure 4.73 and Figure 4.71).

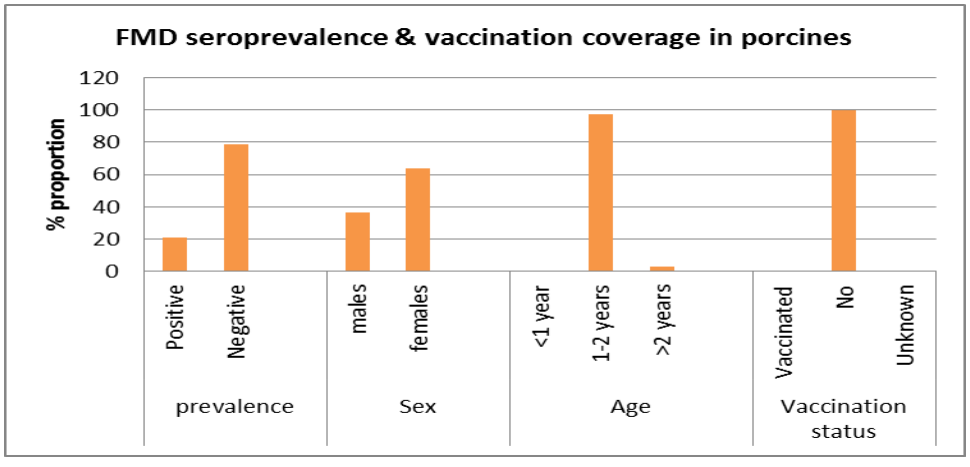


Figure 4.73: Porcine prevalence in Nyeri

4.6.3 Embu County

All the animals sampled from Embu County were females. The reported vaccination coverage was 65.7% (23/35). The seroprevalence of FMD virus was significantly higher at 82.9% (29/35 animals were seropositive) (Figure 4.74).

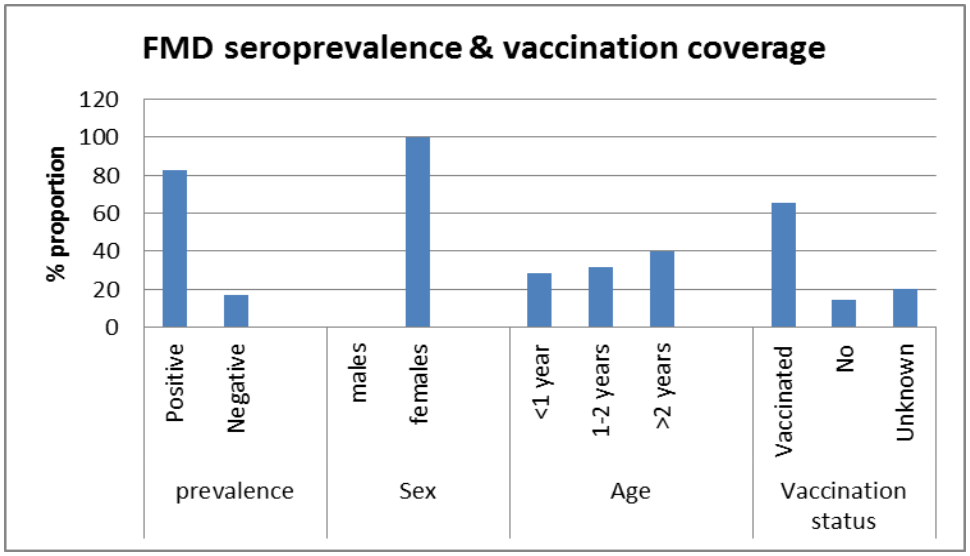


Figure 4.74: FMD seroprevalence and vaccination cover in Embu

27 animals out of 29 (93.1%) had at least one or more serotype detectable. Those with three serotypes were 34.5% (10/29) while those with two serotypes were 27.6%. Of the total serotypes, 44.8%, (13/29) were from adult females >2 years while 62.1%, (8/29) were from the vaccinated group. All the five serotypes were detected with serotype SAT 2 being the most prevalent with 82.8% (24/29) prevalence and was trailed by serotype C, SAT 1 and O with 58.6%, 44.8% and 37.9% prevalence respectively (Figure 4.75).

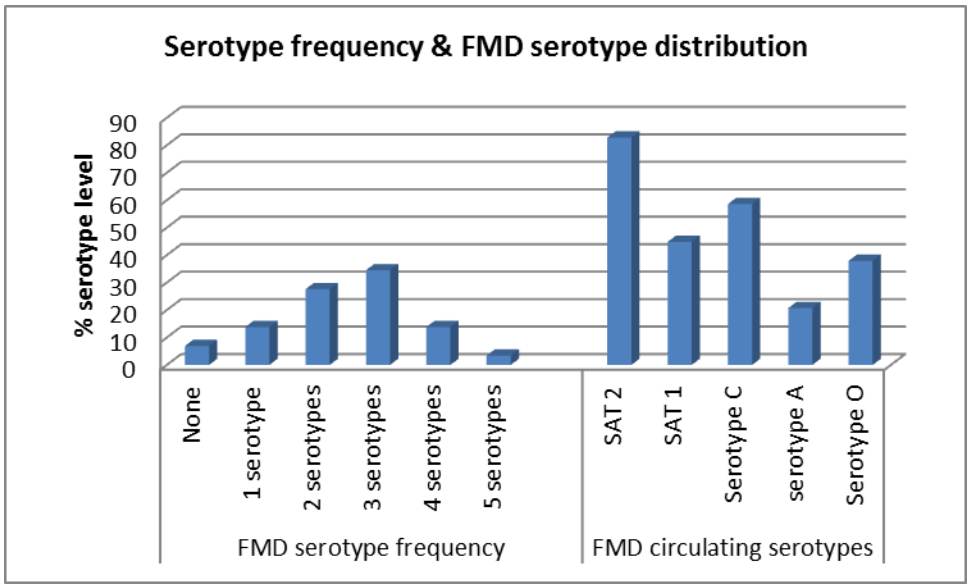


Figure 4.75: serotype frequency and distribution in Embu.

4.6.3.1 Foot-and-mouth disease in porcines

All the 8 porcine samples were females and unvaccinated. FMD seroprevalence was 50% (4/8). The porcine species seroprevalence was however lower than that of bovine species which had 82.9% (Figure 4.76 and Figure 4.74).

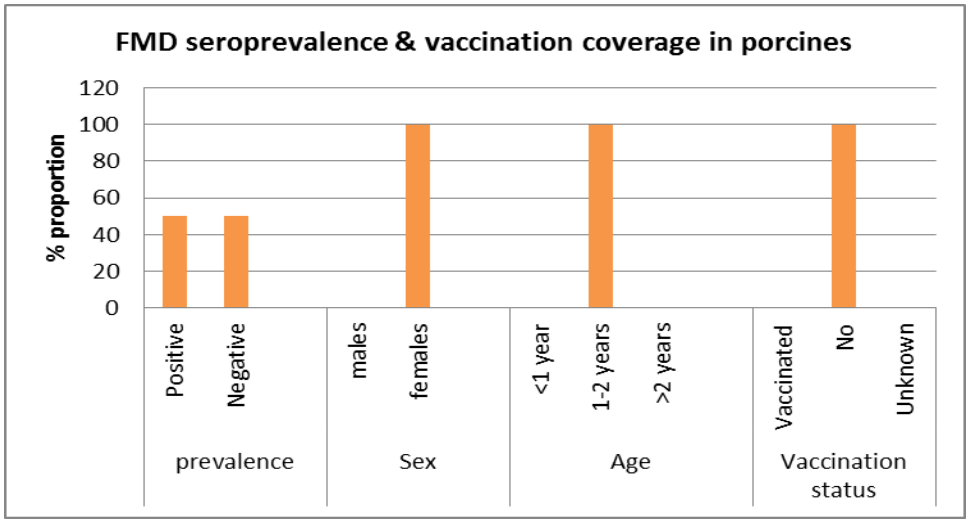


Figure 4.76: Porcine FMD prevalence in Embu

4.6.4 Meru County

A total of 77 animals were sampled. FMD vaccination cover was 41.6% (32/77). The FMD prevalence was lower than the national prevalence of 35% (Figure 4.77).

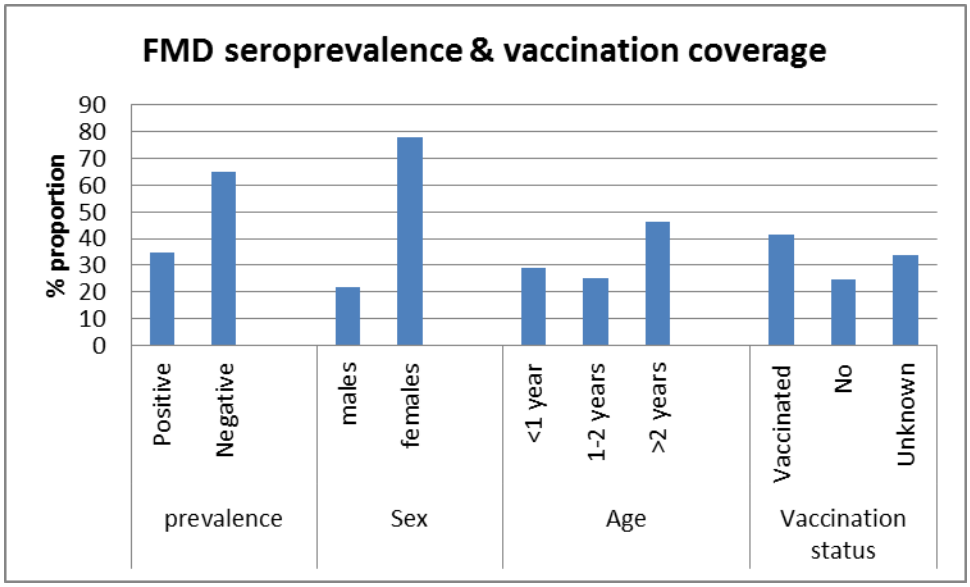


Figure 4.77: FMD seroprevalence and vaccination cover in Meru

Only 16 samples were analyzed for serotype distribution in which 81.2% of them (13/16) were positive to at least one or more serotype. Those with four circulating serotypes were the majority at 31.2% (5/16) followed by those with five serotype 18.8% (3/16). Animals of between 1-2 years contributed highly to the number of serotype frequency (43.8%, 7/16). All the five serotypes were detected in the county with the most prevalent being serotype O with 68.8% (11/16) (Figure 4.78).

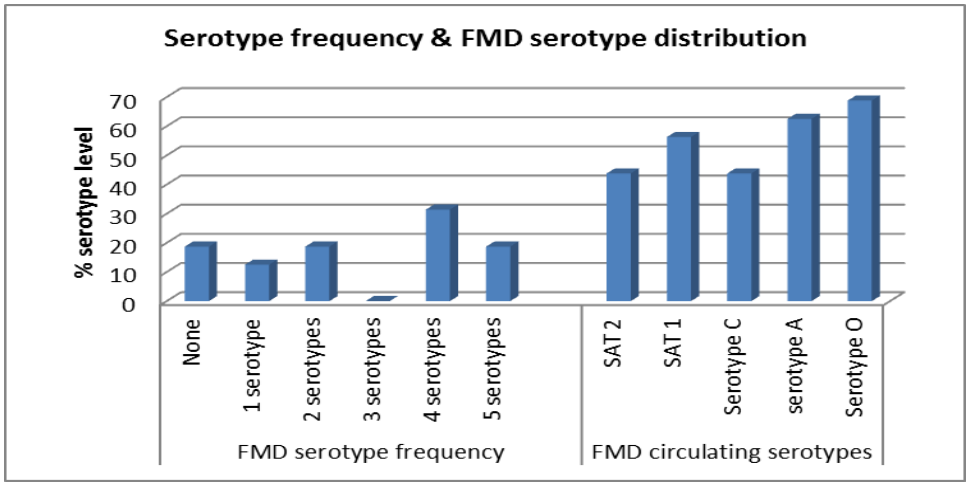


Figure 4.78: serotype frequency and distribution in Meru

4.6.4.1 Foot-and-mouth disease in porcines

A total of 8 samples were analyzed for FMD with only one animal detected to be seropositive (12.5%) (Figure 4.79).

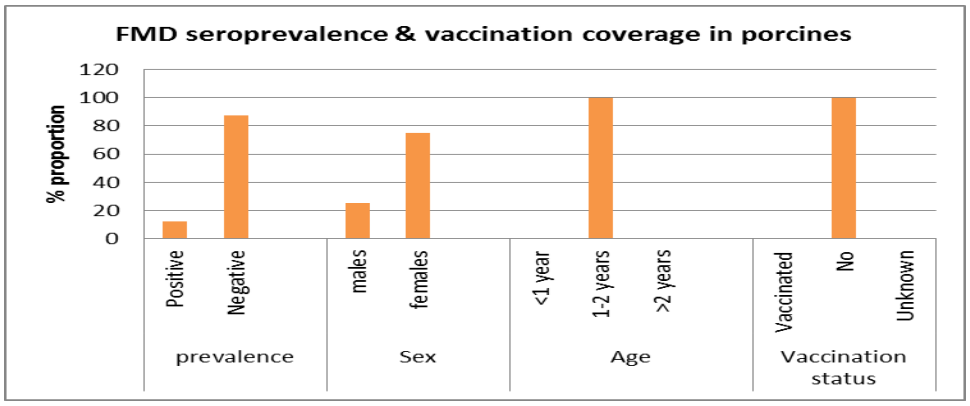


Figure 4.79: Porcine FMD prevalence in Meru

4.6.5 Laikipia County

The proportion of animals that had detectable FMD antibodies on NSP was (49.2%, 30/61). The reported vaccination cover was 63.9% (39/61) (Figure 4.80).

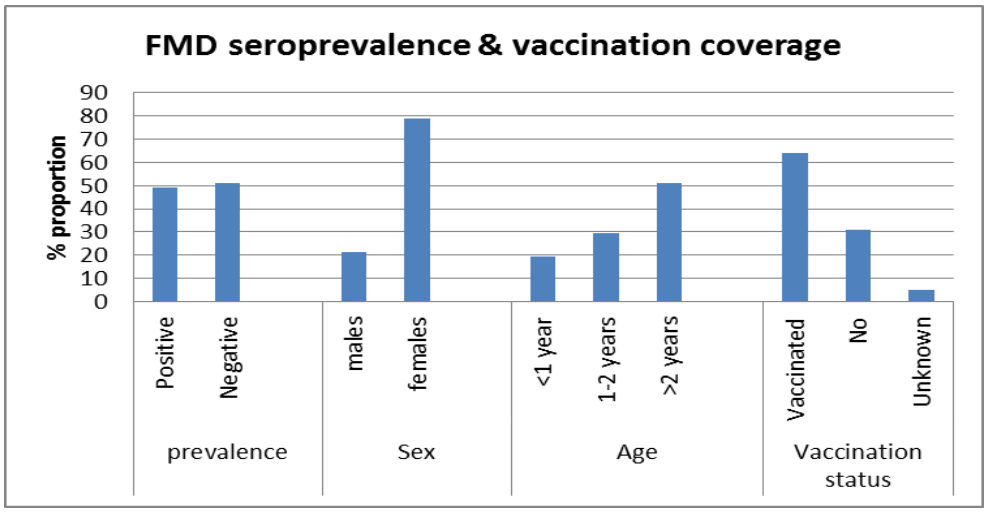


Figure 4.80: FMD prevalence and vaccination cover in Laikipia

83.3% (15/18) had at least one or more serotype detectable. Those with three serotypes were the majority with 33.3% (6/18). Significant number of serotypes came from adults >2 years (61.1%, 11/18). All the five serotypes were detected with serotype SAT 2, SAT 1 and type C being the most prevalent serotypes. All had prevalence of 61.1% (11/18) (Figure 4.81).

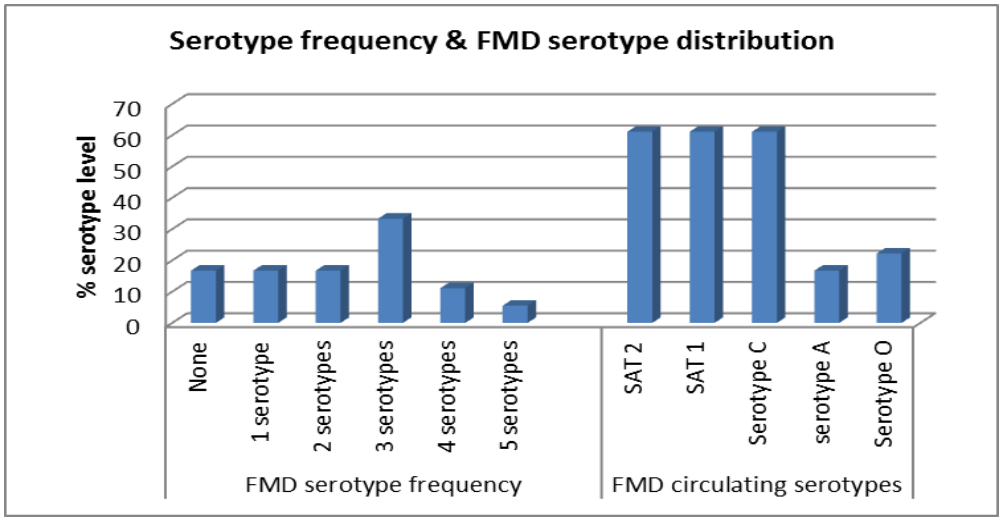


Figure 4.81: Serotype distribution and frequency in Laikipia

4.6.6 Nyandarua County

The number of sampled females and adults >2 years was high at 92.5% (37/40) and 47.5% (19/40) respectively. The reported vaccination coverage was 32.5% (13/40). The FMD seroprevalence within the county was 37.5% (15/40) (Figure 4.82).

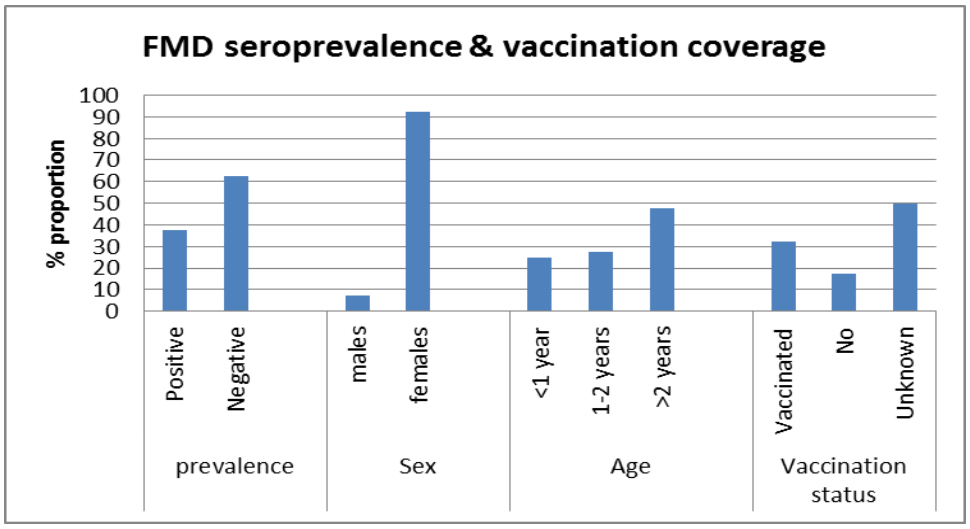


Figure 4.82: FMD seroprevalence and vaccination cover in Nyandarua

86.7% (13/15) had at least one or more serotype detectable. Majority of the animals had three circulating antibodies 26.7% (4/15). The most prevalent serotype among the five serotypes detected was serotype C with prevalence of 66.7% (10/15) and was followed by serotype SAT 2 with prevalence of 60% (9/15). Serotype O was the least prevalent with 20% (Figure 4.83).

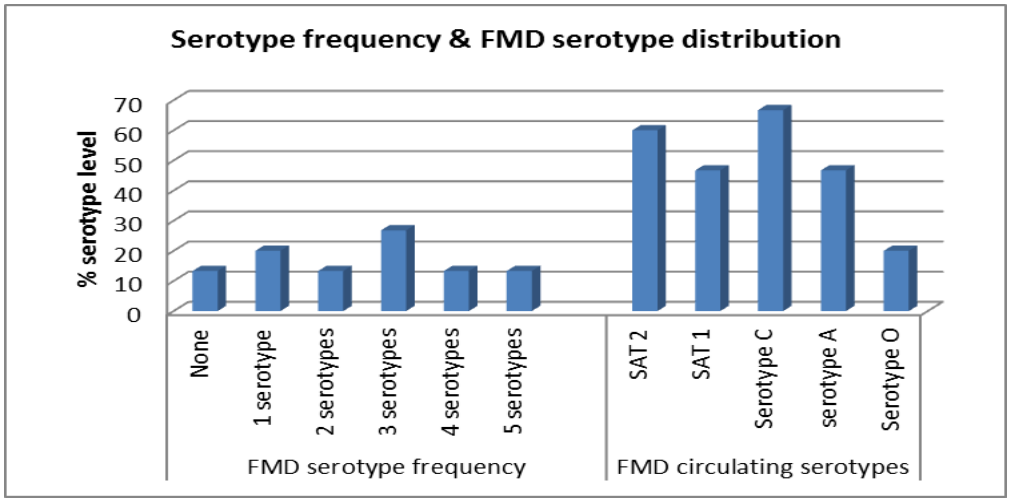


Figure 4.83: Serotype frequency and distribution in Nyandarua

4.6.7 Tharaka Nithi County

The vaccination coverage within the districts of Tharaka Nithi County was 35% (7/20). Of the 20 animals samples subjected to NSP, 8 of them showed seropositivity to FMDV antibodies giving seroprevalence of 40% (Figure 4.84).

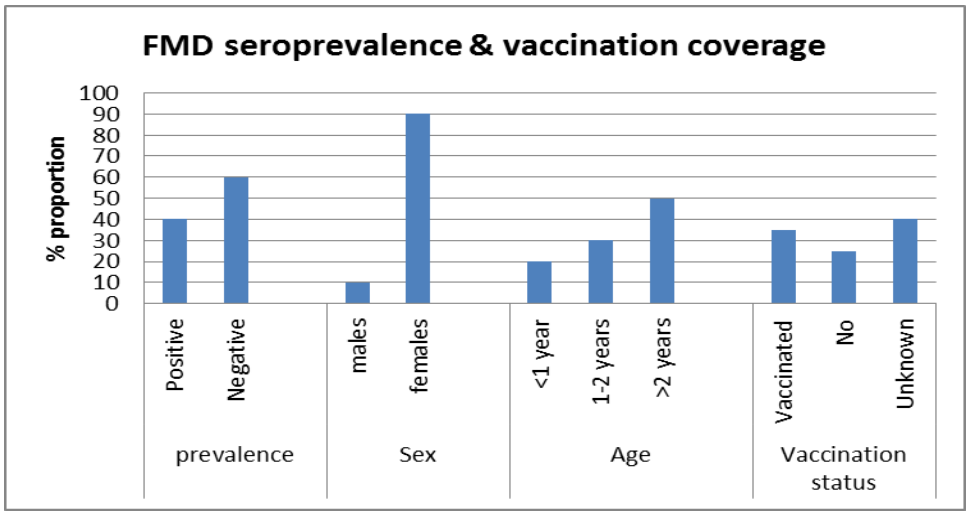


Figure 4.84: FMD seroprevalence and vaccination coverage in Tharaka Nithi

Only 8 samples were assessed for serotype distribution. 87.5% (7/8 indicated presence of at least one or more serotype while those with two serotypes had 62.5% (5/8). Only four serotypes were detected with the most prevalent serotypes being O and A at 50% each (4/8). Serotype C was negative (Figure 4.85).

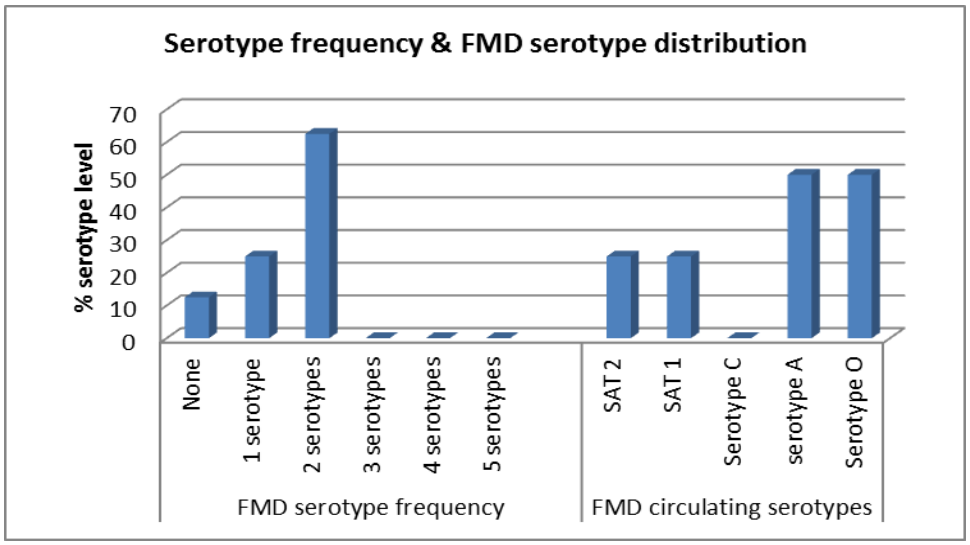


Figure 4.85: Serotype frequency and distribution in Tharaka Nithi

4.6.8 Garissa County

There was no FMD vaccination carried out in Garissa. The FMD prevalence was high compared to other counties of Somali Ecosystem (194/266 animals had FMD antibodies detectable by NSP, seroprevalence of 72.9%) (Figure 4.86).

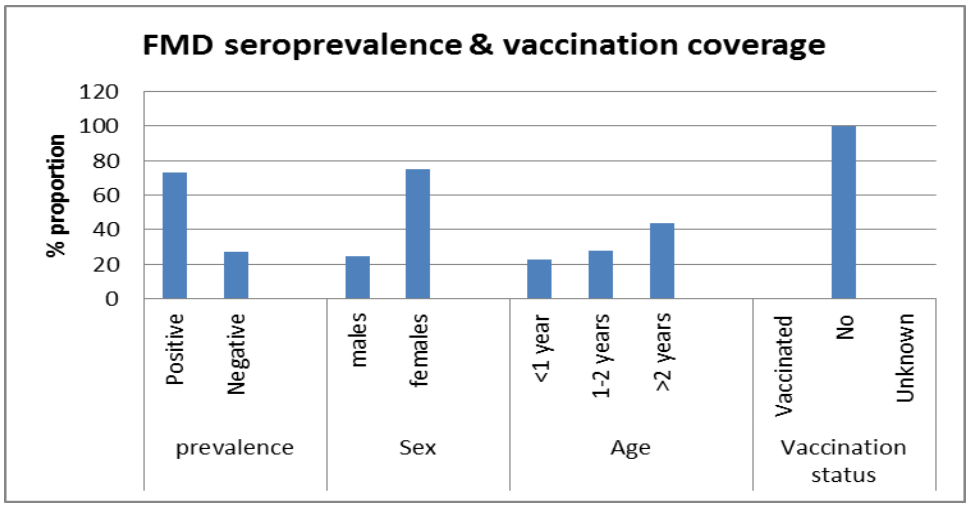


Figure 4.86: FMD prevalence and vaccination cover in Garissa

Out of the 33 samples subjected to serotype titration, 97% (32/33) had at least one or more serotype detectable. Those with three serotypes were the majority at 45.5% (15/33). All the five serotypes were detected with serotype SAT 2 being the most prevalent serotype with prevalence of 81.8% (27/33). Serotypes O and SAT 1 followed with seroprevalence of 69.7% (23/33) and 63.6% (21/33) respectively (Figure 4.87).

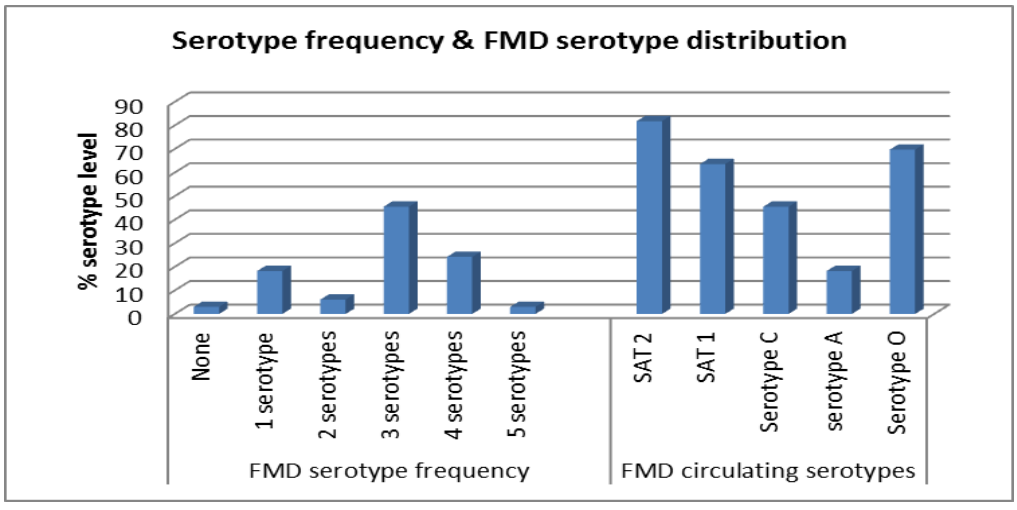


Figure 4.87: Serotype frequency and distribution in Garissa

4.6.9 Tana River County

None of the 220 animals sampled had reported history of vaccination. Among the animals sampled, females and adults >2 years, were 72.7% (160/220) and 50.5% (111/220) respectively. The seroprevalence of FMD within the county was higher than the national prevalence at 65.5% (144/220) (Figure 4.88).

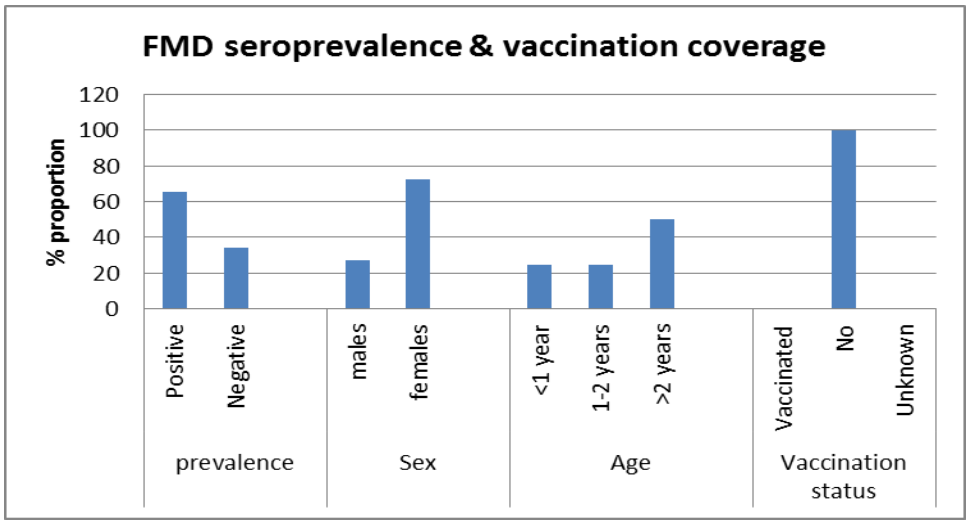


Figure 4.88: FMD seroprevalence and vaccination cover in Tana River

All the animals sampled had at least one or more serotype detectable. Significant number of animals had two serotypes circulating (67.9%, 19/28). Serotype SAT 2 was the most prevalent serotype with prevalence of 82.1% (23/28). It was closely followed by serotype SAT 1 with a prevalence of 67.9% (19/28) and serotype O with 50% prevalence (14/28). Serotype C was the least prevalent with only one animal showing detectable level of antibodies (Figure 4.89).

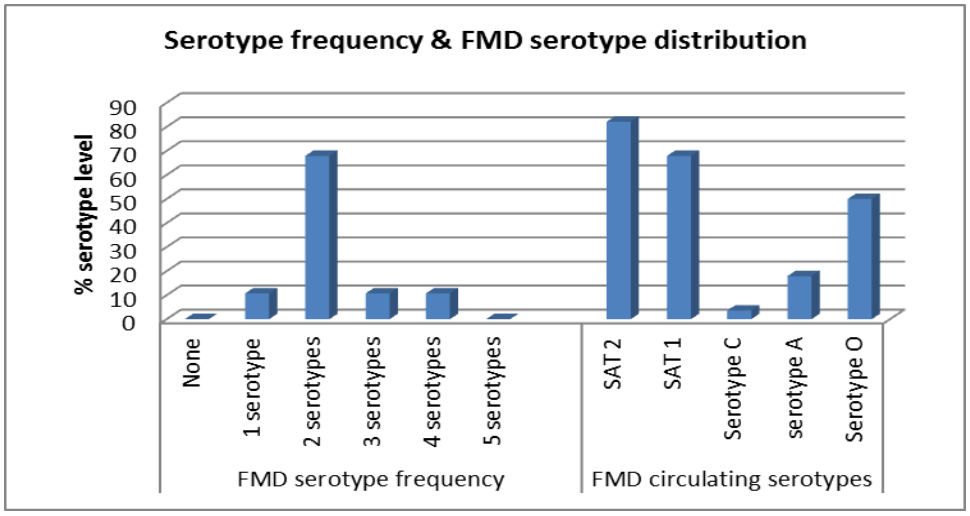


Figure 4.89: Serotype frequency and distribution in Tana River

4.7.0 Lamu County

The county had seroprevalence of 65.7% with 71/108 animals having detectable FMDV antibody levels. There was no reported vaccination within the county (Figure 4.90).

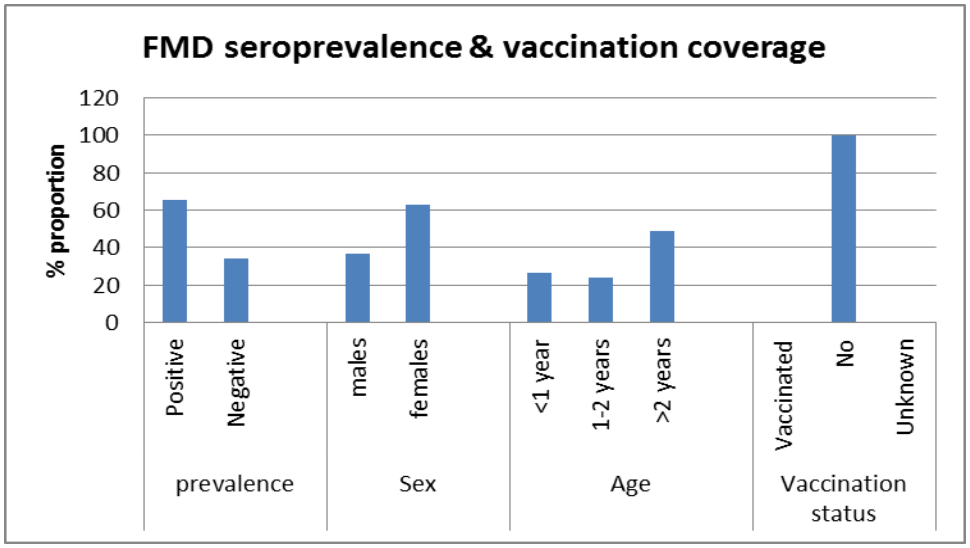


Figure 4.90: FMD seroprevalence and vaccination cover in Lamu

95.7% (22/23) had at least one or more serotype detectable by LPBE. Those with four serotypes were 34.8% (8/23) while those with three FMDV serotypes were 26.1% (6/23). Serotype SAT 2 and SAT 1 were the most prevalent serotypes with 82.6% (19/23) and 87% (20/23) prevalence respectively. It was followed by serotype O with prevalence of 65.2% (15/23). In summary, all the five serotypes were detected (Figure 4.91).

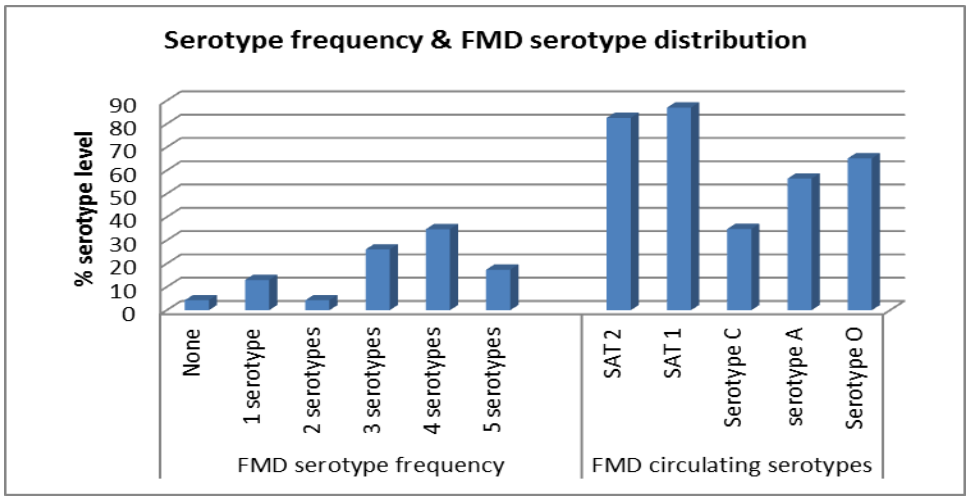


Figure 4.91: serotype frequency and distribution in Lamu

4.7.1 Marsabit County

Of the 361 animals sampled, none had any reported history of vaccination. FMD seroprevalence was low with 65 animals being seropositive on screening test (18%, 65/361) (Figure 4.92).

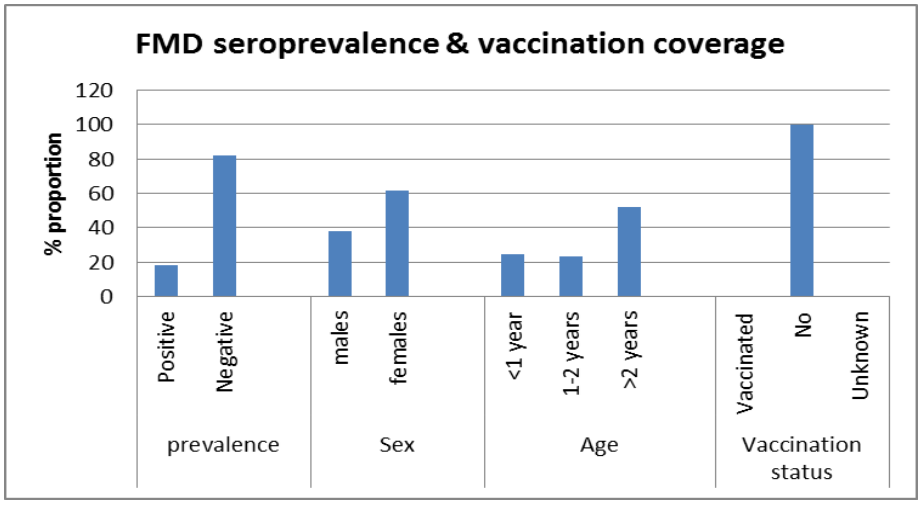


Figure 4.92: FMD seroprevalence and vaccination cover in Marsabit

Only 12 seropositive samples were subjected to serotype titration. 75% (9/12) had at least one or more serotype detectable with majority having single circulating serotype. The highest serotype frequency accounted males and those between 1-2 years of age with 66.7% (8/12) and 50% (6/12) respectively. Only four serotypes were found circulating in the districts of Marsabit County. The most prevalent serotypes circulating were serotype SAT 1 and SAT 2 with 58.3% (7/12) and 41.7% (5/12) respectively. Serotype A was the least prevalent with only one animal having detectable levels of antibodies. Serotype O was not detected (Figure 4.93).

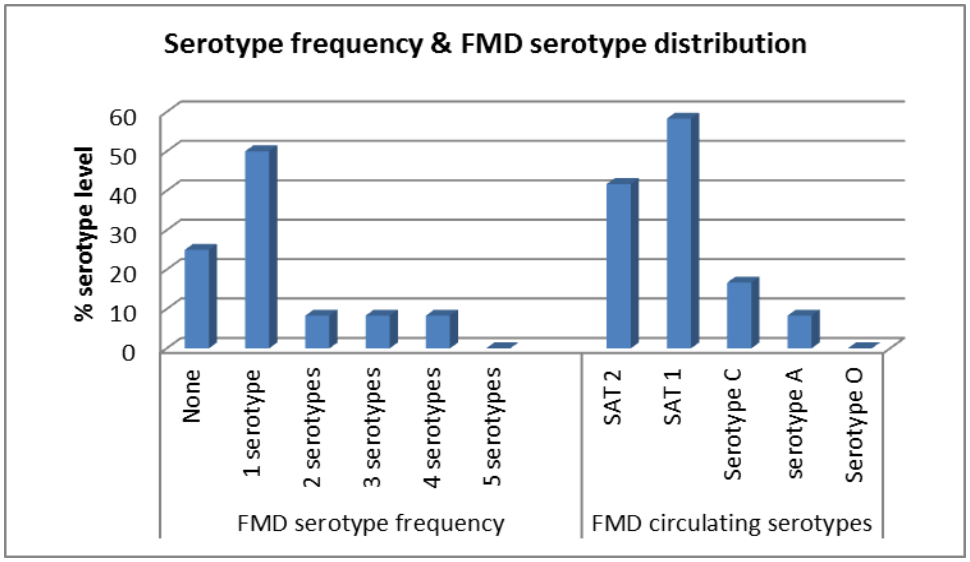


Fig 4.93: Serotype frequency and distribution in Marsabit

4.7.2 Isiolo County

It had FMD seroprevalence of 33.9% with only 20/59 animals showing seropositivity on FMD screening. All animals were unvaccinated (Figure 4.94).

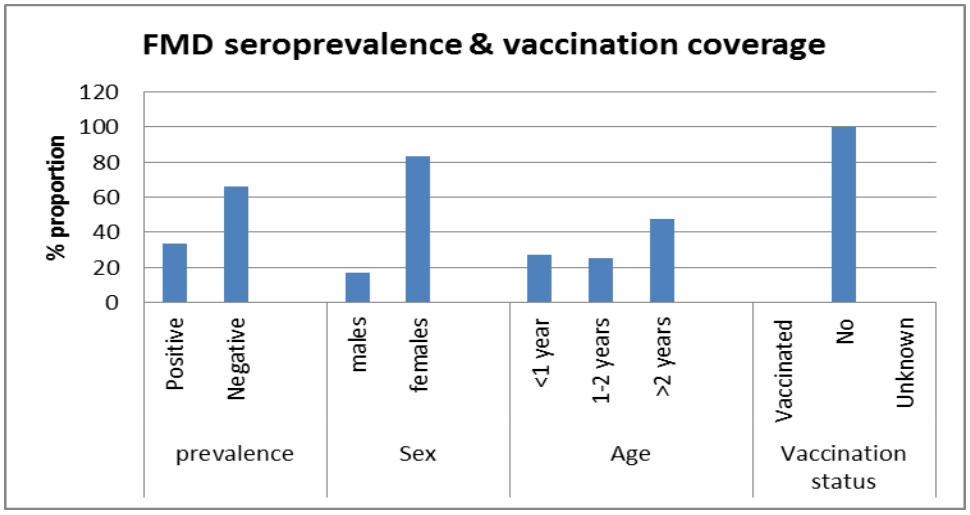


Fig 4.94: FMD prevalence and vaccination cover in Isiolo

All animals had at least one or more serotype detectable, 46.2% (6/13) had two serotypes while 15.4% (2/13) had all the five serotypes circulating. All the five serotypes were detected with serotype SAT 1 being the most prevalent serotype with all the animals having detectable levels of antibodies (100%). It was followed by SAT 2 and serotype O with 76.9% (10/13) prevalence each. Serotype A was the least prevalent serotype 15.4% (2/13) (Figure 4.95).

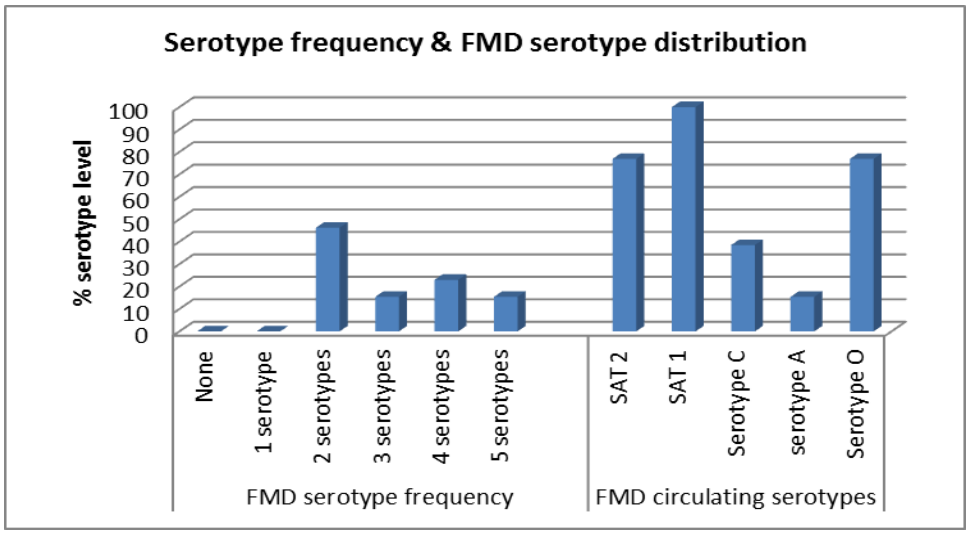


Fig 4.95: Serotype frequency and distribution in Isiolo

4.7.3 Samburu County

Of the 40 animals sampled, 40% turned seropositive to FMD screening with all being reported unvaccinated against FMD virus (Figure 4.96).

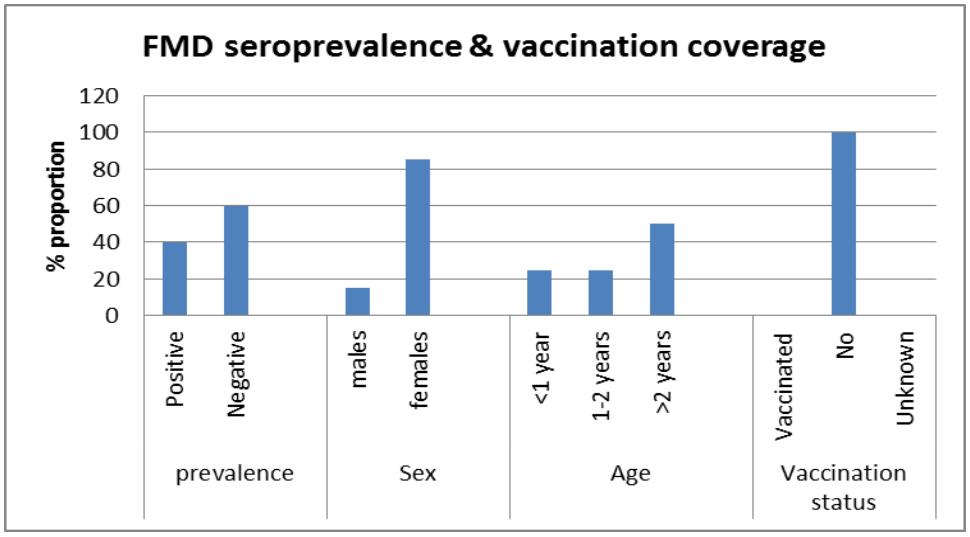


Fig 4.96: FMD seroprevalence and vaccination cover in Samburu

15/16 (93.8%) animals had at least one or more serotype detected during serotype titration. 37.5% (6/16) of them had four circulating serotypes within their bodies. A high number of serotypes came from adult animals >2 years 75% (12/16). Serotypes SAT 1 and SAT 2 were the most prevalent serotypes with 87.5% (14/16) and 81.3% (13/16) respectively. Serotype O had 50% (8/16) prevalence while serotype C was the least prevalent with 12.5% (2/16) (Figure 4.97).

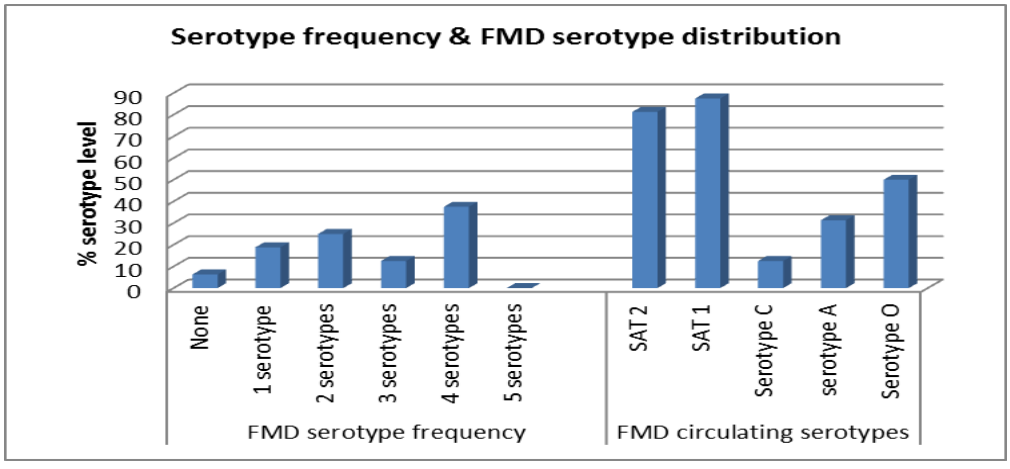


Fig 4.97: FMD serotype frequency and distribution in Samburu

4.7.4 Moyale

A total of 40 animals were sampled with only 3 animals turning seropositive (7.5%). A very high number were seronegative 92.5% (37/40). All animals sampled were reported unvaccinated (Figure 4.98).

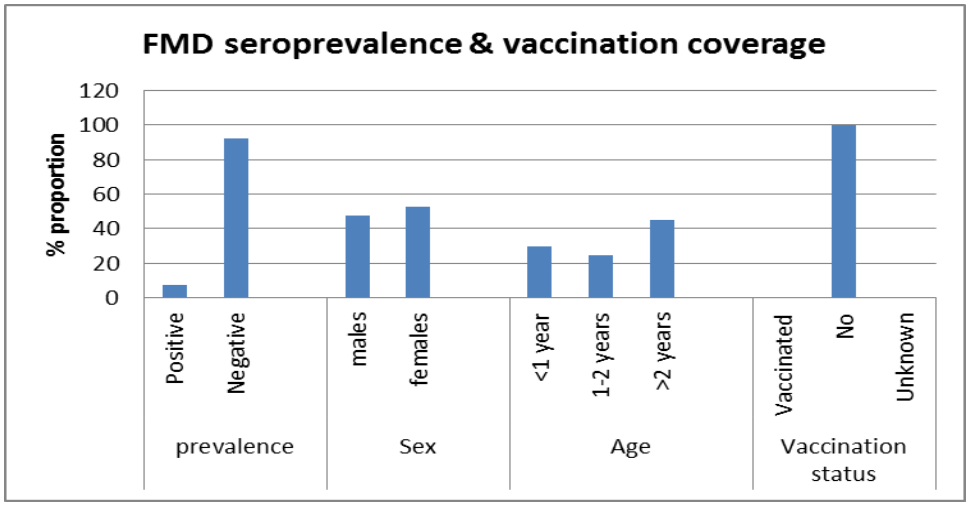


Fig 4.98: FMD seroprevalence and vaccination cover in Moyale

Of the three seropositive samples subjected to FMD serotype titration, only two animals had titratable FMD serotypes. The two animals had four circulating serotypes each. All the five serotypes were detected with serotypes O, SAT 1 and SAT 2 being the most prevalent (Figure 4.99).

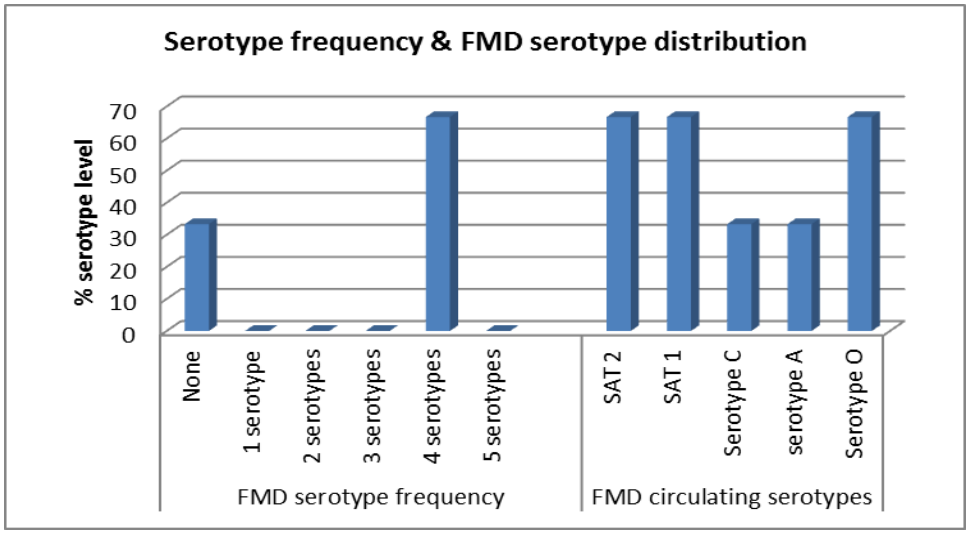


Fig 4.99: Serotype frequency and distribution in Moyale

4.7.5 Kilifi County

Kilifi county had high reported vaccination coverage of 81.3% (75/61) while the FMD seroprevalence was 25.3% (19/75) (Figure 4.10.0).

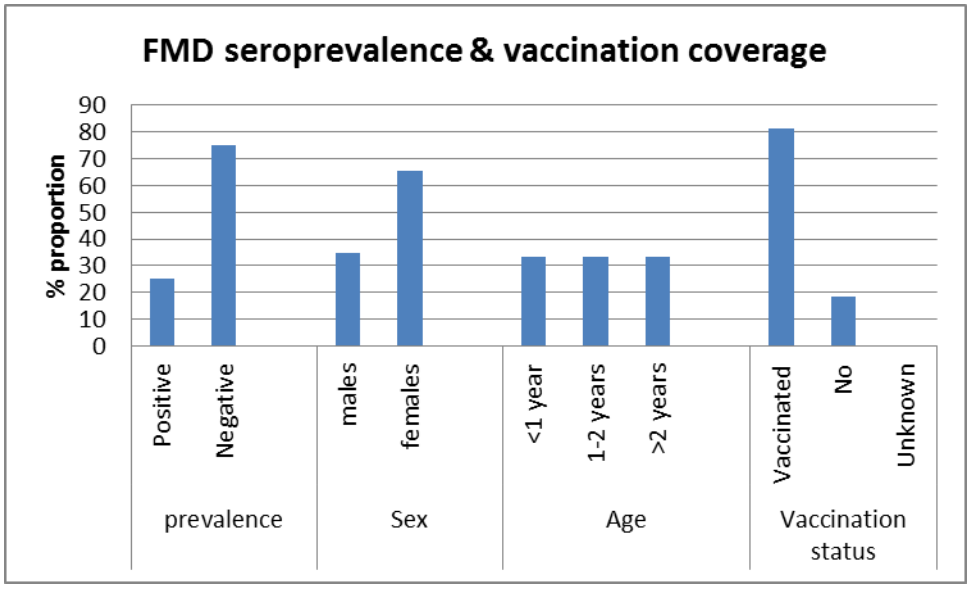


Fig 4.10.0: FMD seroprevalence and vaccination cover in Kilifi

All the five serotypes were found circulating with serotypes SAT 1 and SAT 2 being the most prevalent with 66.7% each (6/9). Serotypes C and A were the least prevalent at 11.1% each (1/9). 7/9 animals (77.8%) had at least one or more circulating serotype. 33.3% (3/9) had three circulating serotypes. A greater number of serotypes came from animals of between 1-2 years (44.4%, 4/9) and those unvaccinated (55.6%, 5/9) (Figure 4.10.1).

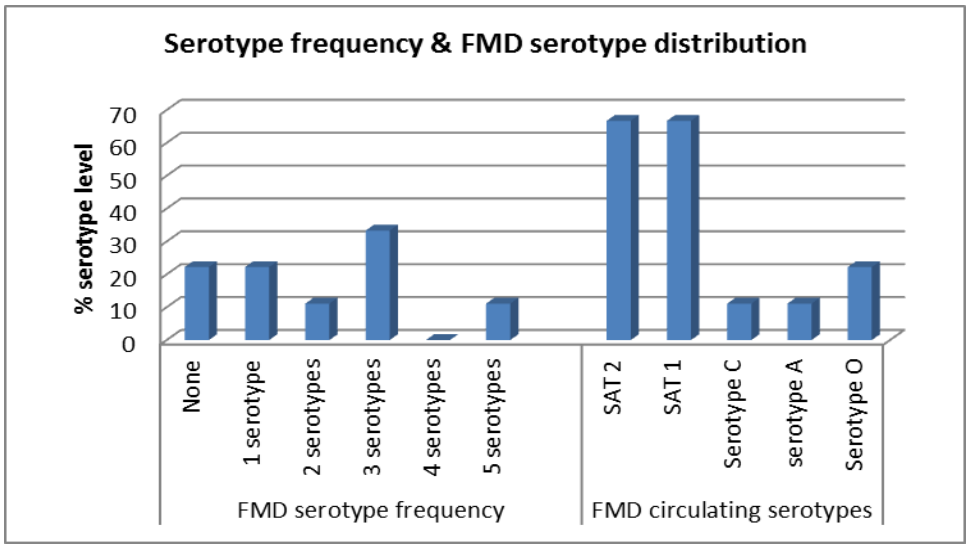


Fig 4.10.1: Serotype frequency and distribution in Kilifi

4.7.5.1 Foot and mouth disease in porcines

All the 16 porcine samples subjected to FMD NSP screening Elisa were seronegative. FMD seroprevalence was higher in bovine species than in porcine species (Figure 4.10.0 and Fig 4.10.2).

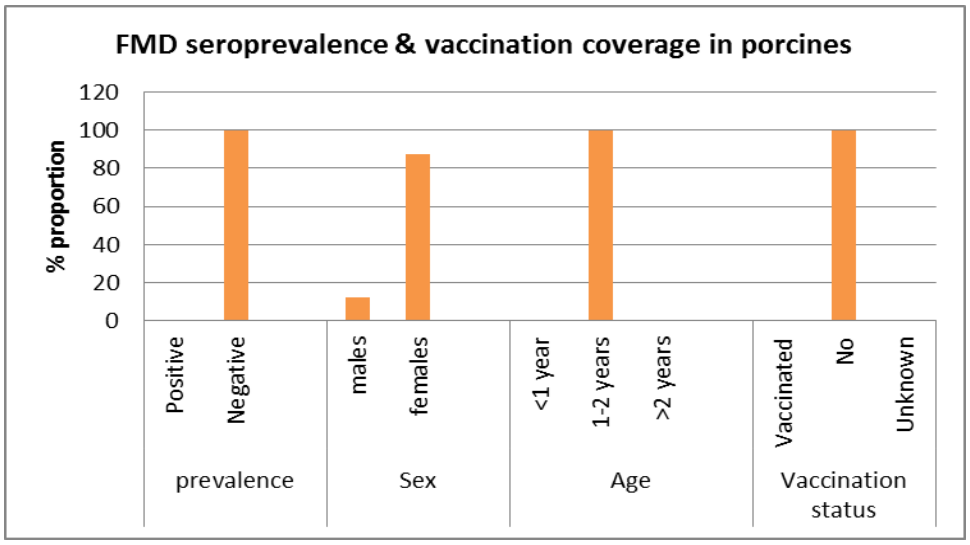


Fig 4.10.2: Porcine seroprevalence in Kilifi

4.7.6 Malindi

All the sampled animals had been reported vaccinated against FMD virus (100%). Only 8 out of the 30 animals sampled were seropositive on NSP screening (26.7% seroprevalence) (Figure 4.10.3).

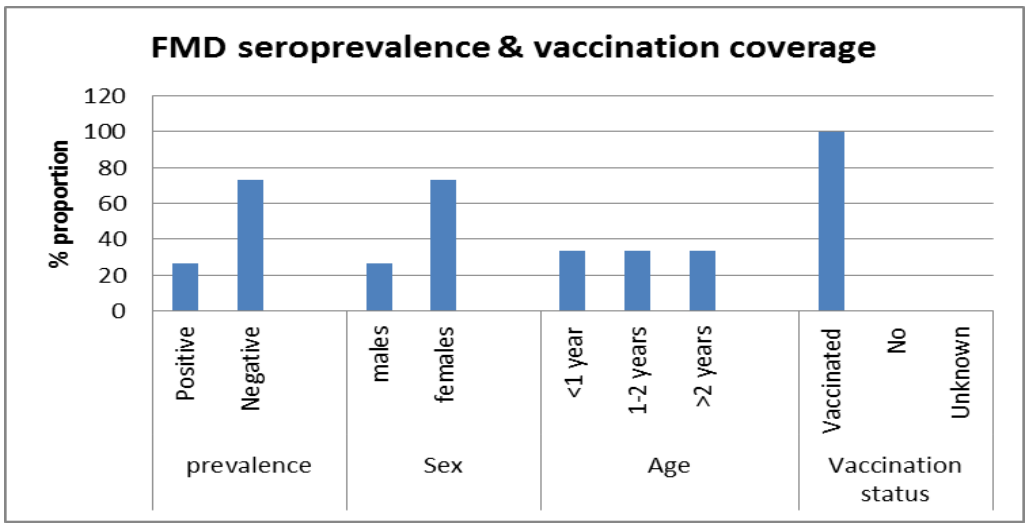


Fig 4.10.3: FMD seroprevalence and vaccination cover in Malindi

Only four animals had at least one or more serotype detected. Three animals had single serotype circulating. Three serotypes namely SAT 1, O and A was detected with SAT 1 being the most prevalent at 37.5% (3/8) (Figure 4.10.4.

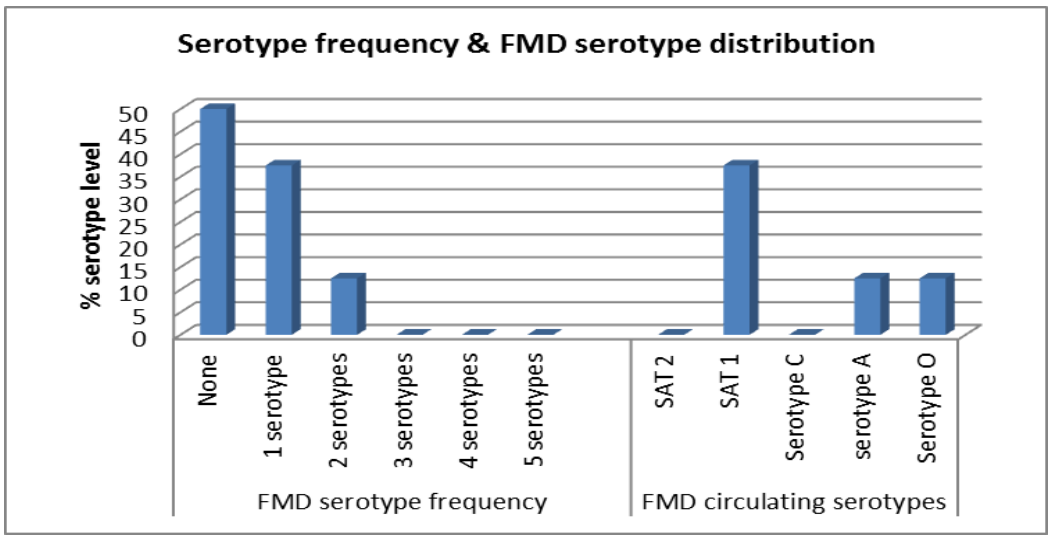


Fig 4.10.4: Serotype frequency and distribution in Malindi

4.7.7 Mombasa County

A total of 14 samples were analyzed. 6/14 animals were seropositive to FMD screening (42.9%) while 8 turned seronegative (57.1%). All the sampled animals were vaccinated against FMD virus (100% vaccination coverage) (Figure 4.10.5).

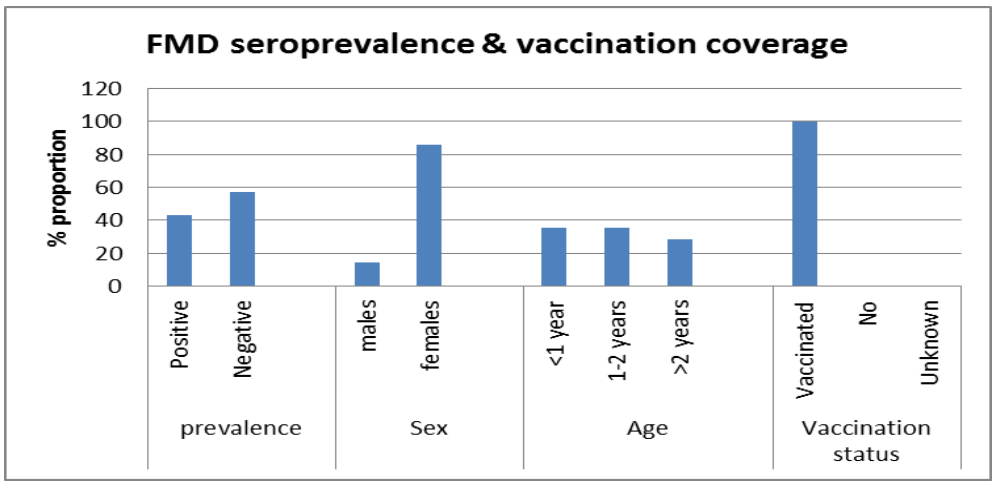


Fig 4.10.5: FMD seroprevalence and vaccination cover in Mombasa

Of the 6 seropositive animals, 3 of them had two circulating serotypes, 2 had four serotypes while one animal had single serotype circulating. All the five serotypes were detected with serotype O and SAT 1 being the most prevalent serotypes at 83.3% (5/6) each. Serotype SAT 2 was the least prevalent with one animal positive to it. All the serotypes were found circulating in female animals (Figure 4.10.6).

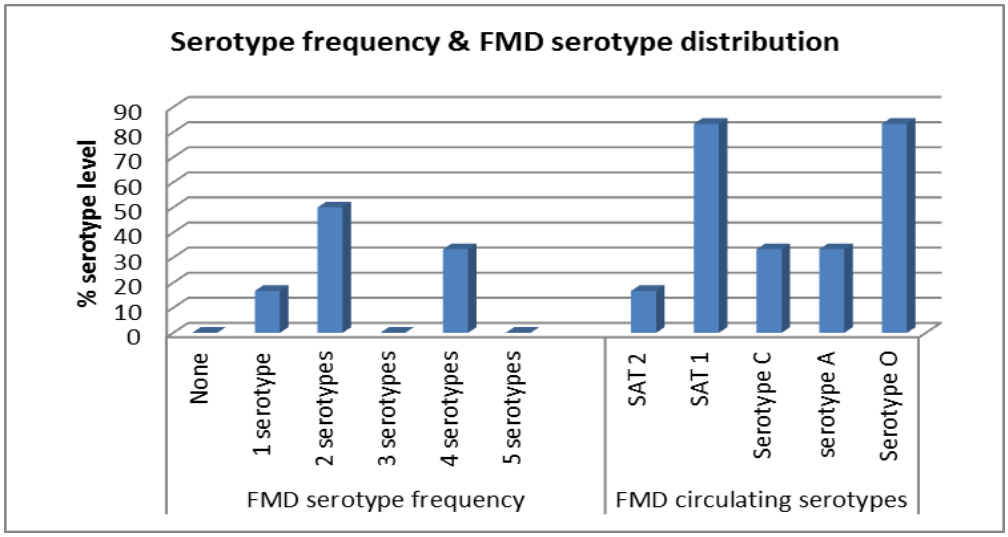


Fig 4.10.6: Serotype frequency and distribution in Mombasa

4.7.7.1 Foot and mouth disease in porcines

All the porcine species sampled were females and unvaccinated. 3/8 animals had detectable levels of FMD antibodies (FMD seroprevalence of 37.5%) (Figure 4.10.7).

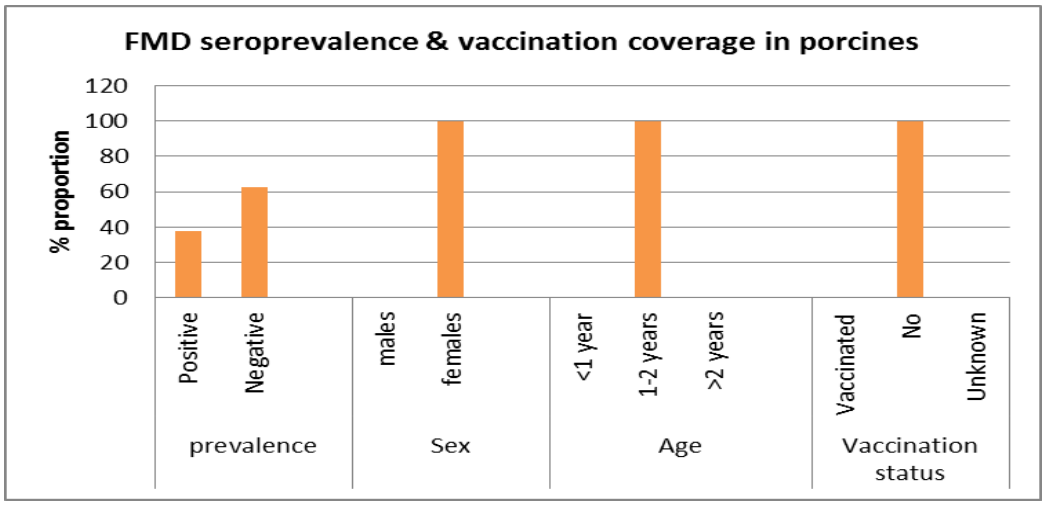


Fig 4.10.7: Porcine FMD prevalence in Mombasa

4.7.8 Kwale County

The FMD seroprevalence was 42.2% (19/45) and with reported vaccination cover of 33.3% (15/45) (Figure 4.10.8.

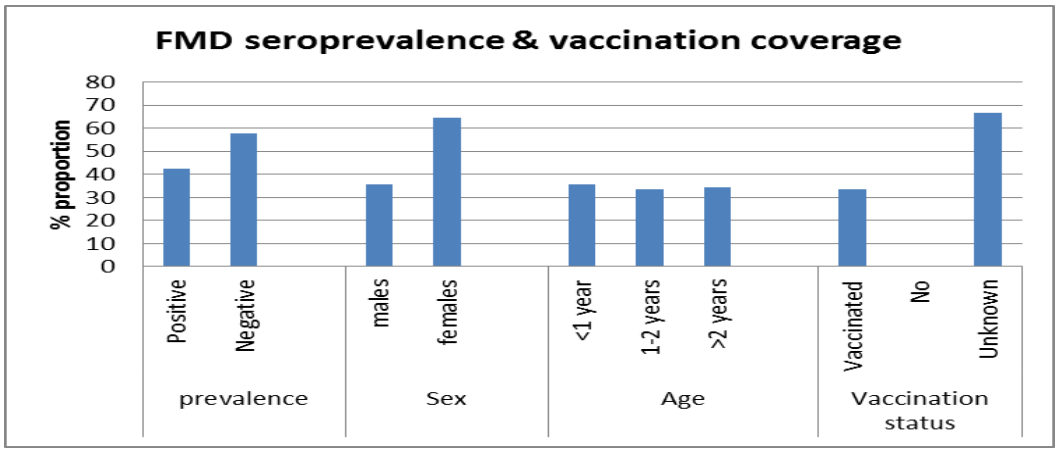


Fig 4.10.8: FMD seroprevalence and vaccination cover in Kwale

17/19 seropositive animals subjected to LPBE had at least one or more serotype detectable. Majority of them had between three and five circulating serotypes. 36.8% (7/19) had three serotypes, 26.3% (5/19) had for serotypes while 21%

(4/19) had all the five serotypes. All the five serotypes were detected with SAT 1 being the most prevalent serotype at 84.2% (16/19). Serotype SAT 2 and serotype O followed with prevalence of 78.9% (16/19) and 68.4% (13/19) respectively. Serotype A was the least prevalent serotype 36.8% (7/19) (Figure 4.10.9).

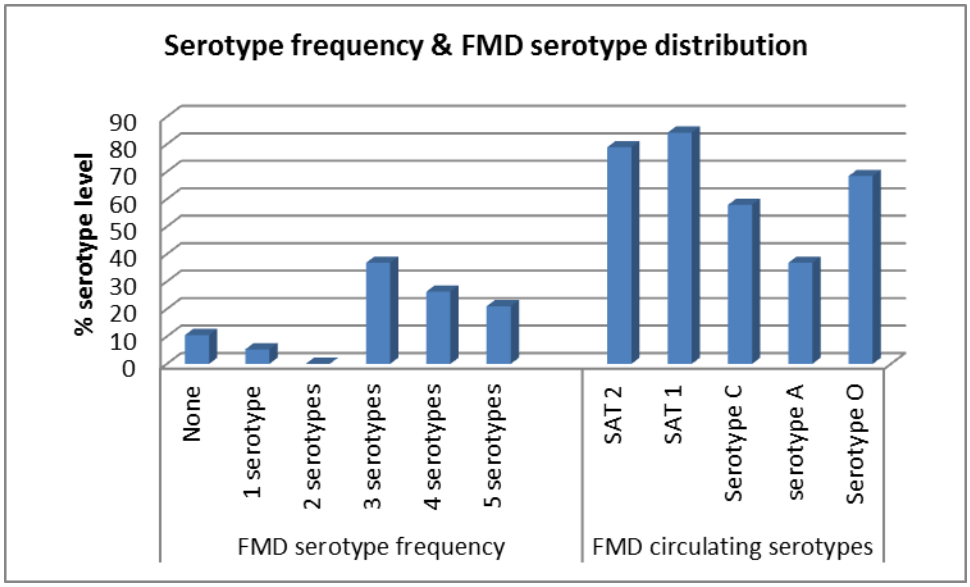


Fig 4.10.9: Serotype frequency and distribution in Kwale

4.7.9 Taita Taveta County

66/164 animals sampled showed detectable levels of FMD antibodies on NSP screening (prevalence 40.2%). The reported vaccination coverage was high at 87.8% (144/164) (Figure 4.11.0)

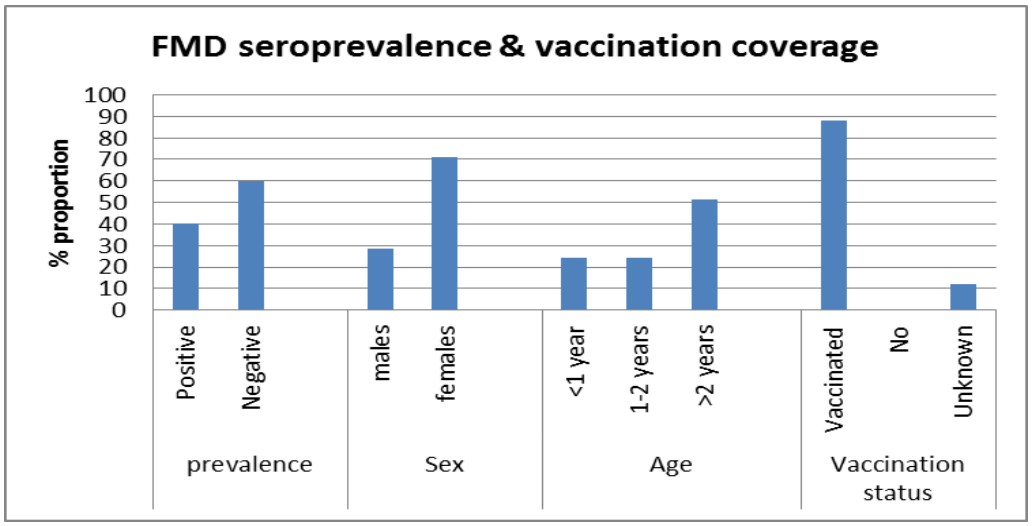


Fig 4.11.0: FMD seroprevalence and vaccination cover in Taita Taveta

13/16 samples subjected to LPBE serotype titration test had at least one or more serotype detectable. A high number of animals had between three and five serotypes. 5/16 animals (31.3%) had three circulating serotypes while other five animals (31.3%, 5/16) had five serotypes. All the five serotypes were detected with serotype O and SAT 2 being the most prevalent serotypes at 81.3% (13/16) each (Figure 4.11.1).

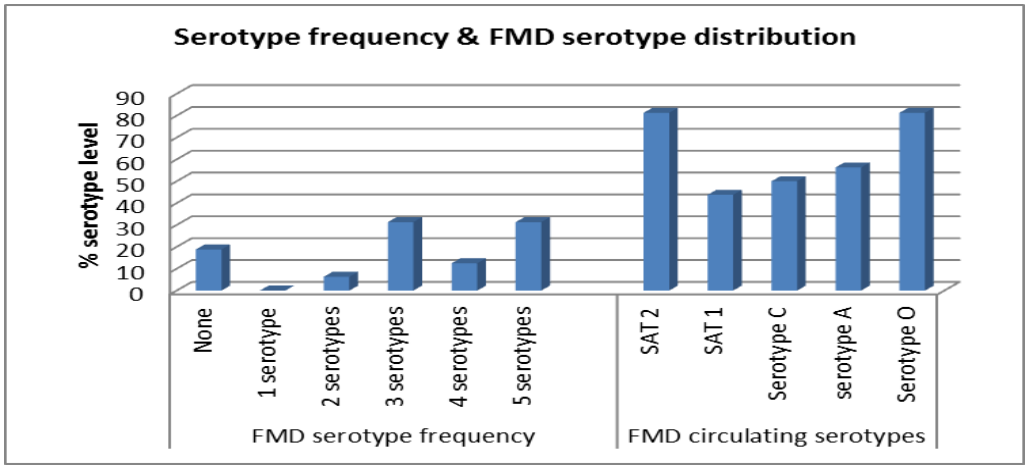


Fig 4.11.1: Serotype frequency and distribution in Taita Taveta

4.7.9.1 Foot-and-mouth in porcines

FMD prevalence was 62.5% (5/8). FMD prevalence in porcine species was higher compared to that of bovine species (40.2%) (Figure 4.11.2 and Figure 4.11.0).

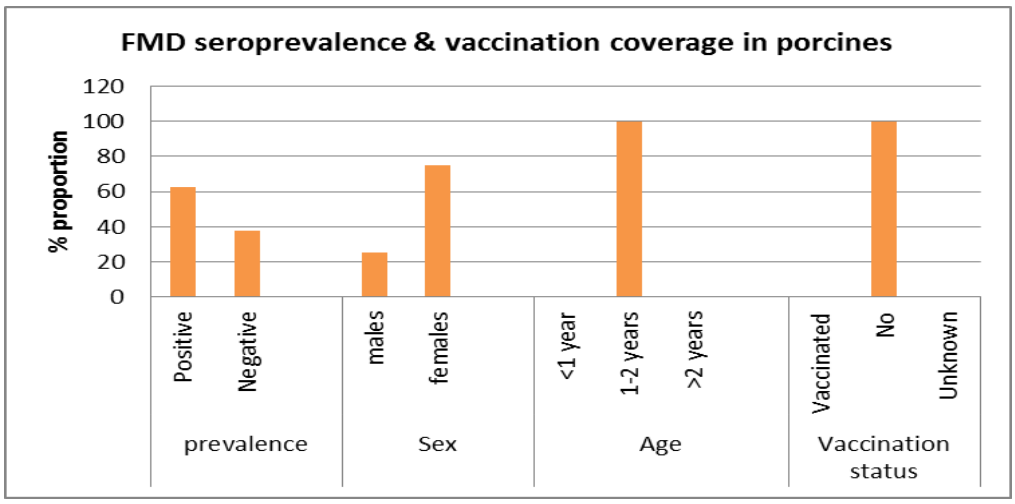


Fig 4.11.2: Porcine FMD prevalence in Taita Taveta

4.8 Foot-and-mouth disease seroprevalence and serotype distribution along borderlands

4.8.1 Kenya/Somalia Border (Somali Ecosystem)

The North Eastern counties that border Somalia include Mandera, Wajir and Garissa. The mean FMD seroprevalence along that border was 38.1% and was lower than the national seroprevalence. The prevalence was highest in Garissa at 72.9% and lowest in Mandera at 8.3% (Figure 4.23). The most prevalent serotypes circulating within the Somali Ecosystem were serotype SAT 1 (found in all the counties), SAT 2 and type O. Serotype C and A was the least prevalent. Mandera county had one detectable serotype (SAT 1). All the animals sampled from the counties bordering Somalia were reported unvaccinated (Figure 4.11.3)

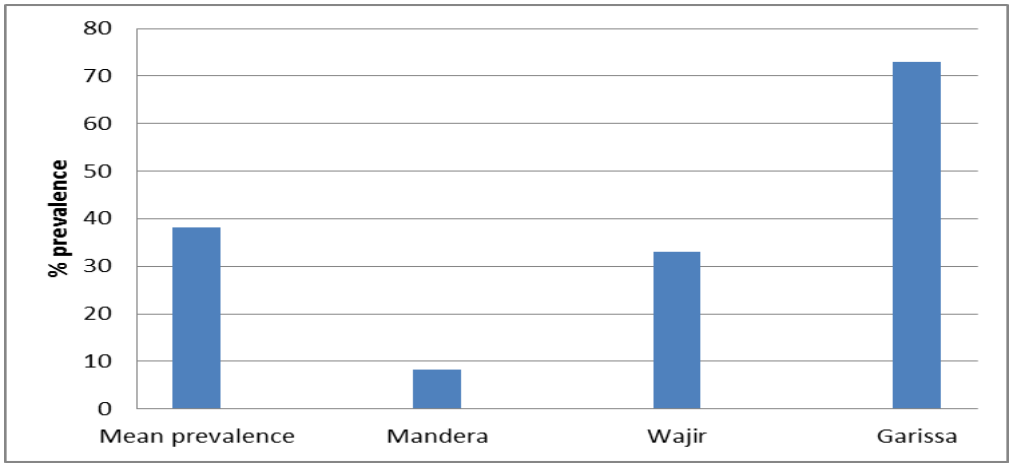


Fig 4.11.3: FMD prevalence along the Kenya-Somalia borderland

4.8.2 Kenya-Ethiopian Border

Turkana, Marsabit, Moyale, Wajir and Mandera counties borders Ethiopia.

The mean seroprevalence was 29.4% and was comparably lower than the national seroprevalence. The seroprevalence for Turkana county was high at 80% while that of Moyale was low at 7.5% (Figure 4.11.4).

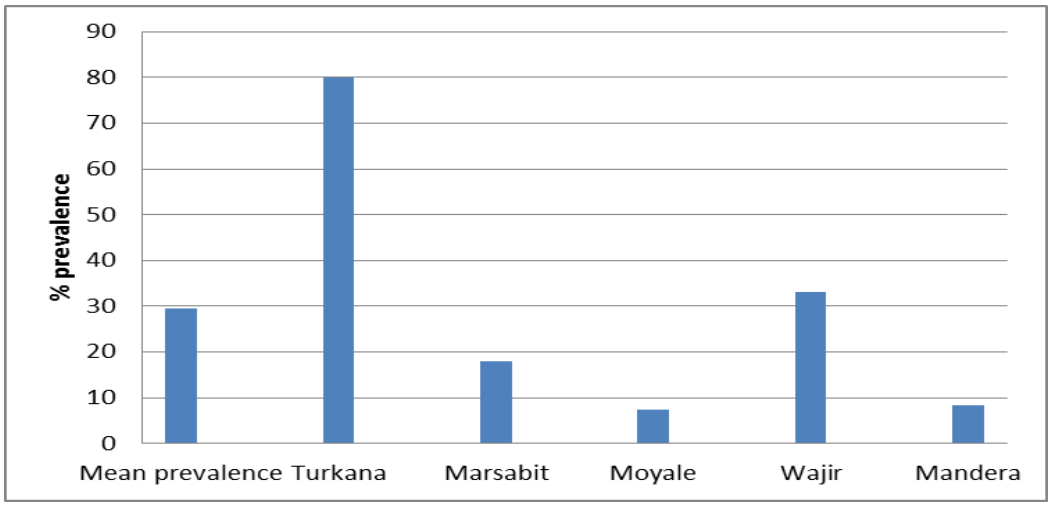


Fig 4.11.4: FMD prevalence along the Kenya-Ethiopia border

Serotype SAT 1 was the most prevalent serotype within the Kenya-Ethiopia border. It was followed closely followed by serotype SAT 2 and type C. Serotype O and A were the least prevalent. There was no reported FMD vaccination carried out in the borderland above.

4.8.3 Kenya-Uganda Border (Turkana, Karamojong, Pokot Ecosystem)

The counties that border Uganda include; Turkana, West Pokot, Trans-Nzoia and Bungoma with mean FMD seroprevalence of 95%. The seroprevalence was highest in West Pokot, Trans-Nzoia and Bungoma at 100% (Fig 4.11.5).

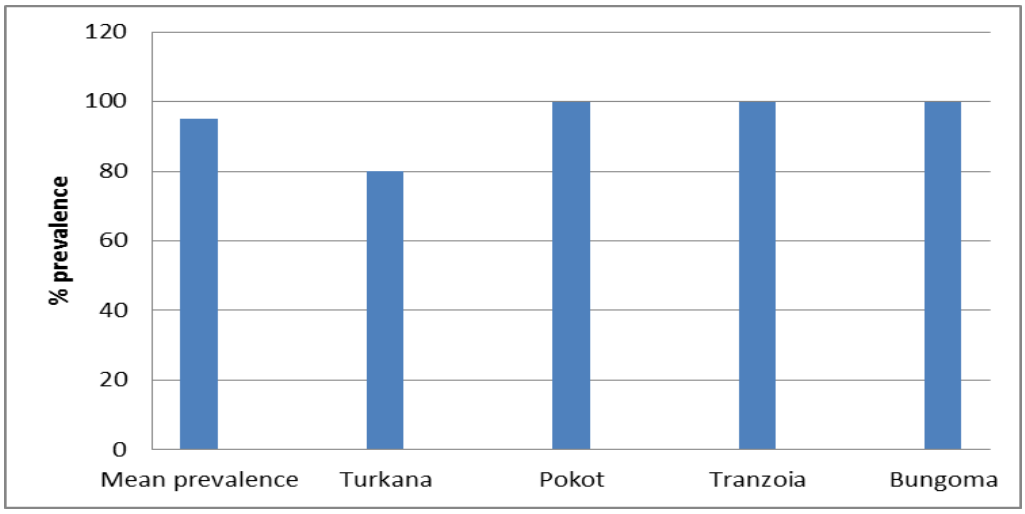


Fig 4.11.5: FMD prevalence along the Kenyan-Ugandan border

Serotype C, SAT 1 and A were responsible for the high FMD seroprevalence. Serotype SAT 2 and O had the lowest presence with SAT 2 not detectable in Trans-Nzoia and Bungoma counties. FMD vaccination was reported done only in Trans-Nzoia (73.3%).

4.8.4 Kenya-Tanzania border (Maasai Ecosystem)

The counties that border Tanzania include; Migori, Narok, Kajiado, Taita Taveta and Kwale. The mean seroprevalence was 63.2% which was higher than the national seroprevalence of 52.4%. The FMD seroprevalence was highest in Narok at 90.4% and lowest in Taita Taveta county 40.2% (Fig 4.11.6).

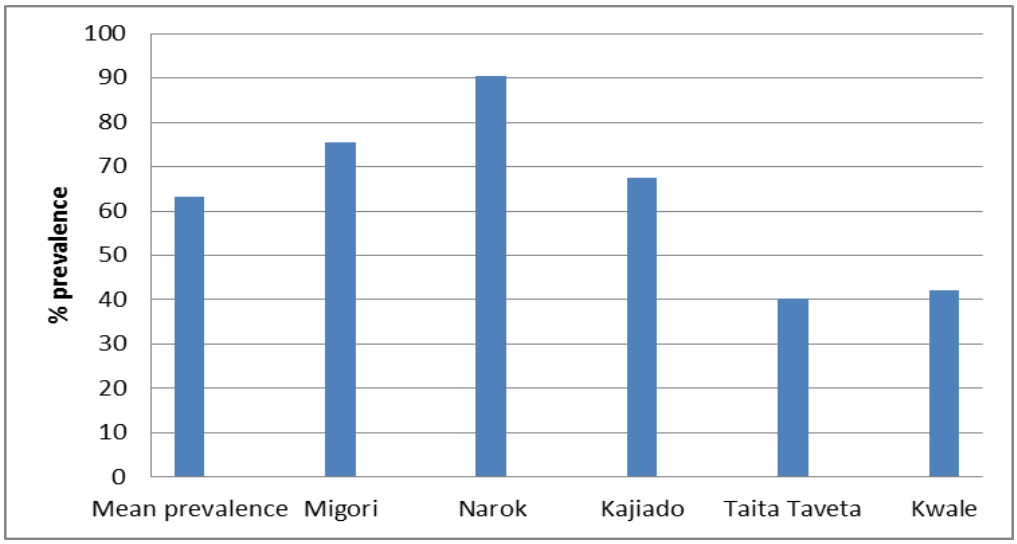


Fig 4.11.6: FMD prevalence along the Kenya-Tanzania border

Serotypes SAT 1, type C and SAT 2 had higher seroprevalence compared to serotype O and type A. The mean vaccination cover at the Kenya Tanzania border (Maasai Ecosystem) was 25.6%. FMD vaccination was reported carried out in Taita Taveta at 87.8% while Kajiado had the lowest vaccination cover at 6.8%. There was no reported vaccination in Narok and Migori counties.

4.9 FMD seroprevalence between pastoral and non-pastoral areas

4.9.1 Pastoral areas (Agro-ecological zone V-VII)

The mean seroprevalence in the pastoral areas was 53% and was closely similar to the national seroprevalence. The FMD prevalence was high in the southern pastoral areas at 58.2% as compared to northern pastoral areas which had 49.4% seroprevalence (Figure 4.11.7).

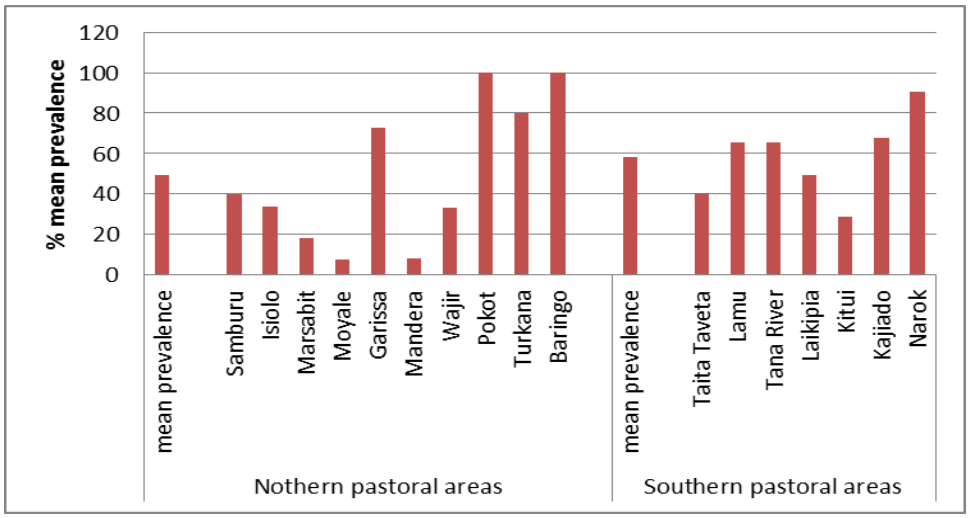


Fig 4.11.7: FMD prevalence in pastoral areas of Kenya

4.9.2 Non-pastoral areas (AEZ I-IV)

The mean seroprevalence of FMD in non-pastoral areas was 58.6% and was higher compared to that of pastoral areas which stood at 53% (Fig 4.11.8).

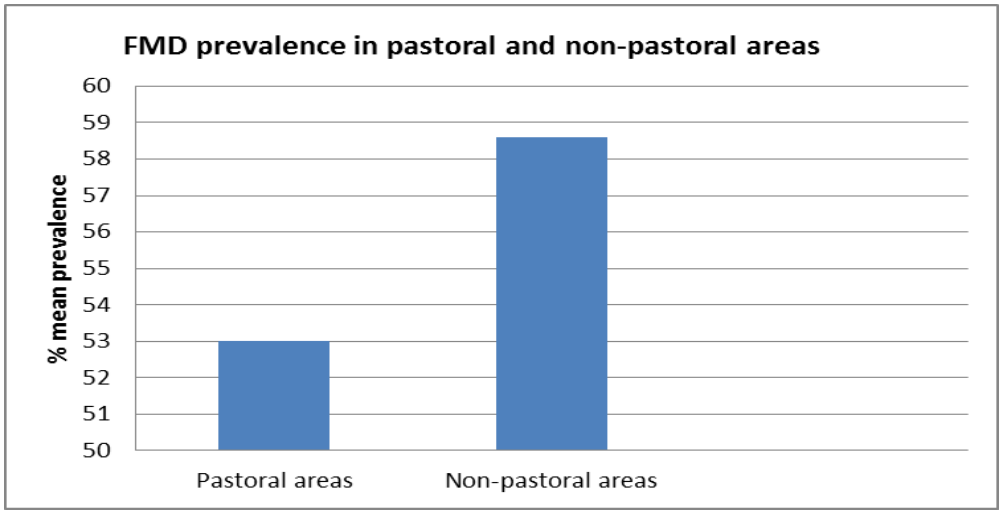


Fig 4.11.8: FMD prevalence in pastoral and non-pastoral regions

4.10 FMD seroprevalence in the proposed disease free zones (DFZ)

4.10.1 Coastal DFZ

The coastal DFZ will act as disease buffer zone for animals in the following counties: Kilifi, Taita Taveta, Kwale, Lamu, Tana River, Malindi, Garissa, Mandera and Wajir. The mean FMD seroprevalence within the coastal DFZ was 42.2% which was slightly lower than the national seroprevalence. Vaccination was reported to be carried out in only four counties (Kilifi 81.3%, Taita Taveta 87.8%, Kwale 33.3% and Malindi 100%) (Fig 4.11.9).

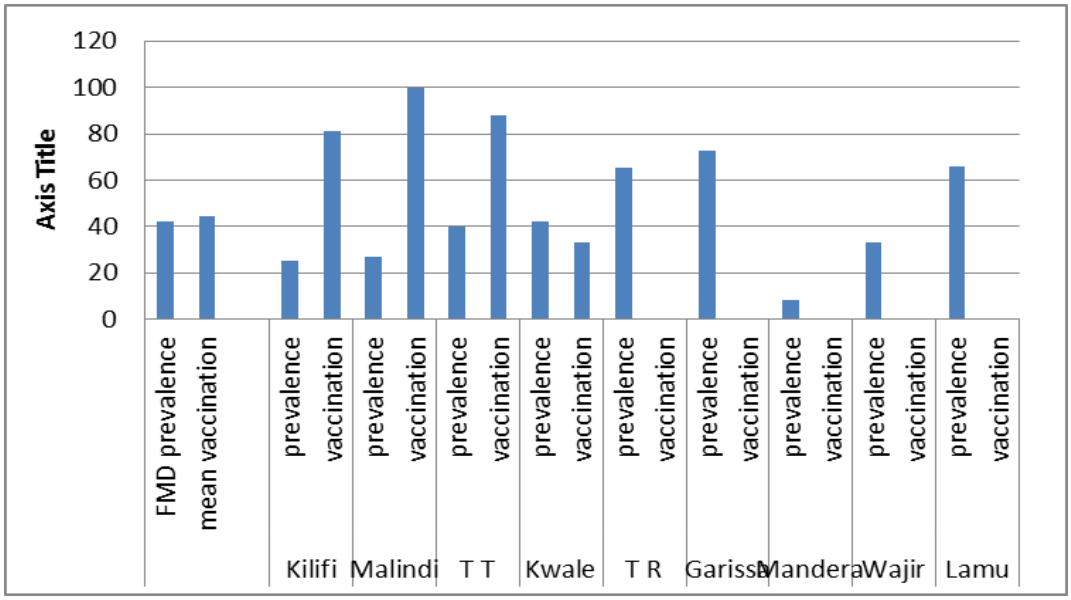


Fig 4.11.9: FMD prevalence and vaccination cover in Coastal DFZ

FMD seroprevalence was highest in Lamu and Tana River counties. Amongst the five serotypes circulating in the coastal DFZ, serotypes SAT 1, SAT 2 and type O were the most prevalent with occurrence across the nine counties that make up the DFZ.

4.10.2 Laikipia/Isiolo complex DFZ

Laikipia/Isiolo complex will act as fattening and disease control zone targeting animals from northern pastoral areas including: Laikipia, Isiolo, Samburu, Meru, Marsabit, Moyale, Mandera and Wajir. The mean FMD seroprevalence within the Laikipia/Isiolo complex was 28.1%. All the five serotypes were found circulating. Serotypes SAT 1, SAT 2 and type O were the most prevalent serotypes. Only two counties (25%) reported to have carried out some level of FMD vaccination (Laikipia 63.9% and Meru 41.6%).

4.10.3 Machakos/Kajiado DFZ

The Machakos/Kajiado disease free zone will act as disease buffer zone for animals from the following counties; Machakos, Makueni, Kajiado, Kitui and Tana River. The mean seroprevalence was 46.4% with the highest FMD seroprevalence witnessed in Kajiado (67.6%). Serotypes SAT 1, SAT 2 and type O were the most prevalent serotypes except in some counties like Kajiado which showed serotype C and A with higher prevalence. Three out of five counties reported to have carried out some FMD vaccination (Kajiado 6.8%, Makueni 1.6%, and Kitui 9.8%).

4.10.4 North Rift DFZ

North rift disease free zone comprises of the following counties; Uasin Gishu, Nandi, Trans Nzoia, Elgeyo Marakwet, Turkana, Pokot, Kakamega and Bungoma counties. The mean seroprevalence was very high at 97.5%. Serotypes C and A were found to have a high level of circulation compared to other serotypes especially SAT 1 and O. Despite the very high level of FMD seroprevalence, the reported FMD vaccination was very low with only three counties 37.5% (3/8) showed to have carried out (Uasin Gishu 33.3%, Nandi 20% and Trans Nzoia 73.3%).

4.10.5 Central Kenya DFZ

It encompasses the following counties: Nyeri, Nyandarua, Nakuru, Baringo and Bomet. The mean FMD seroprevalence was 47.1% with Baringo having the highest level of seroprevalence 100%. Serotypes C, A and SAT 1 were the most

prevalent. O and SAT 2 were undetected in some counties. Four counties reported to have carried out some FMD vaccination (Figure 4.12.0)

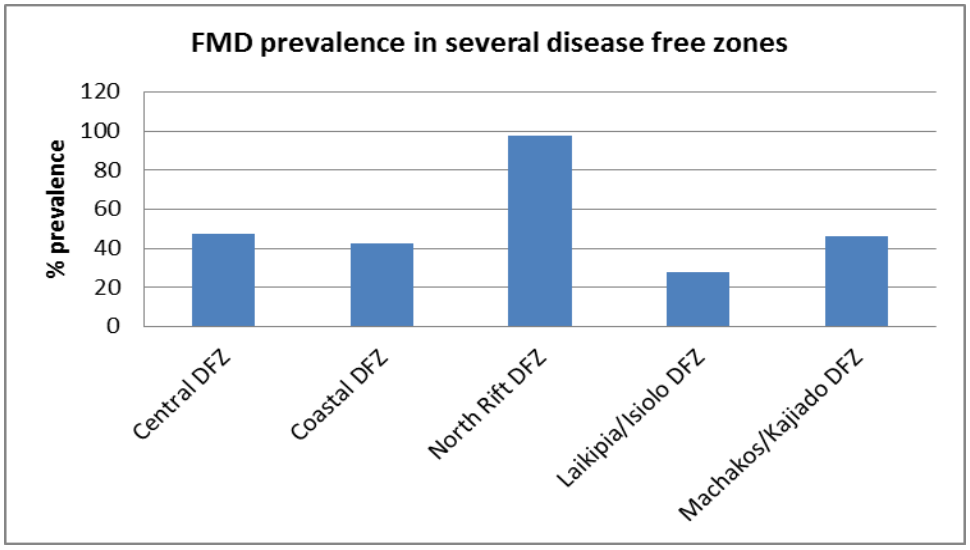


Fig 4.12.0: FMD seroprevalence in several proposed disease free zones

4.11 FMD seroprevalence around national game parks and game reserves

4.11.1 Sibiloi national park

The park is located in Marsabit and borders Turkana to the West. The mean FMD seroprevalence around the park was 49%. Serotypes C and SAT 1 were the most prevalent serotypes.

4.11.2 South Turkana game reserve

The park is located in Southern part of Turkana. It borders two other counties namely Pokot and Baringo. FMD seroprevalence around this game reserve was high and stood at 93.3%. Serotypes C and A were the most prevalent.

4.11.3 Marsabit national game reserve

It's located in Marsabit and borders other two counties namely Wajir and Moyale. The mean seroprevalence of FMD around this reserve was low at 19.5%. SAT 1 and SAT 2 serotypes had notable levels of circulation.

4.11.4 Losai national game reserve

The game reserve is located in Marsabit County. It borders Wajir and Isiolo counties. The mean FMD seroprevalence around this game reserve was 28.3% with serotypes SAT 1 and SAT 2 being the most prevalent.

4.11.5 Samburu national game reserve

The game reserve is located in Samburu County and shares border with other three counties namely Isiolo, Marsabit and Turkana. The mean seroprevalence of FMD was 43% with serotypes SAT 1, SAT 2 and type O being the most prevalent serotypes although C and A were prevalent in Turkana.

4.11.6 Meru national park

The park is located in Meru county. It is surrounded by five other counties namely; Isiolo, Laikipia, Nyeri, Tharaka Nithi and Tana River. It had mean FMD seroprevalence of 38.2%. Serotypes SAT 1, SAT 2, O and type C had significant levels of prevalence.

4.11.7 Mt. Elgon national park

The park is located in Bungoma county which shares border with two other counties namely Trans-Nzoia and Kakamega. The Mean FMD seroprevalence was the high at 100%. Serotypes C and A were the most prevalent foot and mouth disease serotypes.

4.11.8 Mt. Kenya and Aberdare national parks

The national parks are located within Nyeri County which had the lowest FMD seroprevalence in bovine species (5.3%). The parks shares borders with Nyandarua, Laikipia, Meru and Nakuru counties. The mean seroprevalence of FMD was 30%. Serotypes SAT 1, C and SAT 2 were the most common serotypes circulating in bovines around these parks.

4.11.9 Maasai Mara game reserve

The game reserve is located within Narok County which shares border with Kajiado, Nakuru and Migori counties. The FMD seroprevalence around the game reserve was higher than the national prevalence at 64.1%. The serotypes found to highly circulate within this area were SAT 1 and type C.

4.12.0 Lake Nakuru and Hells Gate national parks

The parks are located within Nakuru County which borders Narok, Kajiado, Nyandarua, Laikipia and Baringo counties. The mean FMD seroprevalence was 61.2%. Serotypes C and SAT 1 were the most prevalent serotypes and was followed by serotype A

4.12.1 Amboseli national park

The park is located within the Kenya/Tanzania border in Kajiado County. It borders Narok and Taita Taveta counties. The FMD seroprevalence around the park was 66.1% with serotypes SAT 1, SAT 2 and C being the most prevalent serotypes.

4.12.2 Tsavo national park

The park spans across seven counties namely Kajiado, Makueni, Kitui, Tana River, Taita Taveta, Kwale and Kilifi. The mean FMD seroprevalence around this park was 42.3%. Serotypes SAT 1 and SAT 2 were the most prevalent serotypes circulating with prevalence of 60% and 54.8% respectively (Appendix 10). Figure 4.12.1 below summarizes the mean prevalence of FMD around several national parks and game reserves.

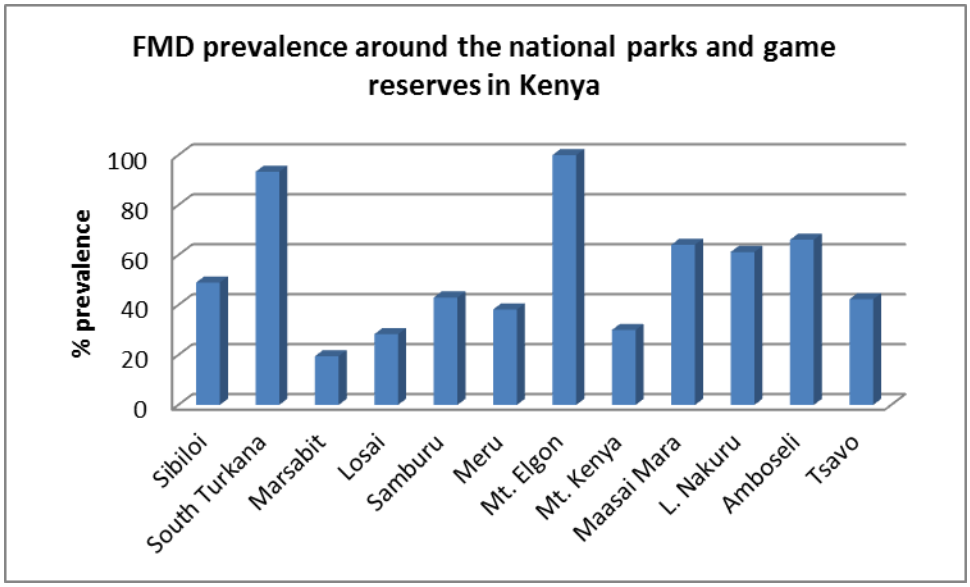


Fig 4.12.1: FMD prevalence around the national parks and game reserves in Kenya

4.13 FMD prevalence along stock routes in Kenya

4.13.1 North Eastern corridor

1. Route 1-*Ethiopian border-Moyale-Marsabit-Isiolo-Meru-Embu-Nairobi*. Animals using this route cross a total of five counties which gives mean FMD seroprevalence of 35.5%.
2. Route 2-*Ethiopian border-Marsabit-Isiolo-Laikipia-Nyeri-Nairobi*. The mean FMD seroprevalence of this stock route was 26.6%.
3. Route 3-*Ethiopia/Somalia/Mandera-Wajir-Garissa-Tana River-Lamu-Kilifi-Mombasa*. Animals using this stock route end up in Mombasa county for sale or fattening in ranches within the coastal DFZ. The mean FMD seroprevalence of this stock route was 44.8%.
4. Route 4-*Mandera-Wajir-Isiolo-Meru-Embu-Nairobi*. The mean FMD seroprevalence of this stock route was 38.6%
5. Route 5-*Somalia/Wajir-Garissa-Tana River-Kitui-Machakos*. This stock route had a mean FMD seroprevalence of 48.7%
6. Route 6-*Wajir-Garissa-Tana River-Lamu-Kilifi-Mombasa*. The mean FMD seroprevalence of this stock route was 50.9%.

4.13.2 Northern corridor

1. Route 1-*Samburu (Baragoi/Maralal)-Laikipia-Nyandarua-Nakuru-Nairobi*. The mean seroprevalence of FMD in this stock route was 37.4%.
2. Route 2-*Turkana (Lokichogio/South Sudan/Lodwar)-Pokot-Trans Nzoia-Uasin Gishu-Nakuru-Nairobi*. The stock route had high FMD seroprevalence of 80.5%.

4.13.3 Southern corridor

1. Route 1-*Migori-Narok-Kajiado-Nairobi*. The mean FMD seroprevalence along this route was quite high at 77.9%.
2. Route 2-*Narok-Kajiado-Taita Taveta-Kwale-Mombasa*; the mean seroprevalence of FMD along this route was 47.2%.

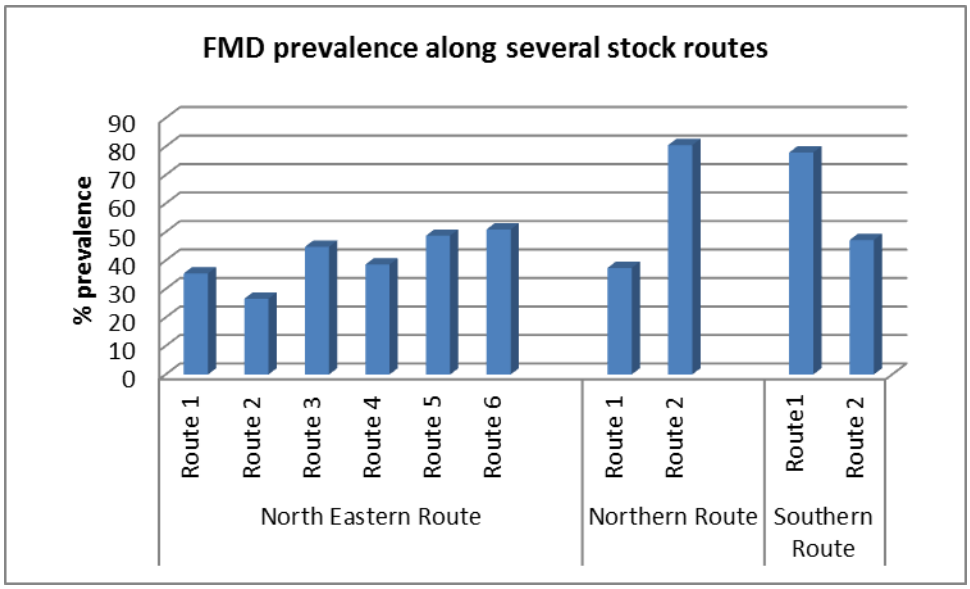


Fig 4.12.2: FMD prevalence along several stock routes in Kenya

4.14 Measurement of agreement between AniGen and Priocheck NSP tests

There was high level of agreement between the AniGen FMD NSP Elisa and Priocheck FMD NSP Elisa of 0.9 (Table 4.9). The observed percentage of agreement between the two tests was 94.8% and the chance percentage agreement was 50%. The agreement between the tests therefore was almost perfect.

Table 4.9: Measurement of agreement between AniGen and Priocheck FMD NSP Antibody ELISA test (kappa)

Measurement of agreement between AniGen & Priocheck tests					
		AniGen (Test 1)			
		positive	negative	Total	Apparent prevalence
Priocheck (Test 2)	positive	102	398	500	0.5
	negative	128	372	500	
		230	770	1000	
	Apparent	0.23			
Observed proportion Agreement					(102+372)/500 = 0.948
Chance proportion agreement (both+)					0.5×0.23 = 0.115
Chance proportion agreement (both-)					0.5x0.77 = 0.385
Chance proportion agreement					0.115+0.385 = 0.5
observed minus chance agreement					0.948-0.5 = 0.448
Maximum possible agreement beyond chance level					1-0.5 = 0.5
Kappa					0.448/0.5 = 0.896

CHAPTER FIVE

5.0 DISCUSSION

5.1 General discussion

The overall mean seroprevalence of bovine FMD from this study was 52.5% slightly similar to the anticipated prevalence used (50%). The prevalence was slightly higher in porcine species at 54.4% compared to bovine species 52.5%. All the five serotypes SAT 1, SAT 2, type O, A and C were found to have circulated or are circulating in Kenya with serotype SAT 1 being the most prevalent at 50.9%. The serotype was found to have countrywide circulation. Serotype C, A, SAT 2 and O also had notable levels of circulation with 48.9%, 37.3%, 36% and 30.9% prevalence respectively.

A high number of animals had at least two serotypes circulating within their bodies (11.9%) and was followed closely with animals that had three serotypes at 11%. The reported vaccination cover across the country was low at 14.1% (522/3709). A number of serum samples were either too small in quantity for LPBE analysis or had visible gross contamination to the extent that the test could not be carried out or the serum vials were empty. At least one of the above reasons explains why 147/1383 (10.6%) of the samples did not show any serotype. Some of the samples subjected to LPBE did not indicate the presence of any serotype.

5.2 FMD prevalence

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, Trans-Nzoia, Bungoma, Pokot and Kakamega recorded 100% seroprevalence while others like the counties of Mandera, Moyale and Nyeri had the lowest seroprevalence of 8.3%, 7.5% and 5.3% respectively. The variation in the seroprevalence may be attributed to the breed of animals kept. The western part of the country largely keeps the exotic type of breeds that is highly susceptible to FMDV infection as compared to the counties in the northern of the country that rears mainly the indigenous type and is less susceptible to FMDV infection. Although exotic breeds are largely kept in Nyeri County, the intensity of FMD vaccination is also quite high and therefore reducing the rates of infection.

Porcine sera from Uasin Gishu, Nandi, Trans-Nzoia, Bungoma and Kakamega had prevalence of 100% similar to bovine species. FMD antibodies were not detected in porcine samples from Bomet and Kilifi. Despite the bovine species showing 70% and 25.3% prevalence in Bomet and Kilifi counties respectively, the porcine sera were negative to FMD antibodies. In some counties such as Nakuru, Siaya, Kisumu, Nyeri and Taita Taveta, porcine sera had a higher prevalence compared to those of bovines (33.3%). This may be attributed to the fact that pigs in western Kenya are known to roam freely intermingling with bovines and therefore acting as source of infection. Domestic pigs are the most efficient excretors of FMDV into the environment (Tesfaye, 2006). In counties like Embu, Meru and Mombasa, the bovine prevalence was higher than those of porcines.

5.3 Reported vaccination coverage

Kenya is endemic with FMD and with five serotypes in circulation; routine vaccination is the only viable method of control (Chema, 1975). 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). Since the country serotype picture is not uniform, the valency of the vaccine should be informed by the prevailing situation at the time. 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). However, the reported national FMD vaccination cover was virtually low at 14.1%. Vaccination cover was highest in Mombasa and Malindi (100%) and lowest in Baringo, Kajiado, Makueni and Kitui at 4.8%, 6.8%, 7.4% and 9.8%. The difference in reporting may be attributed to either the level of awareness of livestock owners on the type of vaccine being administered or in the case of pastoral areas, very low intensity of vaccination that was carried out. 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).

5.4 Association between seropositivity, reported vaccination status, sex and age

The observed significant association between seropositivity and reported vaccination status indicated that the unvaccinated were at high risk of infection than those vaccinated. Maramorosch and Koprowaki (1967) documented that FMD control in developing countries depends primarily on vaccination until the incidence of disease had decreased sufficiently for other approaches to be cost-effective. Therefore with endemicity of the disease, the vaccination cover needs to be increased to a level where herd immunity can be attained. Unfortunately, the use of vaccines is sub-optimal in relation to the size of population and most of the FMD susceptible animal populations are at risk (FAO, 2006).

Although the number of female samples was higher at 70.2%, the level of association between sex and seropositivity was not significant. 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 seroprevalence of FMD among dairy cattle in northwest of Ethiopia, Hailu *et al.,* (2010) documented a higher 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 than in calves <1 year and mid age 1-2 years was consistent with other previous studies largely done in Ethiopia which

include 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 Esayas *et al.,* (2009) who did research in Bench Maji zone, southern of Ethiopia documented no significant association between seropositivity of FMD and age of cattle. 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 porcines and wildlife. Young cattle are herded around homesteads and hence have less chance of exposure. In the zero grazing units, the adult and the young rarely interact.

5.5.1 Serotypes in the Rift valley

The seroprevalence of FMDV was high in counties of the north rift than central or south rift. Animals from Baringo were exposed to all the four serotypes – O, A, SAT 1 and C but no exposure to serotype SAT 2. Serotypes C and A were the most prevalent. This result was consistent to the findings of Wariru, (1994) which found serotype C to highly circulate in the then Koibatek district causing sporadic FMD breakouts.

5.5.2 Serotypes in the North Eastern Region (Somali Ecosystem)

The general herd seroprevalence of FMDV in the Somali Ecosystem was 30.4%. This was almost consistent with the past estimates of 35% that was made in unimproved zebus (Ellis and Putt, 1981). The prevalence was higher compared to similar survey by Abdulahi *et al*., (2011) in the Somali region of Eastern Ethiopia which found a prevalence of 14.6%. The seropositivity finding was higher than

the overall seroprevalence of 21, 26.5 and 8.18% reported by Shale *et al.,* (2004), Rufael *et al*., (2008) and Molla *et al.,* (2010) in Ethiopia. However, the study finding was inconsistent with similar survey done by Chepkwony *et al.,* (2012), who reported a higher prevalence of 45.3% in similar ecosystem.

The findings of Chepkwony *et al.*, (2012) together with the findings of this study confirm that there exists a higher prevalence of FMD in the Kenyan part of the Somali ecosystem compared to both Ethiopian and Somali sides of SES. This high prevalence could be attributable to the fact that there has been no recorded comprehensive vaccination campaign undertaken in the SES. It may also be attributed to the pastoral nature of the communities in the SES in search of water and pasture and the continuous interaction between different herds and wildlife in several places like grazing grounds and watering points therefore increasing the chances of disease spread.

Mandera County only recorded serotype SAT 1 circulating. This was contrary to the findings of Chepkwony *et al.,* (2012) who reported significant exposure to serotype A and mild exposure to SAT 2 and O. Serotype C was not detected in both studies. Moyale had a high circulation of serotype O, SAT 1 and SAT 2 (all had 66.7% prevalence) but only minimal exposure to type A and C. According to studies carried out by (Tesfaye, 2006) in the country of Ethiopia by Elisa tests, serotype O was the most prevalent serotype with 99.2%. There is a lot of cross border movement of livestock between Kenya and Ethiopia by pastoralists in search of pastures and water during dry season or cross border trade and therefore

this explains why serotypes prominent in the border counties of Kenya are also found in Ethiopian side.

The FMD prevalence of Wajir was 33% and was higher compared with the findings of Chepkwony *et al.,* (2012) who reported prevalence of 23.6%. All the five serotypes were detected in Wajir. In a European Union aided survey of FMD in Somalia, serological tests showed exposure of the target unvaccinated cattle herds to FMD virus serotypes O, SAT 1, A and SAT 2 (Jabra, 2010). The border areas are characterized by uncontrolled livestock and human movement where illegal trade and FMD transmission continues to be at risk.

In Garissa County, the FMD seroprevalence was higher compared to other counties of the SES (72.9%) contrary to the findings of Chepkwony *et al.*, (2012) who reported a low seroprevalence of 6.2%. The findings also indicated a high exposure of SAT 2, O and SAT 1 with 81.8, 69.7 and 63.6% prevalence respectively and mild exposure to serotype C and A. The main market for the cattle in the SES is located within Garissa where about 1500-4000 cattle from Somalia, Ethiopia, the SES and other parts of the region are sold every week and then trucked to Mombasa or Nairobi for beef market or fattening in ranches. The high prevalence may be attributed to the fact that cattle meant for markets interact with local cattle or animals bought from the nearby markets being allowed to interact freely with the local cattle without proper disease control and surveillance measures.

Contrary to previous reports (FAO, 2005-2009) which reported serotype C to be extremely rare with the last confirmed case in Kenya being in the year 2005, the findings of this study acknowledges otherwise. Serotype C had slightly high prevalence and therefore there is need to incorporate it in the multivalent vaccine commonly used for the control of FMD in East Africa. The detectable high prevalence of serotype C could also arise due to non-specific cross reactivity in the test therefore giving false picture which therefore necessitates the use of VNT (gold standard for antibody assays) to confirm the results. According to Sangula *et al.*, (2011), the negligible circulation of serotype C in the SES and the low genetic and antigenic diversity contributed to no change in vaccine strain in use.

5.5.3 Serotypes in the Coastal region of Kenya

The mean FMD seroprevalence was 44.1%. This was higher compared to the prevalence of SES. Counties bordering Garissa, Tana River and Lamu, showed consistent prevalence. When livestock are sold in Garissa market, they are transported to either terminal markets of Mombasa and Nairobi or alternatively trekked to coastal ranches for fattening. The continued trekking may act as source of infection to other naïve areas (Mahmoud, 2010).

5.6 FMD and viral serotypes along the Kenyan borders

Kenyan borderlands are predominantly inhabited by pastoral communities characterized by high mobility in search of water and pasture. They have strong cross border linkages with their counterparts on the other side of the border. Borderlands constitute a dynamic livestock trading zone that supports the

livelihoods of thousands of people. Despite tension and numerous border closures that occasionally occur; community interactions, livestock migration and trade have continued unabated. Cross border livestock migration and trade have kept relations at the community level vibrant, while national economies and local authorities have benefitted from taxes on the flourishing trade in livestock and livestock products.

FMD prevalence was highest in the Kenyan/ Uganda border (also known as Karamojong/Turkana/Pokot Ecosystem) with 95% prevalence while the Kenya-Tanzania (Maasai ecosystem) border had prevalence of 63.2%. The main inhabitants, the Maasai, crosses border to Tanzania in search of water and pasture with subsequent interaction between the livestock and wildlife that roam freely in these areas.

The Kenya-Somali border (Somali Ecosystem) was third with 38.1% prevalence with Garissa leading at 72.9%. The high prevalence in Garissa may be attributed to the interaction between local and market livestock which are sourced from as far as Ethiopia and Somali. All the five serotypes were detected with serotype SAT 1 being the most prevalent serotype in the SES with a prevalence of 61.2%. The Kenya-Ethiopian border had the least prevalence of 29.4% compared to other borderlands. All the five serotypes were detected with SAT 1 being the serotype with the highest exposure along the Kenya-Ethiopia border.

5.7 Status of FMD in pastoral and non-pastoral regions

The mean prevalence of FMD was higher in the non-pastoral areas at 58.6% compared to that of pastoral areas which stood at 53%. In the non-pastoral areas, FMD control is largely through vaccination although there exist continuous interaction between bovine and porcine species therefore acting as source of infection to each other. Cows and pigs are more susceptible to FMD and show greater severity compared to sheep and goats (Alexandersen *et al*., 2003). Exotic animals mostly kept in the non-pastoral areas are more susceptible compared to the more resistant (indigenous) livestock kept in the pastoral areas.

According to the findings of this study, no FMD vaccination was carried out in porcines and therefore their role in maintaining FMD within bovines of high potential areas needs to be investigated further. This fact is supported by the high prevalence of FMD in pigs (54.4%). 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).

5.8 FMD status in the proposed disease free zones

In line with Vision 2030, the Ministry of Livestock Development is in the process of establishing disease free zones in coast region, Laikipia/Isiolo complex, North Rift, Machakos/Kajiado and central Kenya. Some activities towards the establishment of coastal DFZ have already been initiated through financing from Africa Development Bank (ADB). DFZ will serve as the last holding points for cattle that have been vaccinated and quarantined en-route. The DFZ's will be

equipped with dips, water and feeding facilities and will be fully fenced. It also aims at building and rehabilitating quarantine centers (Mirtini and Bachuma in coast). The DFZ will target cattle coming from the North and North Eastern provinces of Kenya, Somalia and Ethiopia.

DFZ aims at creating a geographically compact area within which the frequency of vaccination can be safely reduced to once per annum. The zoo-sanitary measures applied will be cost-wise rather being prohibitive to both government and farmers and will be applied at ease (Muriithi, 1976). Benefits accrued from such venture will be immense ranging from free and regular movement of livestock to markets without undue quarantine restrictions, increase in exports earnings from sale of beef and pigs in international markets, improved export marketing possibilities for live animals, Kenya could act as source of disease free breeding stock, improved and regular deliveries of cattle and pigs to slaughterhouses, increased availability of cheaper disease free meat to improved turn over from livestock and livestock products.

However, obtaining recognition of zones free from FMD will be 'logistically difficult', very expensive and socially disruptive with displacement and exclusion of local populations and livestock (Thomson, 2008). The alternative to this would be to upgrade and strengthen veterinary services and animal disease surveillance, reporting and control-key elements. These need to be addressed in the short to medium term with a view to perhaps establishing DFZs in the long term.

North Rift DFZ, which aims at acting as buffer zone for livestock from the Northern Kenya, had the highest mean FMD prevalence of 97.5%. Animals from this zone had high exposure of serotypes C and A. Serotypes SAT 1 and O had mild exposure. Despite the very high level of FMD prevalence, the FMD vaccination cover was very low with only three counties found to have carried out some FMD vaccination.

The central DFZ was second highest in prevalence with 47.1%. Serotypes C, type A and SAT 1 were the most prevalent serotypes. Four of the five counties carried out small scale FMD vaccination. Machakos/Kajiado DFZ had the third highest FMD prevalence with 46.4% exposure. Serotypes SAT 1, SAT 2 and type O were the most prevalent serotypes. Three out of five counties that make up Machakos/Kajiado DFZ carried out some level of FMD vaccination.

The coastal DFZ will serve livestock from Ethiopia, Somalia and Northern Kenya (counties within the Somali ecosystem and those surrounding it). The mean FMD prevalence within the coastal DFZ was 42.2% with only four out of the nine counties carrying out some FMD control through vaccination. All the five serotypes were found circulating within the coastal DFZ with serotypes SAT 1, SAT 2 and type O being the most prevalent serotypes.

The Laikipia/Isiolo complex DFZ had the lowest FMD seroprevalence of 28.1%. The complex once established will serve as disease control zone to animals from the Northern pastoral regions and also as fattening ground. All the five FMD

serotypes were found circulating with Serotypes SAT 1, SAT 2 and type O found to highly circulate within the complex. Only two of the eight counties within the DFZ were found carry out some level of FMD vaccination.

5.9 FMD status around National Parks and Game Reserves

Thomson *et al*., (2003) made an observation that wherever in the world FMD has been eradicated from livestock, it has always disappeared from wildlife in those regions too. Similarly, outbreaks of FMD in zoological gardens have coincided with outbreaks of FMD in domestic animals. In Sub-Saharan Africa, wildlife is clearly involved in the maintenance of FMD. Wildlife in South Africa, particularly the Cape buffalo ((*Syncerus caffer)* has been identified as natural hosts for the SAT serotypes of FMDV, although they may be infected by all serotypes (Hedger, 1976; Vosloo *et al.*, 1996).

The prevalence of FMD was highest 100% in areas around Mt. Elgon National Park. The park extends from Kenya into Uganda and is bisected by the border. Elephants and buffaloes can be found on the lower slopes of the mountain. Livestock are grazed communally on open rangelands with potential contact with several wildlife populations. This wildlife-livestock interface is critical for disease transmission particularly around common watering points and through contamination of pastures. During dry season, livestock herders force their way into wildlife conservancies with subsequent contact with wildlife species.

The disease has been reported in several species of wildlife, such as the African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), Kudu (*Tragelaphus strepsiceros*) species, Warthog (*Phacochoerus aethiopicus*), and African savanna forest elephants (*Loxodanta Africana/Loxodanta cyclotis* respectively) with an ability to both maintain and transmit the disease. The mechanism facilitating SAT-type virus transmission from buffalo appears to occur readily when there is close contact between the two species during acute stage of infection and shedding large amounts of virus.

South Turkana national game reserve, located in the south of Turkana County was the second highest wild life protected area with a very high FMD prevalence of 93.3%. Livestock interact freely with wild life which include; elephants, buffalo, eland, oryx, impala, bushbuck, greater kudus, Grants and Thomson gazelle. The high number of herbivores known to harbor and transmit the disease may be the reason for the high FMD prevalence. Impala (*Aepyceros melampus*) is the most frequent infected species and act as an intermediary in disease transmission between livestock and buffalos (Vosloo *et al.*, 2002). Rare case of FMD has also been reported in Indian elephant (*Elephas maximus*) and in the African elephant (*Loxodo africana*) (Thomson, 1994).

Amboseli national park which spreads across the Kenya/Tanzania border (Kajiado) had FMD prevalence of 66.1%. The park consists of the free ranging African Elephant, Cape buffalo, impala, zebras and wildebeests. Maasai Mara national game reserve had prevalence of 64.1%. The presence of Thomson's

gazelle, wild beasts, African elephant and African buffalo within the reserve may be responsible for maintaining and transmission of FMD.

Both Hells Gate and Lake Nakuru national park had mean prevalence of 61.2%. It harbors the African buffalos, zebras, eland, hartebeest and Thomson's gazelle which thought to be involved in FMD maintenance and transmission.

5.10 FMD status along various stock routes in Kenya

It should be noted that stock routes extend to neighboring countries of Uganda, Sudan, Ethiopia, Somalia and Tanzania. Most of the stock routes terminate in Nairobi and Mombasa markets. Nairobi's Dagoretti market is served by southern corridor (including supplies from Tanzania), Northern corridor from North West Kenya (including supplies from Uganda, South Sudan and Ethiopia) and North Eastern corridor mostly from Garissa. Dandora is served by Northern route (Moyale, Marsabit), western and eastern (Garissa). Mombasa receives most of its animals from North Eastern and Tana River. The chains are based on trekking (mostly from pastoral areas to primary and secondary markets) and trucking from secondary markets to terminal markets in Nairobi and Mombasa. In some cases trekking is also done from the secondary to the terminal market as in the case of Garissa-Tana River-Mombasa route (AU-IBAR, 2006). Livestock sale contribute directly or indirectly towards food security and therefore disruption of the stock routes (i.e. through insecurity) impact negatively on livelihood.

As animals (sick/carriers) are trekked, they disseminate the virus along their journeys through saliva and other excretions. Kenyan borders are porous and therefore disease surveillance by the veterinary personnel may not be effective or achieved. The contact between market and local animals at watering points or pastures act as an important avenue of transmission. As animals are trekked, they interact with wild life such impala, African buffalo, Kudu species, Warthog and African savanna and forest elephants, known to maintain or transmit the disease. Some of the trekking routes cross game reserves and national parks.

In the North Eastern corridor, the highest FMD prevalence was witnessed in route 6 (Wajir-Garissa-Tana River-Lamu-Kilifi-Mombasa) with 50.9%. Animals using this route are largely trekked to their destinations for purposes of either selling or fattening. Route 5 (Somalia/Wajir-Garissa-Tana River-Kitui-Machakos) had mean prevalence of 48.7%. Animals from the primary sources (Somalia/Wajir) are trekked to the secondary market (Garissa) where majority of them are trucked to terminal markets (AU-IBAR-2006).

In the Northern corridor, route 2 (Lokichogio/South Sudan/Lodwar)-Pokot-Trans Nzoia-Uasin Gishu-Nakuru-Nairobi had high mean prevalence of 80.5%. The livestock crosses the South Turkana game reserve where they interact with wild life.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

- The study was able to achieve all the target objectives. The study was able to show that the national FMD seroprevalence in Kenya was 52.5% and 54.4% in bovine and porcine species respectively. The study was also able to determine the proportion of the national reported vaccination coverage which was at 14.1% in addition to the reported vaccination cover across the 39 counties. It was also able to show variation in prevalence of FMD in various counties with the highest prevalence being in counties located in western and north rift regions of Kenya. It was able to show prevalence of FMD along the Kenyan borders with the Kenya-Uganda border leading in seroprevalence at 95%

- The study was also able to show FMD seroprevalence across several proposed disease free zones and several stock routes. In addition, it was able to determine FMD seroprevalence in several agro-ecological zones and around several national parks and game reserves.

- The study was able to show the serotypes circulating within the country and in counties under consideration. All the five serotypes SAT 1, SAT 2, type O, type A and type C were shown to be in circulation with serotype SAT 1 being the most prevalent serotype (50.9%). Major serotypes in circulation within the proposed disease free zones, borderlands and various national parks and game

reserves were also determined. The study managed to show a high number of the animals exposed to between two and three serotypes.

- The study was able to show that there existed no association between the seropositivity and sex. On the contrary, the study was able to indicate that there existed association between seropositivity and age group and also between seropositivity and vaccination status.

- In conclusion, study was able to compare the level of agreement between two NSP Elisa tests namely AniGen (FMD NSP Ab Elisa test kit from Korea) and Priocheck (FMD NSP Ab Elisa kit from Netherlands). The results pointed out to a very high level of agreement (0.9) between the two tests and can be used for the purposes of screening FMD.

- Under the new constitution [Schedule 4, Part 2 (functions of the county governments)], the county governments will be responsible for veterinary services and disease control. There is need to have well trained personnel to oversee the necessary for disease control policy formulation at the county government.

- The high seroprevalence of FMD in porcine species underlines the need for inclusion of porcines into FMD vaccination programs in order to reduce transmission to bovines.

- The results indicated all the five serotypes of FMD circulating in Kenya and is therefore recommended that control of FMD should employ the use of multivalent vaccines containing all the five serotypes (Serotype O, A, C, SAT 1 and SAT 2). Further tests on vaccine matching should be done on the current and future field isolates to ensure that the vaccine strains produced locally by the manufacturers are able to give adequate protection from outbreak challenge.

- There is need to increase the number of animals under FMD protection in order to attain high herd immunity. Pastoral areas falls under the non-compulsory vaccination areas for FMD vaccination policy of the government but with the current development of disease free zones (DFZ) and the global framework for eradication of FMD it will be important to initiate such programs in this region.

- There is need to strengthen border surveillance and control to ensure that carrier or diseased animals from the neighboring countries are not allowed into the country or are quarantined at the border or at the markets. There is also need to educate the farmers and traders on the importance of reducing contact between local and market animals to reduce the level of transmission. The government needs to seek bilateral agreements with neighboring countries in order to come up with workable policy aimed at controlling animal movement and increased surveillance of FMD along our porous borders.

- It is also recommended that seroprevalence be undertaken for wild life and small stock so as to determine their role in the transmission of FMD. Such surveys should be part of a systematic disease surveillance and data collection. Further studies needs to be undertaken to ascertain the high prevalence of serotype C including ascertaining cross-reactivity.

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APPENDICES

APPENDIX 1: TABLE SHOWING SERUM SAMPLE DISTRIBUTION AND SELECTION IN BOVINES

	SUMMARY OF SAMPLE DISTRIBUTION AND SELECTION					
	COUNTY	TOTAL SAMPLES	MISSING	PORCINE SAMPLES	CATTLE SAMPLES	NO SELECTED
1	Baringo	63	0	0	63	40
2	Keiyo-Marakwet	15	1	0	14	14
3	Uasin Gishu	79	0	19	60	40
4	Nandi	25	0	10	15	15
5	Tranzoia	23	0	8	15	15
6	Bungoma	22	0	7	15	15
7	Kakamega	24	0	9	15	15
8	Turkana	220	0	0	220	54
9	West Pokot	100	0	0	100	33
10	Narok	228	0	0	228	45
11	Kajiado	281	0	0	281	40
12	Bomet	33	0	3	30	30
13	Migori	45	0	0	45	40
14	Nakuru	65	2	19	44	39
15	Siaya	61	0	16	45	41
16	Kisumu	53	0	8	45	40
17	Mandera	131	71	0	60	38
18	Wajir	382	155	0	227	45
19	Kitui	266	2	0	264	44
20	Machakos	65	0	0	65	39
21	Makueni	127	0	0	127	42
22	Nyeri	131	22	33	76	38
23	Embu	63	20	8	35	35
24	Meru	85	0	8	77	39
25	Laikipia	61	0	0	61	40
26	Nyandarua	40	0	0	40	40
27	Tharaka Nithi	40	20	0	20	20
28	Garissa	287	21	0	266	44
29	Tana River	220	0	0	220	43
30	Lamu	108	0	0	108	36
31	Marsabit	380	19	0	361	40
32	Isiolo	59	0	0	59	41
33	Samburu	40	0	0	40	40
34	Moyale	40	0	0	40	40
35	Kilifi	112	21	16	75	37
36	Malindi	30	0	0	30	30
37	Mombasa	24	2	8	14	14
38	Kwale	45	0	0	45	40
39	Taita Taveta	189	17	8	164	42
	TOTAL	**4262**	**373**	**180**	**3709**	**1383**

APPENDIX 2: TABLE SHOWING FMD SEROPOSITIVITY IN PORCINES

RESULTS SUMMARY TABLE														
			Sex		Age			Vacination History			**Anigen Results**		% Proportion (100%)	
No.	County	**No. of San**	Male	Female	<1 years	1-2 years	>2 years	Yes	No	Unknown	**Positive**	**Negative**	% Positivity	% Negativit
1	Uasin Gish	**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	Tranzoia	**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 Tave	**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**		

APPENDIX 3: 3 ABC FMD NSP ELISA PLATE LAYOUT READING AND INTERPRETATION

Measurement count: 1	Filter: 450											
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.015	1.182	1.394	1.129	0.43	1.265	1.242	0.971	0.629	0.048	1.068	1.162
B	1.79	1.862	0.615	1.666	1.241	1.205	0.388	0.673	1.506	0.355	0.227	1.009
C	0.045	0.366	1.168	1.37	1.067	1.488	1.345	1.515	1.094	1.293	0.215	1.615
D	0.052	1.572	0.268	1.621	1.093	1.441	0.369	1.504	0.76	0.422	1.203	0.612
E	1.73	1.616	0.394	0.283	1.07	1.547	0.199	1.507	0.437	0.199	0.949	1.253
F	1.177	1.852	0.907	1.671	1.129	1.454	0.26	1.402	0.417	0.153	1.298	0.886
G	1.681	1.585	1.771	1.422	1.131	0.185	0.45	1.207	0.112	1.603	1.027	1.802
H	0.601	1.477	1.733	1.275	1.137	0.15	1.254	1.416	1.165	1.602	1.631	1.908
Mean NC	1.903			VALID								
Mean PC	0.049	PI PC	97	VALID								
	1	2	3	4	5	6	7	8	9	10	11	12
A	-6	38	27	41	77	34	35	49	67	97	44	39
B	6	2	68	12	35	37	80	65	21	81	88	47
C	98	81	39	28	44	22	29	20	42	32	89	15
D	97	17	86	15	43	24	81	21	60	78	37	68
E	9	15	79	85	44	19	90	21	77	90	50	34
F	38	3	52	12	41	24	86	26	78	92	32	53
G	12	17	7	25	41	90	76	37	94	16	46	5
H	68	22	9	33	40	92	34	26	39	16	14	0
	1	2	3	4	5	6	7	8	9	10	11	12
A	neg	neg	neg	neg	POS	neg	neg	neg	POS	POS	neg	neg
B	neg	neg	POS	neg	neg	neg	POS	POS	neg	POS	POS	neg
C	POS	POS	neg	neg	neg	neg	neg	neg	neg	neg	POS	neg
D	POS	neg	POS	neg	neg	neg	POS	neg	POS	POS	neg	POS
E	neg	neg	POS	POS	neg	neg	POS	neg	POS	POS	POS	neg
F	neg	neg	POS	neg	neg	neg	POS	neg	POS	POS	neg	POS
G	neg	neg	neg	neg	neg	POS	POS	neg	POS	neg	neg	neg
H	POS	neg	neg	neg	neg	POS	neg	neg	neg	neg	neg	neg
	PLATE 25											
	NSP SCREENING OF SERECU SAMPLES											
	1	2	3	4	5	6	7	8	9	10	11	12
	NC	2223	2231	2239	2247	2255	2263	2271	2279	2287	2295	2303
	NC	2224	2232	2240	2248	2256	2264	2272	2280	2288	2296	2304
	PC	2225	2233	2241	2249	2257	2265	2273	2281	2289	2297	2305
	PC	2226	2234	2242	2250	2258	2266	2274	2282	2290	2298	2305
	2219	2227	2235	2243	2251	2259	2267	2275	2283	2291	2299	2306
	2220	2228	2236	2244	2252	2260	2268	2276	2284	2292	2300	2307
	2221	2229	2237	2245	2253	2261	2269	2277	2285	2293	2301	2308
	2222	2230	2238	2246	2254	2262	2270	2278	2286	2294	2302	2309

APPENDIX 4: LPB ELISA PLATE READING AND INTERPRETATION

PLATE LAY-OUT II

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	C++	2018	2018	2138	2138	2236	2236	2296	2296	2444	2444
B	C++	C++	2072	2072	2158	2158	2266	2266	2297	2297	2445	2445
C	C+	C+	2084	2084	2185	2185	2269	2269	2301	2301	2446	2446
D	C+	C+	2118	2118	2188	2188	2272	2272	2307	2307	2447	2447
E	C-	C-	2119	2119	2200	2200	2284	2284	2343	2343	2448	2448
F	C-	C-	2121	2121	2206	2206	2285	2285	2441	2441	2449	2449
G	Ca	Ca	2126	2126	2212	2212	2291	2291	2442	2442	2450	2450
H	Ca	Ca	2127	2127	2215	2215	2292	2292	2443	2443	2451	2451

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.183	0.135	0.46	0.496	2.489	2.452	2.122	2.146	2.702	2.693	2.146	2.271
B	0.174	0.124	2.175	2.167	2.346	2.403	2.316	2.037	2.113	2.188	1.97	1.934
C	0.702	0.637	2.128	2.027	1.808	2.092	0.764	0.765	2.272	2.31	2.145	2.289
D	0.756	0.722	0.987	0.935	2.568	2.568	2.21	2.207	2.25	2.469	2.173	2.408
E	1.577	1.412	1.013	0.954	1.73	1.796	1.594	1.476	1.657	1.715	0.954	1.007
F	1.691	1.551	1.564	1.607	0.138	0.16	1.453	1.712	1.96	2.321	0.996	1.508
G	1.847	1.719	2.748	2.757	2.361	2.283	0.692	0.621	0.86	0.861	2.153	2.481
H	1.913	1.823	0.62	0.589	2.388	2.28	0.942	0.841	1.799	1.799	1.99	2.438

CONTROL DATA

	OD1	OD2	OD3	OD4	PI1	PI2	PI3	PI4
C++	0.183	0.135	0.174	0.124	90	93	91	93
C+	0.702	0.637	0.756	0.722	62	65	59	61
C-	1.577	1.412	1.691	1.551	14	23	8	15
Ca	1.847	1.719	1.913	1.823	-1	6	-4	1

Median intermediate values: 1.835

INTERPRETATION CONTROL DATA

THREE OR FOUR OUT OF FOUR SHOULD BE VALID!!!

					CRITERIA	UCL	LCL
Ca (OD)	VALID	VALID	INVALID	VALID	Ca (OD)	1.9	0.8
Ca (PI)	VALID	VALID	VALID	VALID	Ca (PI)	25	-25
C++	VALID	VALID	VALID	VALID	C++ (PI)	100	85
C+	VALID	VALID	VALID	VALID	C+ (PI)	85	50
C-	VALID	VALID	VALID	VALID	C- (PI)	49	-10

SAMPLE DATA

LAB. NO.	ANI.ID	OD1	OD2	PI1	PI2	MEAN PI	RESULT	LAB. NO.	ANI.ID	OD1	OD2	PI1	PI2	MEAN PI	RESULT
2018	4BA112	0.460	0.496	75	73	74	POS	2200	1BL18	1.594	1.476	13	20	16	N
2072	1BA8	2.175	2.167	-19	-18	-18	N	2206	3BL158	1.453	1.712	21	7	14	N
2084	3BA86	2.128	2.027	-16	-10	-13	N	2212	3BL217	0.692	0.621	62	66	64	POS
2118	3BA97	0.987	0.935	46	49	48	N	2215	1BL44	0.942	0.841	49	54	51	POS
2119	1BA39	1.013	0.954	45	48	46	N	2296	1BL17	2.702	2.693	-47	-47	-47	N
2121	8BA142	1.564	1.607	15	12	14	N	2297	3BL202	2.113	2.188	-15	-19	-17	N
2126	1BA57	2.748	2.757	-50	-50	-50	N	2301	1BL29	2.272	2.310	-24	-26	-25	N
2127	4BA111	0.620	0.589	66	68	67	POS	2307	2BL119	2.250	2.469	-23	-35	-29	N
2138	IBA18	2.489	2.452	-36	-34	-35	N	2343	2BL103	1.657	1.715	10	7	8	N
2158	11BA204	2.346	2.403	-28	-31	-29	N	2441	1BL4	1.960	2.321	-7	-26	-17	N
2185	1B6	1.808	2.092	1	-14	-6	N	2442	2MD54	0.860	0.861	53	53	53	POS
2188	1B38	2.568	2.568	-40	-40	-40	N	2443	1BL12	1.799	1.799	2	2	2	N
2200	5BA12	1.730	1.796	6	2	4	N	2444	3BL228	2.146	2.271	-17	-24	-20	N
2206	11BA176	0.138	0.160	92	91	92	POS	2445	1BL19	1.970	1.934	-7	-5	-6	N
2212	2BA106	2.361	2.283	-29	-24	-27	N	2446	2IB67	2.145	2.289	-17	-25	-21	N
2215	1BL68	2.388	2.280	-30	-24	-27	N	2447	1BL1	2.173	2.408	-18	-31	-25	N
2138	1BL30	2.122	2.146	-16	-17	-16	N	2448	2BL127	0.954	1.007	48	45	47	N
2158	1BL35	2.316	2.037	-26	-11	-19	N	2449	1BM1	0.996	1.508	46	18	32	N
2185	3BL214	0.764	0.765	58	58	58	POS	2450	BP22	2.153	2.481	-17	-35	-26	N
2188	1BL32	2.210	2.207	-20	-20	-20	N	2451	BP18	1.990	2.438	-8	-33	-21	N

APPENDIX 5: FMD SEROPOSITIVITY IN BOVINES

			sex		age			vaccination history			Anigen		% Seroprevalence	
no	COUNTY	No. of sample	male	female	< 1 years	1-2 years	> 2 years	yes	no	uknown	no. positv	no. negati	% seropositivity	% Seronegativity
1	Baringo	63	18	45	23	20	20	3	8	52	63	0	100	0
2	Elgeyo Marakw	14	6	8	4	5	5	0	0	14	14	0	100	0
3	Uasin Gishu	60	25	35	21	19	20	20	7	33	60	0	100	0
4	Nandi	15	2	13	5	5	5	3	2	10	15	0	100	0
5	Tranzoia	15	5	10	5	5	5	11	0	4	15	0	100	0
6	Bungoma	15	5	10	5	5	5	0	0	15	15	0	100	0
7	Kakamega	15	6	9	5	5	5	0	0	15	15	0	100	0
8	Turkana	220	91	129	55	55	110	0	2	218	176	44	80	20
9	West Pokot	100	36	64	25	24	51	0	0	100	100	0	100	0
10	Narok	228	36	192	61	59	108	0	45	183	206	22	90.4	9.6
11	Kajiado	281	52	229	80	79	122	19	0	262	190	91	67.6	32.4
12	Bomet	30	13	17	10	10	10	0	15	15	21	9	70	30
13	Migori	45	16	29	15	15	15	0	45	0	34	11	75.6	24.4
14	Nakuru	44	18	26	18	16	10	14	30	0	10	34	22.7	77.3
15	Siaya	45	12	33	15	16	14	0	30	15	28	17	62.2	37.8
16	Kisumu	45	8	37	15	14	16	0	45	0	23	22	51.1	48.9
17	Mandera	60	2	58	13	19	28	0	60	0	5	55	8.3	91.7
18	Wajir	227	15	212	50	57	120	0	227	0	75	152	33	77
19	Kitui	264	132	132	85	98	82	26	195	43	76	188	28.8	71.2
20	Machakos	65	41	24	22	21	22	0	53	12	28	37	43.1	56.9
21	Makueni	127	65	62	42	44	41	2	96	29	34	93	26.8	73.2
22	Nyeri	76	21	55	24	27	25	46	30	0	4	72	5.3	94.7
23	Embu	35	0	35	10	11	14	23	5	7	29	6	82.9	17.1
24	Meru	77	17	60	22	19	35	32	19	26	27	50	35	65
25	Laikipia	61	13	48	12	18	31	39	19	3	30	31	49.2	50.8
26	Nyandarua	40	3	37	10	11	19	13	7	20	15	25	37.5	62.5
27	Tharaka Nithi	20	2	18	4	6	10	7	5	8	8	12	40	60
28	Garissa	266	66	200	60	74	132	0	266	0	194	72	72.9	27.1
29	Tana River	220	60	160	55	54	111	0	220	0	144	76	65.5	34.5
30	Lamu	108	40	68	29	26	53	0	108	0	71	37	65.7	34.3
31	Marsabit	361	138	223	88	85	188	0	361	0	65	296	18	82
32	Isiolo	59	10	49	16	15	28	0	59	0	20	39	33.9	66.1
33	Samburu	40	6	34	10	10	20	0	40	0	16	24	40	60
34	Moyale	40	19	21	12	10	18	0	40	0	3	37	7.5	92.5
35	Kilifi	75	26	49	25	25	25	61	14	0	19	56	25.3	74.7
36	Malindi	30	8	22	10	10	10	30	0	0	8	22	26.7	73.3
37	Mombasa	14	2	12	5	5	4	14	0	0	6	8	42.9	57.1
38	Kwale	45	16	29	16	15	14	15	0	30	19	26	42.2	57.8
39	Taita Taveta	164	47	117	40	40	84	144	0	20	66	98	40.2	59.8
	Total	3709	1098	2611	1022	1052	1635	522	2053	1134	1947	1762		
	% Proportion		29.6	70.4	27.6	28.4	44	14	55.4	30.6	52.5	47.5		

APPENDIX 6: FMD PREVALENCE COMPARISON BETWEEN BOVINE AND PORCINE SPECIES

	BOVINE	PORCINE
COUNTY	Prevalence	Prevalence
Uasin Gishu	100	100
Nandi	100	100
Trans Nzoia	100	100
Bungoma	100	100
Kakamega	100	100
Bomet	70	0
Nakuru	22.7	36.8
Siaya	62.2	75
Kisumu	51.1	75
Nyeri	5.3	21.2
Embu	82.9	50
Meru	35	12.5
Kilifi	25.3	0
Mombasa	42.9	37.5
Taita Taveta	40.2	62.5

APPENDIX 7: FMD SEROTYPE DISTRIBUTION AND FREQUENCY ACROSS THE COUNTIES

		SEROTYPE DISTRIBUTION					SEROTYPE FREQUENCY					
COUNTY	NO. OF SA	SAT 2	SAT 1	TYPE C	TYPE A	TYPE O	5	4	3	2	1	0
Baringo	41	0	16 (39%)	35 (85.4%)	32 (78%)	5 (12.2%)	0	1	17	13	7	3
Elgeyo Ma	14	0	7 (50%)	11 (78.6%)	10 (71.4%)	0	0	0	6	2	6	0
Uasin Gish	40	7 (17.5%)	20 (50%)	34 (85%)	14 (35%)	18 (45%)	0	8	6	18	7	1
Nandi	15	0	6 (40%)	12 (80)	2 (13.3)	5 (33.3)	0	0	2	7	5	1
Tranzoia	15	0	3 (20)	10 (66.7)	3 (20)	2 (13.3)	0	0	1	5	6	3
Bungoma	15	0	1 (6.7)	7 (46.7)	6 (40)	1 (6.7)	0	0	1	5	2	7
Kakamega	15	1 (6.7)	4 (26.7)	3 (20)	9 (60)	0	0	1	1	2	6	5
Turkana	44	3 (6.8)	25 (56.8)	26 (59.1)	36 (81.8)	17 (38.6)	3	5	14	10	9	3
West Pok	33	2 (6.1)	8 (24.2)	17 (51.5)	15 (45.5)	2 (6.1)	0	2	4	9	6	12
Narok	40	12 (30)	24 (60)	22 (55)	20 (50)	12 (30)	5	4	12	4	7	8
Kajiado	28	15 (53.6)	18 (64.3)	20 (71.4)	17 (60.7)	11 (39.3)	6	8	3	3	4	4
Bomet	21	4 (19)	5 (23.8)	11 (52.4)	12 (57.1)	1 (4.8)	0	2	5	2	7	5
Migori	30	2 (6.7)	13 (43.3)	9 (30)	5 (16.7)	1 (3.3)	0	0	3	7	7	13
Nakuru	10	1 (10)	1 (10)	1 (10)	2 (20)	1 (10)	0	1	0	0	2	7
Siaya	24	6 (25)	8 (33.3)	11 (45.8)	0	0	0	0	5	4	2	13
Kisumu	21	1 (4.8)	5 (23.8)	4 (19)	1 (4.8)	2 (9.5)	0	1	0	3	3	14
Mandera	3	0	1 (33.3)	0	0	0	0	0	0	0	1	2
Wajir	16	7 (43.8)	13 (81.3)	12 (75)	7 (43.8)	4 (25)	1	5	3	3	2	2
Kitui	14	6 (42.9)	7 (50)	6 (42.9)	0	2 (14.3)	0	1	1	5	4	3
Machakos	15	8 (53.3)	7 (46.7)	5 (33.3)	4 (26.7)	1 (6.7)	0	4	1	3	0	7
Makueni	11	3 (27.3)	6 (54.5)	3 (27.3)	0	4 (36.4)	0	1	2	3	0	5
Nyeri	1	1 (100)	1 (100)	1 (100)	0	0	0	0	1	0	0	0
Embu	29	24 (82.8)	13 (44.8)	17 (58.6)	6 (20.7)	11 (37.9)	1	4	10	8	4	2
Meru	16	7 (43.8)	9 (56.3)	7 (43.8)	10 (62.5)	11 (68.8)	3	5	0	3	2	3
Laikipia	18	11 (61.1)	11 (61.1)	11 (61.1)	3 (16.7)	4 (22.2)	1	2	6	3	3	3
Nyandaru	15	9 (60)	7 (46.7)	10 (66.7)	7 (46.7)	3 (20)	2	2	4	2	3	2
Tharaka N	8	2 (25)	2 (25)	0	4 (50)	4 (50)	0	0	0	5	2	1
Garissa	33	27 (81.8)	21 (63.6)	15 (45.5)	6 (18.2)	23 (69.7)	1	8	15	2	6	1
Tana River	28	23 (82.1)	19 (67.8)	1 (3.6)	5 (17.9)	14 (50)	0	3	3	19	3	0
Lamu	23	19 (82.6)	20 (87)	8 (34.8)	13 (56.5)	15 (65.2)	4	8	6	1	3	1
Marsabit	12	5 (41.7)	7 (58.3)	2 (16.7)	1 (8.3)	0	0	1	1	1	6	3
Isiolo	13	10 (76.9)	13 (100)	5 (38.5)	2 (15.4)	10 (76.9)	2	3	2	6	0	0
Samburu	16	13 (81.3)	14 (87.5)	2 (12.5)	5 (31.3)	8 (50)	0	6	2	4	3	1
Moyale	3	2 (66.7)	2 (66.7)	1 (33.3)	1 (33.3)	2 (66.7)	0	2	0	0	0	1
Kilifi	9	6 (66.7)	6 (66.7)	1 (11.1)	1 (11.1)	2 (22.2)	1	0	3	1	2	2
Malindi	8	0	3 (37.5)	0	1 (12.5)	1 (12.5)	0	0	0	1	3	4
Mombasa	6	1 (16.7)	5 (83.3)	2 (33.3)	2 (33.3)	5 (83.3)	0	2	0	3	1	0
Kwale	19	15 (78.9)	16 (84.2)	11 (57.9)	7 (36.8)	13 (68.4)	4	5	7	0	1	2
Taita Tave	16	13 (81.3)	9 (56.3)	8 (50)	7 (43.8)	13 (81.3)	5	2	5	1	0	3
Total	738	0	0	0	0	0	39	97	152	168	135	147
% proportion (x/738		36	50.9	48.9	37.3	30.9						

APPENDIX 8: MAP SHOWING VARIOUS NATIONAL PARKS AND GAME RESERVES

APPENDIX 9: NATIONAL SEROTYPE PREVALENCE

	SEROTYPE DISTRIBUTION				
	SAT 2	SAT 1	TYPE C	TYPE A	TYPE O
NO OF SAMPLES	266	376	361	276	228
738					
% Proportion	36	50.9	48.9	37.3	30.9

APPENDIX 10: PROPOSED DISEASE FREE ZONES IN KENYA

APPENDIX 11: SERUM SAMPLE VERIFICATION