The epidemiology of foot-and-mouth disease at the wildlife-livestock interface in northern Tanzania

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Summary

Foot-and-mouth disease (FMD), a disease of cloven hooved animals caused by FMD virus (FMDV), is one of the most economically devastating diseases of livestock worldwide. The global burden of disease is borne largely by livestock-keepers in areas of Africa and Asia where the disease is endemic and where many people rely on livestock for their livelihoods and food-security. Yet, there are many gaps in our knowledge of the drivers of FMDV circulation in these settings.

In East Africa, FMD epidemiology is complicated by the circulation of multiple FMDV serotypes (distinct antigenic variants) and by the presence of large populations of susceptible wildlife and domestic livestock. The African buffalo (*Syncerus caffer*) is the only wildlife species with consistent evidence of high levels of FMDV infection, and East Africa contains the largest population of this species globally. To inform FMD control in this region, key questions relate to heterogeneities in FMD prevalence and impacts in different livestock management systems and to the role of wildlife as a potential source of FMDV for livestock. To develop FMD control strategies and make best use of vaccine control options, serotype-specific patterns of circulation need to be characterised.

In this study, the impacts and epidemiology of FMD were investigated across a range of traditional livestock-keeping systems in northern Tanzania, including pastoralist, agro-pastoralist and rural smallholder systems. Data were generated through field studies and laboratory analyses between 2010 and 2015. The study involved analysis of existing household survey data and generated serological data from cross-sectional livestock and buffalo samples and longitudinal cattle samples. Serological analyses included non-structural protein ELISAs, serotype-specific solid-phase competitive ELISAs, with optimisation to detect East African FMDV variants, and virus neutralisation testing. Risk factors for FMDV infection and outbreaks were investigated through analysis of cross-sectional serological data in conjunction with a case-control outbreak analysis. A novel Bayesian modeling approach was developed to infer serotype-specific infection history from serological data, and combined with virus isolation data from FMD outbreaks to characterise temporal and spatial patterns of serotype-specific infection.

A high seroprevalence of FMD was detected in both northern Tanzanian livestock (69%, [66.5 - 71.4%] in cattle and 48.5%, [45.7-51.3%] in small ruminants) and in buffalo (80.9%, [74.7-86.1%]). Four different serotypes of FMDV (A, O, SAT1 and SAT2) were isolated from livestock. Up to three outbreaks per year were reported by households and active surveillance highlighted up to four serial outbreaks in the same herds within three years. Agro-pastoral and pastoral livestock keepers reported more frequent FMD outbreaks compared to smallholders. Households in all three management systems reported that FMD outbreaks caused significant impacts on milk production and sales, and on animals' draught power, hence on crop production, with implications for food security and livelihoods.

Risk factor analyses showed that older livestock were more likely to be seropositive for FMD (Odds Ratio [OR] 1.4 [1.4-1.5] per extra year) and that cattle (OR 3.3 [2.7-4.0]) were more likely than sheep and goats to be seropositive. Livestock managed by agro-pastoralists (OR 8.1 [2.8-23.6]) or pastoralists (OR 7.1 [2.9-17.6]) were more likely to be seropositive compared to those managed by smallholders. Larger herds (OR: 1.02 [1.01-1.03] per extra bovine) and those that recently acquired new livestock (OR: 5.57 [1.01 – 30.91]) had increased odds of suffering an FMD outbreak. Measures of potential contact with buffalo or with other FMD susceptible wildlife did not increase the likelihood of FMD in livestock in either the cross-sectional serological analysis or case-control outbreak analysis.

The Bayesian model was validated to correctly infer from ELISA data the most recent serotype to infect cattle. Consistent with the lack of risk factors related to wildlife contact, temporal and spatial patterns of exposure to specific FMDV serotypes were not tightly linked in cattle and buffalo. In cattle, four serial waves of different FMDV serotypes that swept through southern Kenyan and northern Tanzanian livestock populations over a four-year period dominated infection patterns. In contrast, only two serotypes (SAT1 and SAT2) dominated in buffalo populations.

Key conclusions are that FMD has a substantial impact in traditional livestock systems in East Africa. Wildlife does not currently appear to act as an important source of FMDV for East African livestock, and control efforts in the region should initially focus on livestock management and vaccination strategies. A novel modeling approach greatly facilitated the

interpretation of serological data and may be a potent epidemiological tool in the African setting. There was a clear temporal pattern of FMDV antigenic dominance across northern Tanzania and southern Kenya. Longer-term research to investigate whether serotype-specific FMDV sweeps are truly predictable, and to shed light on FMD post-infection immunity in animals exposed to serial FMD infections is warranted.

To Jason and my parents.

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Finally, and importantly, I would like to thank my husband Jason and my parents, Tom and Breda. Their unwavering support has made all the difference.

Declaration

I declare that this thesis and the research contained within it is my own work unless otherwise stated, and no part of it has been submitted as part of any other degree or qualification.

Miriam Casey

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Abbreviations

AIC	Akaike information criterion
CI	Confidence interval
DIC	Deviance information criterion
ECF	East Coast Fever (Theleriosis)
EuFMD	The European Commission for the control of Foot-and-Mouth Disease
FAO	Food and Agriculture Organisation of the United Nations
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
ELISA	Enzyme linked immunosorbent assay
GFRA	Global Foot-and-Mouth Disease Research Alliance
GLM	Generalised linear model
GLMM	Generalised linear mixed model
HPD	Highest posterior density
IQR	Inter quartile range
LRT	Likelihood ratio test
MCF	Malignant catarrhal fever
PCR	Polymerase chain reaction
OD	Optical density measured by an ELISA plate reader
OIE	Office International des Epizooties
OR	Odds ratio
SAT	Southern African Territories FMD serotypes
SPCE	Solid phase competition ELISA
VNT	Virus neutralisation testing
WRL-FMD	World Reference Laboratory for Foot-and-Mouth Disease

Chapter 1: Introduction[1]

1.1 Introduction to foot-and-mouth disease

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hooved animals caused by FMD virus (FMDV) of the family *Picornaviridae* (genus *Aphthovirus)*. The virus comprises seven serotypes (distinct antigenic variants, O, A, C, Asia 1, South-African-Territories [SAT] 1, SAT 2 and SAT 3). Whilst the disease has been reported since the 16th century (Mahy, 2005), FMD poses a continuing challenge to the international community with circulation of highly divergent virus serotypes and strains that have great potential for trans-boundary spread. High genetic and antigenic variability is evident in FMDV (Carrillo, 2012; Vosloo *et al.*, 2010) with a spectrum of variants suited to very different epidemiological conditions; it can infect over 70 different species (Shimshony, 1988; Arzt et al. 2011; Karesh, 2012; Hedger 1981; Bengis & Erasmus 1988; Pinto 2004) and it is highly infectious in the acute stages of infection, but can also survive sub-clinically for years in persistently infected animals, so-called "carriers" (Alexandersen et al., 2002; Bengis et al., 1986; Burrows, 1966).

Due to the diversity of FMDV and its hosts, FMD has a complex epidemiology. Clinical signs range from no observed signs, as is reported in many cases for the African buffalo (*Syncerus caffer*) (Thomson, 1994), to severe clinical signs and even animal deaths. Examples of severe outbreaks include serotype SAT2 emergence in Egyptian livestock (Ahmed *et al.*, 2012), serotype O outbreaks in Israeli mountain gazelle (*Gazella gazella* (Shimshony, 1988) and pigs in Taiwan (Dunn & Donaldson, 1997). Morbidity at animal level is difficult to measure in FMD outbreaks in countries that are normally FMD free due to the rapid implementation of control measures (Gibbens *et al.*, 2001), but FMD is recognised to be highly contageous (OIE, 2012). At farm level, analyses of the FMD outbreak in the United Kigdom highlighted that the number of secondary cases per infected premesis could vary widely depending on local and climatic conditions and on the lag time between infection and control measures (Haydon *et al.*, 1997; Hugh-Jones & Wright, 1970; Keeling *et al.*, 2001; Tildesley & Keeling, 2010). Similarly, the reported animal level morbidity (proportion of animals with clinical signs in an outbreak) ranges from 4- 100%

[1] **Part of the material used in the introduction chapter is published as an Elsevier book chapter: Casey, M.B., Lembo, T.,**

in endemic countries (Gonzales *et al.*, 2014; Govindaraj *et al.*, 2015) and further work is necessary to understand the drivers of this variation.

Clinical findings associated with FMD in domestic livestock include fever with vesicles on the feet, in and around the mouth and sometimes on the teats. After eroding or ulcerating, the vesicles heal within 2-3weeks (Kitching & Hughes, 2002; Kitching, 2002). Death occasionally occurs through myocarditis in young-stock (Arzt *et al.*, 2010, 2011a; Kitching & Hughes, 2002; Kitching, 2002). Pigs may suffer severe foot lesions and separation of the hoof horn from the underlying tissues (Alexandersen *et al.*, 2003). Animals with FMD lose weight and produce less milk, and these may continue as a long-term sequel to FMD infection (Bayissa *et al.*, 2011; Catley *et al.*, 2004).

1.2 Virus characteristics

The FMD virus consists of an icosahedral capsid made of protein, without an envelope, that encloses positive-sense single stranded RNA encoding a genome of approximately 8.4 kilobases in length (Carrillo *et al.*, 2005; Grubman & Baxt, 2004) (Figure 1.1).

The RNA is translated from one long open reading frame into a polyprotein that is cleaved by viral proteases into structural and non-structural proteins. The FMD genome consists of P1, P2 and P3 regions (Figure 1.1). The P1 region contains 1A, 1B, 1C and 1D genes encoding 60 copies each of four structural capsid proteins, (VP4, VP2, VP3 and VP1 respectively). Structural proteins VP1, VP2 and VP3 are involved in cell receptor binding and antigenicity. In contrast, VP4 is not generally exposed on the outside surface of the capsid (Acharya *et al.*, 1989). The P2 region encodes non-structural proteins 2A, 2B and 2C. The P3 region encodes 3A, three copies (1-3) of 3B (Vpg), 3C (protease) and 3D (RNA polymerase) (Grubman & Baxt, 2004; Longjam *et al.*, 2011).

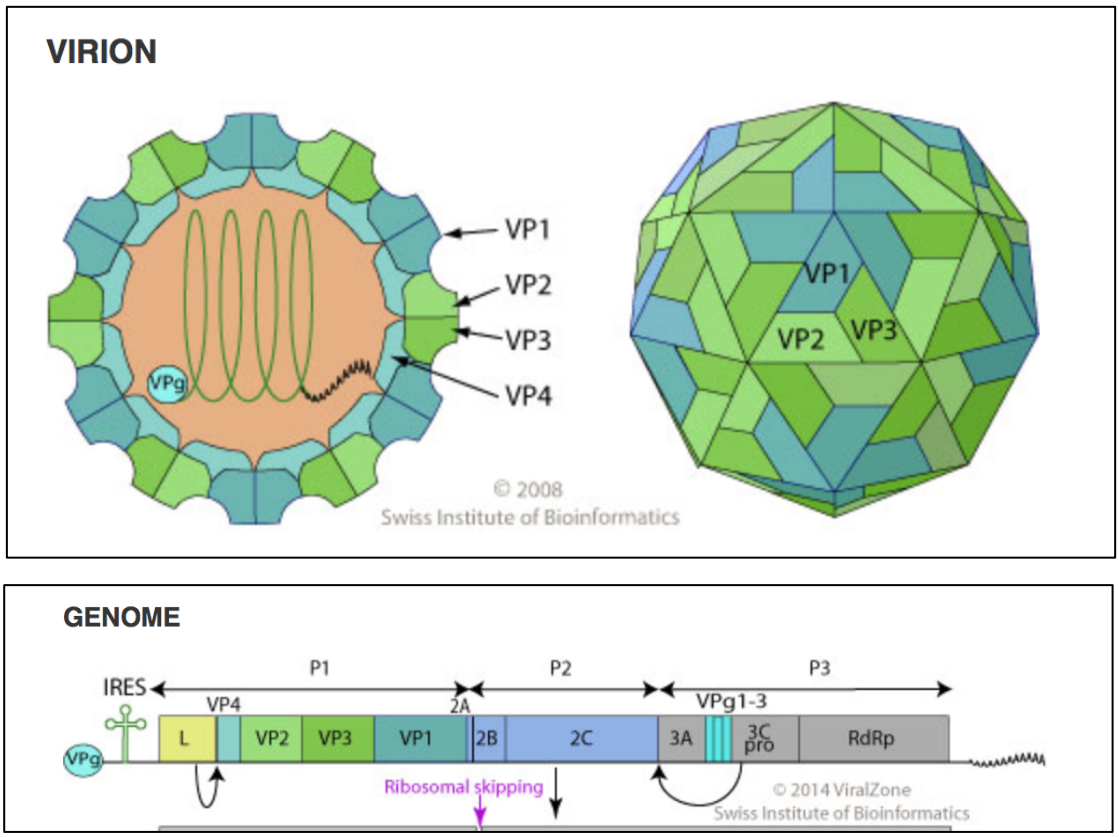

Figure 1.1: A schematic diagram of the FMDV structure (top) and genome (bottom). Image with permission from the Swiss Institute of Bioinformatics https://www.isb-sib.ch

Structural protein VP1 is the most immunogenic protein of the FMDV capsid, making up 54% of the viral surface (Morrell *et al.*, 1987). Based on a comparative genomics study of 103 FMDV whole genomes from of all seven serotypes (Carrillo *et al.*, 2005), VP1 was the least conserved of the structural proteins (Table 1.1). Work is ongoing to establish a repeatable and easy-to use measure of FMD genetic and antigenic diversity (Ludi *et al.*, 2014a; Reeve *et al.*, 2010). It is generally accepted that a relatively higher degree of genetic and antigenic variation occurs within each of the SAT serotypes, especially SAT2, and serotype A compared to lower diversity within serotypes O, C and Asia 1 (Carrillo *et al.*, 2005; Wekesa *et al.*, 2013b). This variation means that a multitude of specific tailored vaccines are necessary for different strains, particularly for the SAT and A serotypes (Sumption *et al.*, 2012). Evidence for genetic recombination between different strains of FMDV has also been reported (Carrillo *et al.*, 2005).

Table 1.1: Function and amino acid conservation of FMDV proteins (Carrillo, 2012; Grubman & Baxt, 2004).

Region of FMDV genome	% Invariant Amino Acids (Carrillo *et al.*, 2005)	Comment	Function (Grubman & Baxt, 2004)
Lpro	44		Translation
1A (VP4)	81		Capsid protein
1B (VP2)	47		Capsid proteins: Adsorption and penetration
1C (VP3)	39		
1D (VP1)	24	Most variable and commonly used for genotyping	
2A	65		Non – structural proteins: RNA replication
2B	76		
2C	72		
3A	37	The 3ABC antigen is used for a pan serotypic serological assay	
3B	50		
3C	76		
3D	74	Used for pan-serotypic PCR testing	

1.3 Transmission of foot-and-mouth disease

The passing of FMDV between animals depends on 1) shedding of infectious virus from an infected animal; 2) transfer of virus to the tissues of another animal; and 3) infection of the other animal.

Periods of shedding of infectious virus

Conventionally, the length of the latent period (exposure to infectiousness) was estimated from the length of time from experimental exposure to first detection of FMDV in infected animals' secretions. Figure 1.2 summarises meta-analyses of 19 experiments measuring the FMD latent period, incubation period (from exposure to clinical signs), and period of virus shedding (termed "infectiousness") (Mardones *et al.*, 2010).

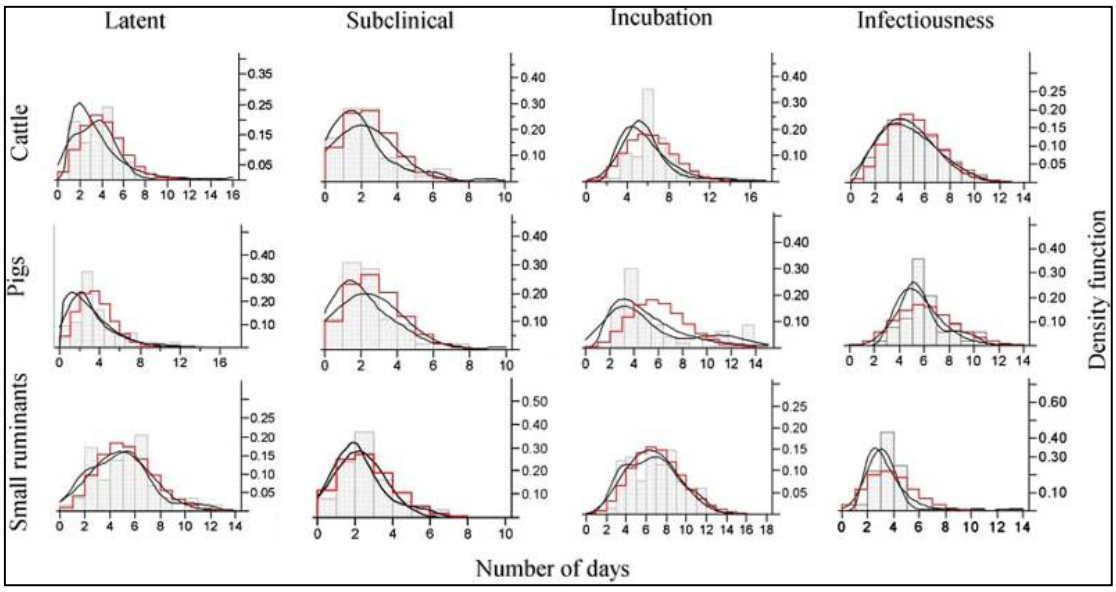

Figure 1.2: Latent, subclinical, incubation and virus shedding ("infectious") periods of FMD.
The plot is based on 19 experiments with serotype O. Frequency distributions and probability density functions fit to continuous (grey boxes) and discrete (red) data for experimental animals and FMD stage. Non-parametric density estimation using the kernel standard deviation (dashed line) was estimated for smoothing the distribution. N= 19 experiments, 295 animals (64 cattle, 149 sheep, 72 pigs, and 10 goats). From (Mardones *et al.*, 2010).

Levels of virus shedding

Levels of virus shedding may be measured by quantifying FMDV in secretions and excretions of infected animals. Meta-analysis of 32 experiments suggests that most FMDV is found in upper respiratory secretions from cattle, followed by the breath of pigs, probang samples from cattle and blood from pigs (Bravo de Rueda *et al.*, 2014) (Figure 1.3). The amount of FMDV released into the environment is also species-dependent (e.g. pigs excrete higher amounts of FMDV by the airborne route than cattle). Virus excretion levels are positively associated with the presence of clinical signs. Higher levels are excreted around the onset of clinical signs as opposed to later in the course of disease. When variation due to different experiments was taken into account, (Bravo de Rueda *et al.*, 2014) reported that FMDV serotype or route of infection did not help explain the quantities of FMDV shed.

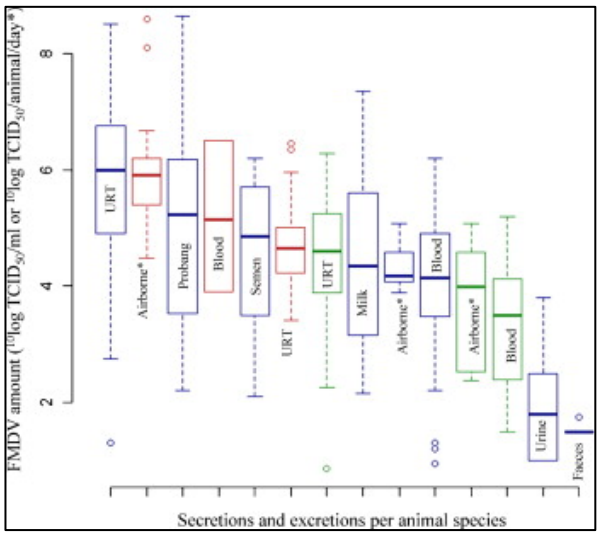

Figure 1.3: Boxplot of FMDV amounts in secretions and excretions. Cattle are represented by blue, swine by red and small ruminants by green. In airborne excretion (*), ^{10}log TCID$_{50}$/animal/day is reported. When applicable, each column contains the extreme of the lower whisker, the lower hinge, the median, the upper hinge and the extreme of the upper whisker for one plot. N = of 32 experiments involving 220 cattle, 71 pigs and 36 small ruminants. URT = upper respiratory tract secretions and excretions. From (Bravo de Rueda *et al.*, 2014).

The association between virus shedding, clinical signs and infectiousness

Whilst FMDV levels in secretions have been reported in multiple experiments, infectiousness is more difficult to measure, and carefully structured transmission studies are required. A recent study with serotype O (N = 9 infected cattle, 28 transmission attempts, and 8 transmission events), showed that, whilst virus is present in secretions prior to clinical signs, transmission events are most likely to occur in the first two days after onset of clinical signs (Charleston *et al.*, 2011). However, in contrast to this study (Charleston *et al.*, 2011), which allowed eight-hour transmission windows only, studies of animals in contact for extended periods of time showed that transmission is possible also prior to observation of clinical signs (Orsel *et al.*, 2007, 2009).

Transfer of virus and infection of another animal

FMDV can be transferred from an infected animal in close proximity, but can also survive in the environment for up to 14 weeks (for example in manure) (Bøtner & Belsham, 2012; Turner *et al.*, 2000), with higher relative humidity promoting virus survival and infectiousness (Donaldson, 1973). There are reports of windborne spread of FMDV under particular climatic conditions (Hugh-Jones & Wright, 1970). FMDV can survive in animal

products, such as untreated meat or milk, which may then be ingested by susceptible animals (Donaldson, 1997; Hartnett *et al.*, 2007). FMDV can also be mechanically transferred via people, non-susceptible animals and objects.

Infection routes in different species have been determined using experiments and field observations, with inhalation of aerosols infected with virus being most common for cattle and small ruminants, and ingestion of contaminated material for swine (Alexandersen *et al.*, 2003). These infection routes suggest that FMDV will be transmitted more rapidly in denser host populations, where more animals will be exposed to high levels of infectious virus from an infected individual. Transmission can also occur through insemination with semen from an infected animal (Cottral *et al.*, 1968) and intra-mammary inoculation (Burrows *et al.*, 1971). Transfer is theoretically possible thorough injection with FMDV contaminated materials and incisions with FMDV contaminated instruments (expert opinion from Prof. David Paton). The ability of FMDV to survive in biting flies has been recently demonstrated, but this potential route of transmission has never been proven (University of Edinburgh & Pirbright Institute, 2016).

Long-term FMD transmission cycles

Transmission from acutely infected animals has been relatively well documented, and extensive studies have investigated outbreaks occurring in countries that are normally FMD free (Boender *et al.*, 2010; Bouma *et al.*, 2003; Cottam *et al.*, 2008a, b; Gibbens & Wilesmith, 2002; Gibbens *et al.*, 2001; Haydon *et al.*, 2004). Conversely, many questions remain about long-term transmission cycles of FMDV in endemic regions. For example, FMDV is reported to have a high reproduction ratio (secondary cases for each infected unit), and, after infection, animals are immune to the variant of FMDV that infected them for several years (Doel, 1996, 2005). Therefore, it would be expected that a high level of herd immunity would cause extinction of FMDV variants after they have circulated through a large enough proportion of the population. However, this does not always happen, and very similar variants have been observed to recur over many decades. Potential explanations for these observations include that:

- There is a large enough connected community of susceptible hosts capable of maintaining a pathogen in the long term. In other words, as a portion of the community reaches a high level of immunity, the FMDV variant is maintained by

the other susceptible hosts within the community. Once immunity decays in the initial section (due to birth of naïve animals and possibly loss of acquired immunity), the variant can cause disease again in this original subset. The concept of a maintenance community (Haydon *et al.*, 2002; Viana *et al.*, 2014) is more fully described in Section 1.7.

- FMDV may have a lower reproduction rate in partially immune populations, particular species or populations with lower density or contact rates. This might result in slower development of herd immunity and longer persistence of the FMDV variant in the population.

- The FMDV variant remains in persistently infected animals long enough for the immunity levels in the population to decay. Transmission may then be achieved from the persistently infected host to a susceptible animal, causing the variant to continue circulating.

In relation to explanation 2, the estimated reproduction ratio of FMDV in a partially immune (vaccinated) population of cattle is lower than in unvaccinated cattle (Gonzales *et al.*, 2014). Similarly, a sheep transmission experiment suggested, that, whilst FMD can potentially persist in a sheep population, the reproduction ratio is relatively low (1.14, 95% CI: 0.3-3.0)), which might result in slower development of herd immunity (Orsel *et al.*, 2007). Research is ongoing to understand transmission parameters and persistence mechanisms of FMDV in African buffalo populations, that may also have lower reproduction ratios compared to cattle (Maree *et al.*, 2016). These populations potentially allow particular strains of FMDV to persist for longer, through lower transmission rates and subsequently slower development of protective immunity.

For explanation 3, there is scant evidence of FMD transmission from persistently infected animals. Milk and semen have respectively been demonstrated to contain FMDV, or at least its genome, for as long as 51 days (Burrows *et al.*, 1971) and five months (Sharma *et al.*, 2012) after infection, meaning that these are two possible transmission routes from animals with no apparent clinical signs. Trans-placental transmission of FMDV in sheep has been demonstrated, raising the possibility of expulsion of FMDV contaminated

foetuses and fluids many months after initial infection (Ryan *et al.*, 2007). Despite these experiments showing that animals can shed FMDV after clinical signs have subsided, no unequivocal reports of transmission from persistently infected livestock exist (Thomson, 1996). As discussed in more detail in Section 1.6, out of seven different experiments attempting to achieve transmission through protracted contact between persistently infected African buffalo and uninfected cattle, only two were successful (Dawe *et al.*, 1994; Vosloo *et al.*, 1996), whereas the other five did not demonstrate transmission (Anderson *et al.*, 1979; Bengis *et al.*, 1986; Condy & Hedger, 1974; Gainaru *et al.*, 1986; Maree *et al.*, 2016). Therefore, the role of persistently infected livestock and wildlife in FMDV transmission has yet to be fully understood.

As well as persistence mechanisms that may be employed by individual variants of FMDV, a further mechanism that might explain persistence is antigenic variation. Pathogens that have high reproduction rates may persist in the face of rapid development of herd immunity by antigenic variation to circumvent the immune response of the host population. As an RNA virus, the FMDV genome has a high replication error rate (Domingo *et al.*, 2006), facilitating rapid evolution of antigenic variants. This is reflected by the large number of FMDV serotypes and variants within serotypes. However, FMDV antigenic variance has a limit. Human rhinovirus for example, a related picornavirus, appears to have relatively more antigenic variation, comprising at least 102 serotypes (Savolainen *et al.*, 2002). This limit to antigenic variation in FMDV may possibly reflect the balance between the benefit of antigenic variation, and the cost of loss of important functions through changes in essential viral genes that may be unique for the ecological niche of each pathogen (Eigen, 2002; Grande-Pérez *et al.*, 2002). This might explain why only seven serotypes of FMDV have been identified.

1.4 Pathogenesis and the carrier state

The pathogenesis of FMD has been studied most extensively in domestic ruminants, and for serotype O. Upon initial infection, the virus replicates in the nasopharynx (Arzt *et al.*, 2010; Burrows *et al.*, 1981; Sellers *et al.*, 1968). This initial replication occurs in the epithelial cells of the mucosa associated lymphoid tissue (Arzt *et al.*, 2011b). There is subsequent widespread replication in pneumocytes in the lungs (Arzt *et al.*, 2010). Viraemia is detectable one or two days before the animal becomes pyrexic, distributing the

virus to multiple tissues and organs. Whilst there are high viral loads in all parts of the skin, the mouth, feet, teats, prepauce, and rumen are the areas that commonly vesiculate. High viral loads, without vesiculation, have been reported in the lungs, lymph-nodes and myocardium (Arzt *et al.*, 2011a).

After the acute signs of FMDV infection have subsided, some animals develop a persistent infection (carrier state). This is defined as recovered or vaccinated and exposed animals in which FMDV persists in the oropharynx for more than 28 days (OIE, 2015a). However, this definition of "carrier" conflicts with the epidemiological understanding of the word, which refers to an asymptomatic animal that can transmit a pathogen to another animal (Thomson, 1996). An example of a carrier in epidemiological terms would be a cow with no clinical signs but that sheds *Brucella abortus* during calving or in milk and infects other animals or people. In contrast to this, asymptomatic livestock with FMDV retrievable from their oropharynx have never been shown to infect other animals. Transmission from carrier buffalo has been demonstrated only in a minority of experiments (Dawe *et al.*, 1994; Vosloo *et al.*, 1996). Roughly 50% of domestic ruminants become persistently infected (Arzt *et al.*, 2011b). Cattle have been reported to carry the virus in their oropharynx for up to 3.5 years, sheep for at least 9 months and goats for 4 months (Alexandersen *et al.*, 2002). Ascertaining the role of persistently infected animals in FMD epidemiology is particularly relevant to African countries that contain the African buffalo, as this species is recognized to harbor FMDV in its oropharynx for up to five years (Condy *et al.*, 1985).

After ulceration of FMD lesions, secondary infections and mastitis may occur (Saini *et al.*, 1992). Chronic lameness due to secondary infections in the hooves is also an issue (Alexandersen *et al.*, 2003). Hirsutism, heat-intolerance and chronic loss of productivity are also reported as long-term sequelae of FMD infection in cattle (Bayissa *et al.*, 2011; Catley *et al.*, 2004; Ghanem & Abdel-Hamid, 2010). The pathophysiology of heat-intolerance is not fully understood, but there is evidence of FMDV replication in the pituitary gland, pathology in the pituitary, thyroid and adrenal glands, and reduced cortisol levels, (original reference (Minnett, 1949), further work reviewed by Arzt *et al.*, (2011a). This suggests metabolic derangement due to a dysfunction in the hypothalmo-pituitary-endocrine axis. Previous research has highlighted FMD related heat-intolerance syndrome as a problem for East African livestock keepers (Bayissa *et al.*, 2011; Catley *et al.*, 2004), but more information is required about the incidence of the syndrome.

1.5 Global distribution of FMD and occurrence in Africa

Whilst FMD was eradicated in most of Western Europe by the late 1980s, five out of the seven known FMDV serotypes (O, A, SAT 1, SAT 2 and SAT 3) are present in Africa, whereas A, O and Asia 1 serotypes are found in Asia and serotypes O and A are present in parts of South America. Serotypes A and O have the widest global distribution. Conversely, serotype C has been very rarely reported over the past 15 years, the last confirmed outbreaks occurring in Brazil and Kenya in 2004 (Rweyemamu et al., 2008). In Asia, South America and Africa, FMDV can be further divided into seven major pools of infection within which transmission tends to cluster (Paton et al., 2009) (Figure 1.4).

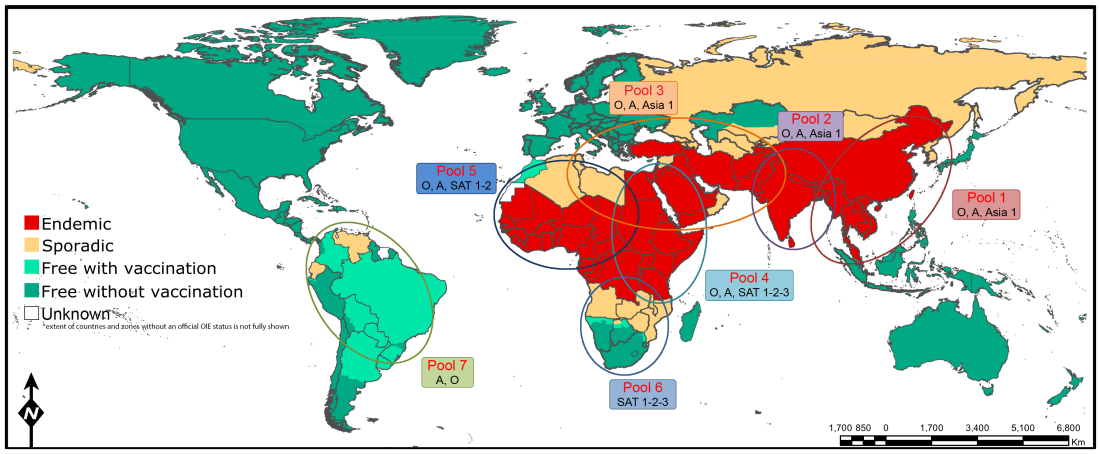

Figure 1.4: The conjectured status and distribution of FMD, showing regional virus pools.
Dr. Antonello di Nardo generated this map. Data for the map came from the World Reference Laboratory for Foot-and-Mouth Disease.

The escape of FMDV strains from their endemic pools into other regions is a matter of great concern due to the potential for disease emergence in new areas previously naïve to those strains. The recent outbreaks of SAT 2 in the Middle East and North Africa (Ahmed *et al.*, 2012) or the Pan-Asia strain of serotype O in the UK in 2001 (Knowles *et al.*, 2001) are examples of this. These introductions can have considerable consequences in terms of disease spread and severity even if resident FMDV strains are already present, because of poor cross-protection against exotic viral strains (Vosloo *et al.*, 2010). Host vulnerability to new strains was evident in a recent incursion of SAT 2 into Egypt, where mortality rates as high as 20% were reported in livestock (Ahmed et al., 2012).

It is generally believed that FMDV originated in Africa due to the long-term subclinical infection of African buffalo and the greater genetic diversity of the SAT serotypes compared to the Eurasian types (Vosloo et al., 2002). However, the earliest available descriptions of FMD come from Europe leading others to speculate that its origin lies on that continent (Tully & Fares, 2008). Additionally, it has been suggested that FMD was present in India in the 11th century (Ayangarya, 2006).

Human activity has had major impacts on the epidemiology of FMD. This is particularly evident in Africa, largely as a consequence of movements of animals and infectious diseases following European colonisation. The rinderpest pandemic, which swept across Africa in the late 19th century following the importation of livestock from India into Ethiopia, decimated more than 90% of cattle, buffalo and other susceptible species in eastern and southern Africa. The pandemic played a central role in the social and political history of Africa, in the epidemiology of many livestock and wildlife diseases present on the continent today (including FMD), and in shaping African ecosystems (Reid *et al.*, 2005; Sinclair *et al.*, 2007; Sinclair, 1979).

Reports of animals with FMD in southern Africa are as old as 1795 (reviewed by Knowles, 1990). However, the rinderpest pandemic largely removed populations susceptible to FMD and it is hypothesised that FMD occurrence declined around the turn of the century, with cases in southern Africa only being reported again in 1931 (Thomson, 1995). It is likely that currently circulating lineages of SAT serotypes re-emerged from small numbers of buffalo or livestock that survived the rinderpest pandemic once numbers of susceptible hosts had recovered.

Anthropogenic factors are also likely to have been critical in the introduction and spread of other serotypes in Africa, for example phylogenetic analyses are consistent with the interpretation that Eurasian FMDV serotypes (O, A and C) were re-introduced through trade and restocking of livestock from Asia or Europe following the ravages of rinderpest. There is evidence for a relatively recent (within the past 100 years) common ancestral history between FMDV O variants that are currently present in Africa, Asia and South America (Data from Knowles, N.J. reviewed by Casey *et al.*, 2014), consistent with emergence of O strains into susceptible animal populations of Africa as a result of introduction with cattle. Serotype O originating from India and Bhutan have recently

emerged in North Africa (Knowles *et al.*, 2014), highlighting the on-going escape of FMDV variants from their endemic pools due to human activities.

1.6 The role of wildlife in FMD epidemiology in Africa

Large populations of FMD susceptible wildlife, especially the African buffalo, complicate FMD epidemiology in Africa. Of all wildlife, African buffalo are thought to play distinctive roles as hosts for FMDV. They are the only wildlife species consistently shown to have high prevalence of FMDV infection in both southern (Jori et al., 2016; Miguel *et al.*, 2013; Thomson, 1995; Thomson *et al.*, 1992) and eastern Africa (Anderson *et al.*, 1979; Ayebazibwe *et al.*, 2010a; Bronsvoort *et al.*, 2008; Hamblin *et al.*, 1990; Mkama *et al.*, 2014). This species is present in wildlife-protected areas throughout Africa, with the highest population in Tanzania (Figure 1.5 and Table 1.2).

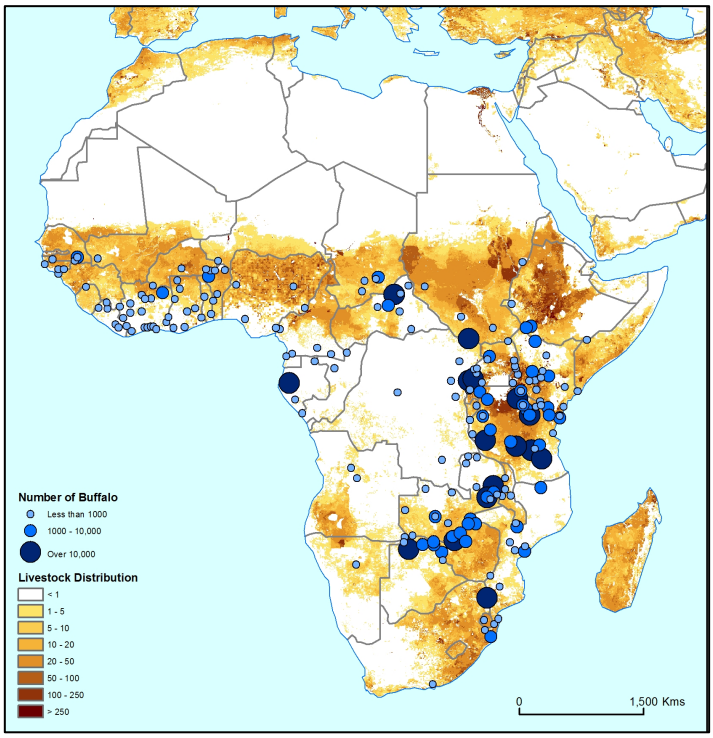

Figure 1.5: Cattle density (Robinson et al., 2007) and buffalo numbers (East, 1999) in Africa.
This map was made with ArcGIS software (ESRI, 2011) with the support of Dr. Mike Shand, University of Glasgow.

Table 1.2: The ten African countries with the highest populations of African buffalo as estimated by East (1999).
The most recent Tanzanian buffalo census indicates that buffalo numbers are increasing in Tanzania but numbers are not yet available for the whole country (TAWIRI, 2014).

Country	Estimated total number of African buffalo in 1998
Tanzania	>342,450*
Zimbabwe	>50,330
Zambia	>40,090
Democratic Republic of Congo	>39,180
South Africa	>30,970
Botswana	>26,890
Uganda	>20,220
Kenya	>19,560
Gabon	>20,000
Central African Republic	>19,000

In contrast to buffalo, the role of other African wildlife in FMD epidemiology is limited to being spillover hosts and very occasional intermediaries of transmission (Hargreaves *et al.*, 2004; Karesh, 2012; Vosloo *et al.*, 2009). Several studies have reported low FMD seroprevalence in non-buffalo wildlife (Anderson *et al.*, 1993; Bronsvoort *et al.*, 2008; Di Nardo *et al.*, 2015).

African buffalo are of particular concern where they may act as potential sources of FMDV for livestock, and as persistently infected animals where antigenic diversity may be generated (Vosloo *et al.*, 1996). Across southern Africa, for example, where FMD is well controlled in livestock, buffalo are implicated as the likely source of many new livestock outbreaks. (Caron et al., 2013; Hargreaves et al., 2004; Jori et al., 2009; Miguel et al., 2013; Thomson et al., 2003; Vosloo et al., 2002a, 2010). Only the SAT serotypes that are conventionally associated with buffalo are present in southern Africa. The lack of serotypes O and A that are typically associated with livestock reflects the strict control policies in keeping livestock FMD free. However, much less is known about the role of

buffalo elsewhere in Africa, and about the importance of buffalo-to-livestock transmission in triggering new outbreaks and sustaining endemic cycles of infection in livestock.

Acutely infected buffalo can develop FMD lesions that shed virus, albeit in quantities lower than cattle (Gainaru *et al.*, 1986). Buffalo calves become infected with FMD between three and six months (Condy & Hedger, 1978), with the proportion of persistently infected animals peaking in the one to three-year age group (Juleff *et al.*, 2012). It is speculated that acutely infected buffalo calves may be a source of virus for other animals (Thomson *et al.*, 2003). However, clear experimental evidence for FMDV transmission from artificially infected buffalo to livestock has been elusive. In the two experiments where transmission was achieved, cattle only became infected 5 and 10 months after the acute stage of the disease in the buffalo (Dawe *et al.*, 1994; Vosloo *et al.*, 1996). A further five studies reported absence of infection in cattle despite protracted contact with persistently infected buffalo (Anderson *et al.*, 1979; Bengis *et al.*, 1986; Condy & Hedger, 1974; Gainaru *et al.*, 1986; Maree *et al.,* 2016). In the studies where transmission occurred, male buffalo were mixed with female cattle, and cattle became infected only after the buffalo reached sexual maturity. This led to the hypothesis that FMD can be transmitted by the sexual route (Vosloo *et al.*, 1996). However, FMD virus was retrieved from semen and sheath wash from only one out of twenty FMDV seropositive male buffalo (Bastos *et al.*, 1999), and therefore the importance of possible sexual transmission of FMD from buffalo to cattle remains inconclusive.

It has also been questioned whether impala (*Aepyceros melampus*) play a role as spill-over hosts for FMD from buffalo (Bastos et al., 2000; Vosloo et al., 2009), and even as potential intermediaries that carry FMD between buffalo and cattle in southern Africa (Hargreaves et al., 2004). Whilst many studies have reported low FMD seroprevalence in impala (Bronsvoort *et al.*, 2008; Hamblin *et al.*, 1990; di Nardo *et al.*, 2012), a study in Kruger National Park suggested higher levels of FMDV infection in dense populations of impala (Vosloo et al., 2009). Experiences in Zimbabwe indicated that impala or kudu could have facilitated the transmission of FMDV from within a fenced wildlife conservancy to cattle (Hargreaves et al., 2004). Whether transmission intermediaries are involved or not, there is substantial evidence from southern African studies that buffalo, that have high levels of infection, are an important source of FMDV for southern African cattle, where the disease

is tightly controlled (Caron et al., 2013; Jori et al., 2009; Miguel et al., 2013; Thomson et al., 2003; Vosloo et al., 2002a, 2010).

In East Africa, where FMD is prevalent in both livestock and buffalo, the epidemiology is likely to differ. As FMD is endemic in the livestock population, and serotypes A and O are present, it is likely that FMD circulation can occur in the livestock population without the need for contact with wildlife. The degree to which buffalo- or livestock-related factors drive FMD circulation, particularly that of the SAT serotypes, is unknown.

A study to understand drivers of FMD infection in livestock in Cameroon, a region with a forest buffalo (*Syncerus caffer nanus*) population, highlighted that livestock movement and mixing were important risk factors for FMD (Bronsvoort et al., 2004a). Herd owners who moved their livestock farther saw more buffalo, confounding conclusions about the significance of buffalo contact as a risk factor. Livestock movement-related risk factors rather than wildlife-related risk factors have also been highlighted by a study in Tanzania, albeit with potential reporting bias (Picado et al., 2011). A study in Ethiopia also reported that larger herds were more likely to have FMD infected cattle (Bayissa et al., 2011). The predominance of livestock-related risk factors in other parts of Africa further demonstrates the potential contrast between FMD epidemiology in southern Africa and other regions on the continent. There is therefore a need to clarify the relative importance of livestock- and wildlife-related drivers of FMD infection in livestock in order to devise appropriate FMD control strategies.

Such differences in the epidemiology of FMD across Africa may relate to distinct livestock management practices. Much livestock management in East Africa relates to movement to reach the best grazing and water (Butt et al., 2009), the best market prices (FAO, 2013a) and avoidance of livestock diseases (Lankester et al., 2015a, b). This contrasts with ranch-based livestock management systems in southern Africa where fencing restricts movement. Therefore, many East African livestock are likely to move farther and contact a greater variety of livestock from different origins compared to buffalo. In contrast to livestock, buffalo movements are limited. They have a preference for availability of high volumes of grass and proximity to water (Hopcraft et al., 2012), and they rarely travel far from water sources (Naidoo et al., 2012). Their visits to water sources occur at predictable times and they will avoid people if possible (Prof. Tony Sinclair, personal communication). Further,

people (and subsequently the livestock they are herding) will avoid them, as they are dangerous animals. For example, injuries from buffalo were the fourth most common animal-related injury or illness in a hospital in a Tanzanian pastoral area (after brucellosis, dog and snake bites, (Hampson *et al.*, 2015). Therefore, if buffalo can shed sufficient FMDV to be a source of infection for livestock, a further question is when contact occurs for this transmission to take place.

1.7 Potential reservoirs of FMD

Given the selection of different potential FMD host populations in East Africa, and the contrast in conditions between eastern and southern Africa, the concepts of Haydon *et al.* (2002) and Viana *et al*. (2014) relating to reservoirs of infection can be used to clarify potential drivers of FMD transmission. Using this framework, domestic livestock are the population of interest, or the "target population." Control of FMD in this group is desired. For appropriate control policies, it is necessary to understand the relative importance of different potential sources of FMDV for livestock. Reservoirs comprise an ecologic system (i.e. a range of epidemiologically connected populations or environments) in which an infectious agent survives indefinitely, and from which infection is transmitted to the target population (Haydon *et al.*, 2002; Viana *et al*., 2014). Examples include wildebeest as a reservoir of Malignant Catarrhal Fever for cattle in East Africa (Plowright *et al.*, 1960), or domestic dogs as a reservoir of rabies for humans in many developing countries . Potential reservoirs of FMDV for East African livestock are connected systems of wildlife and / or livestock. For example, the East African livestock population is highly connected, with movements for grazing and markets meaning that a very large number of animals have connections for potential pathogen transmission. The system of potential wildlife hosts for FMD is more fragmented as it is confined to wildlife areas, but has been shown to be capable of maintaining pathogens such as MCF, and, in southern Africa, FMDV (Caron et al., 2013; Hargreaves et al., 2004; Jori et al., 2009; Miguel et al., 2013; Thomson et al., 2003; Vosloo et al., 2002a, 2010). Furthermore, the wildlife population may be connected with the livestock population in interface areas, giving it the potential to be a component of a maintenance community.

For potential wildlife and livestock reservoirs, key questions are:

i. Can FMDV persist indefinitely in the system without the need for transmission from another system?
ii. Is FMDV transmitted from the system to the target population (livestock)?

The answers to these questions may differ for different serotypes. For example, While SAT 1 and SAT 2 are known to be maintained in buffalo, and buffalo are a reservoir for livestock in southern Africa, these serotypes have also been able to "escape" from sub-Saharan Africa to cause extended livestock outbreaks in North Africa, the Middle East and Europe without involvement of buffalo or other wildlife species (Ahmed *et al.*, 2012; Bastos, 2003; Dimitriadis & Delimpaltas, 1992). This suggests that SAT 1 and SAT 2 can be maintained independently in both livestock and buffalo populations (Figure 1.6A). In the wildlife-rich rangelands of East Africa, the degree to which SAT1 and SAT2 outbreaks in livestock are sustained by re-introduction from buffalo is still unclear, and is a key question addressed in later chapters.

In contrast to SAT 1 and SAT 2, southern African research suggests that serotype SAT 3 is mainly confined to buffalo with only a small number of outbreaks reported in domesticated species (Figure 1.6B) (Thomson, 1995; Bastos et al., 2003; Thomson et al., 2003). There are no reports of SAT3 causing clinically evident FMD outbreaks in livestock in East Africa, but a recent report from Uganda provided strong evidence for transmission of SAT3 FMDV from buffalo to cause a subclinical infection in a domestic bovine (Dhikusooka *et al.*, 2015).

Conversely, although maintenance hosts for SAT serotypes, buffalo are not believed to be reservoirs of Eurasian FMDV serotypes for livestock (Anderson 1979; Ayebazibwe et al. 2010). Outside of experimental infection (Anderson *et al.*, 1979), serotypes A, O, C or Asia1 have never been isolated from an African buffalo. Rather, these serotypes are maintained in domestic livestock populations (Figure 1.6C).

Further wildlife species, whilst possibly not functioning as maintenance populations in their own right, may make up part of a maintenance community for FMDV (Table 1.3, Figure 1.6D). Abundant and mobile species such as impala or other antelope that may have close contact with both buffalo and livestock could be integral for disease transmission (Figure 1.6D) (Hargreaves *et al.*, 2004; Vosloo *et al.*, 2009). However, further evidence

about FMDV prevalence and infectiousness in these species would be necessary to substantiate such a hypothesis. Alternatively, non-buffalo wildlife could function as reservoirs capable of maintaining FMDV circulation without the need for contact with other hosts (Figure 1.6E), but there is no evidence to support this scenario.

Table 1.3: Explanation of terms associated with disease maintenance in populations.

Term	Definition	Reference
Critical community size	The minimum size of a closed population within which a pathogen can persist indefinitely	Bartlett (1960)
Maintenance population	A population larger than the critical community size: disease will be maintained within the population even if transmission into the population from the outside is prevented. A combination of non-maintenance hosts can still combine to make a maintenance community.	Haydon *et al.* (2002) Viana et al. (2014)
Reservoir	One or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined population of interested (target population).	Haydon *et al.* (2002) Viana et al. (2014)
Spill-over transmission	Inter-species transmission from a maintenance host to a non-maintenance host	Daszak et al. (2000); Power & Mitchell (2004)

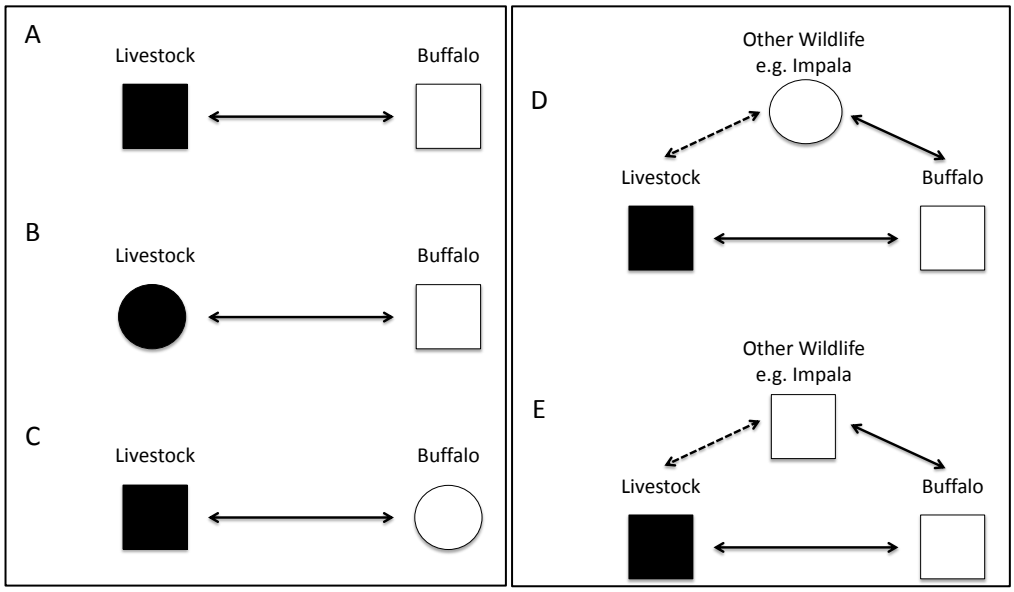

Figure 1.6: Simple models that outline possible FMDV reservoir systems in Sub-Saharan Africa.
Squares represent maintenance populations and circles show non-maintenance populations.
Schematics show different scenarios where:

A. Livestock and buffalo can both maintain FMDV independently of one another, as is thought to be the case for SAT 2 in different parts of Africa;

B. Buffalo, but not livestock can maintain FMDV independently, for example in the case of SAT serotypes in South Africa where livestock control measures are in place;

C. Livestock, but not buffalo can maintain FMDV independently, as is thought to be the case for serotypes A and O;

D. Livestock and buffalo can both maintain FMDV independently of one another. FMDV may spill over to other susceptible animals such as impala but cannot be maintained independently in this other wildlife population, as is the case in most non-buffalo wildlife in Africa; and

E. Livestock, buffalo and other wildlife can all maintain FMDV independently of one another but can also transmit it between each other, as is proposed for some high-density impala populations in South Africa.

1.8 Control strategies for FMD

Improved insight into the relative importance of livestock and wildlife populations as reservoirs of FMD for East African livestock would greatly facilitate decision making on the most appropriate control strategies in this region. In the context of reservoir dynamics, controls could involve measures to protect the target population and blocking tactics to separate the target population and the reservoir.

Target based controls could include vaccination of livestock, as has been used with success in concerted regional campaigns Europe and South America (Naranjo & Cosivi, 2013; Sumption *et al.*, 2012). However, control of endemic FMD in developing countries presents several several key challenges to current FMDV vaccination approaches that are based on using inactivated FMDV. These include short duration of immunity, the requirement for a vaccine cold-chain, poor immunogenicity, issues with vaccine strain selection due to high antigenic variation, and a shortage of the doses required for control (Parida, 2009; Paton *et al.*, 2009).

Some of these challenges have been reflected in recent field effectiveness trials. For example, a trial in Turkey suggested that vaccination alone was unlikely to produce the high levels of herd immunity needed to control the FMDV variants circulating in that country, especially serotype Asia1, without additional control measures (Knight-Jones *et al.*, 2014). The current emergence of a serotype A in the Middle East that matches poorly to any candidate vaccine strain adds difficulties in this region (ProMED, 2015). In Kenya, a locally produced SAT2 vaccine also lacked effectiveness (Lyons et al., 2015).

The requirement for a vaccine cold-chain up until the point of vaccination is a major obstacle to the success of vaccination programmes in developing countries (Paton *et al.*, 2009). Furthermore, rapid waning of vaccine-induced immunity (Woolhouse *et al.*, 1996), means frequent booster vaccinations at 4-12 month intervals are advised (Paton *et al.*, 2009). These logistic hurdles, in combination with the vast diversity of strains circulating in endemic areas such as East Africa, present a formidable challenge to vaccination strategies. For a successful approach, an understanding of which strains and serotypes are circulating, as well as the exposure risks and patterns in the target livestock population is critical.

As well as measures focussed on vaccinating the target population, blocking tactics to separate the target and the reservoir are widely used in FMD control. For example, FMD free countries apply strict border and import controls for livestock and their products to block contact between the FMD reservoirs of livestock in endemic countries and the target population of livestock in FMD free regions (OIE, 2015a). In South Africa where FMD is absent from the livestock population but present in the buffalo population, veterinary cordon fences (Figure 1.7) and a "barrier" of vaccination for livestock have been established between wildlife areas and FMD free areas (Brückner *et al.*, 2002).

Figure 1.7: A game fence to separate wildlife and livestock in South Africa. (http://www.chemvet.co.za)

For a successful blocking strategy, the reservoir of disease from which to separate the target needs to be identified. In East Africa, where FMD is endemic in both livestock and buffalo, it is not clear if buffalo are an important reservoir of FMD for livestock. For this reason, it is critical to understand the potential differences between FMD epidemiology in eastern Africa compared to southern Africa to determine if blocking tactics may be appropriate. In addition, veterinary fencing (Figure 1.7) could be devastating for the rangeland ecosystems of East Africa, where freedom to move is essential for both wildlife conservation and livestock management (Ferguson et al., 2013) (Figure 1.8).

Figure 1.8: A grazing area in Ngorongoro Conservation Area shared by wildlife and livestock.
Photo credit: Jason Bryars

As well as target and blocking tactics, a third FMD control strategy is to reduce the virus burden in the reservoir population, which also requires an understanding of reservoir dynamics. The continental vaccination efforts in South America to reduce FMD in the livestock reservoir are a successful example of this strategy (Naranjo & Cosivi, 2013). Reducing the global burden of FMD, as well as conferring clear benefits to livestock keepers suffering FMD outbreaks, is considered beneficial for FMD free countries, due to a reduced reservoir of disease threatening their target populations (Sumption *et al.*, 2012). If livestock are the main reservoir of FMD for East African livestock, the approach of reducing the FMD burden in the reservoir may be feasible. In contrast to livestock, reduction of FMD circulation in buffalo is not currently technically or logistically feasible (Thomson & Penrith, 2011). This further highlights the motivation to understand the importance of buffalo as sources of FMDV for livestock in East Africa, which would drive the selection of control options.

1.9 Diagnosis of FMD in endemic areas

To understand the epidemiology of FMD and to inform control options, the disease needs to be diagnosed. The developing countries where FMD is endemic are also those that present most challenges in terms of surveillance logistics (Namatovu *et al.*, 2013b).

1.9.1 Diagnosis when animals have acute FMD lesions

In endemic countries, FMD is often diagnosed from clinical signs alone. Due to the range of differential diagnoses for FMD, agreement between livestock keepers' recognition of FMD clinical signs and laboratory results requires investigation. In a study in Cameroon, there was good agreement between herdsmen reported FMD prevalence and seroprevalence of FMD at district level (Bronsvoort *et al.*, 2006b), and 69% of livestock keepers were able to differentiate FMD lesions from lumpy skin disease (LSD) lesions from photographs alone (Bronsvoort *et al.*, 2003). As well as LSD, other differential diagnoses for FMD include:

- Other vesicular diseases (swine vesicular disease, vesicular exanthema, vesicular stomatitis)
- Contagious ecthyma ('Orf')
- Infectious bovine rhinotracheitis
- Bluetongue
- Malignant Catarrhal Fever
- Bovine Papular Stomatitis
- Mucosal disease
- Peste des petits ruminants
- Mycotic stomatitis
- Phototoxic dermatitis
- Footrot
- Chemical irritants and scalding
- Traumatic lesions of mouth and feet

As well as livestock owner reports and clinical examination, FMD can be confirmed by a variety of diagnostic tests. Table 1.4 summarises the benefits and difficulties associated with each of these methods in developing countries.

Table 1.4: The diagnostic aids commonly used for FMD diagnosis available in European laboratories and for pen- side usage.
The table highlights which tests are appropriate for diagnosis of acute and previous FMDV infection. Y = Yes, N = No, Y/N = the test may lack sensitivity. The Immuno transfer blot assay is not included as it is not widely available.

Test	Acutely infected	Previously or persistently infected	Serotype specific	Benefit for East Africa	Limitation for East Africa	Reference
Pen-side tests						
Clinical signs of FMD	Y	N	N	No equipment needed	Mis-diagnosis possible	Kitching, (2002)
Immuochromatographic antigen lateral flow devices	Y	N	N	Straightforward and easy to use	Can lack sensitivity	Ferris et al., (2009)
Portable PCR	Y	N	N	Sensitive	Expensive	Callahan et al., (2002)
Loop-mediated isothermal amplification	Y	N	N	Performance comparable to PCR but easier and cheaper		Howson et al., (2015); Waters et al., (2014)
Laboratory tests						
Virus isolation	Y	Y/N	N	Gold standard	Time consuming, needs cell culture. Lesion material needs to be maintained at -70 C VI and genotyping from probang less successful due to lower virus level	OIE, (2012); Snowdon, (1966)
Genotyping	Y	Y/N	Y	Maximum information about virus	Needs sequencing facilities VI and genotyping from probang less successful due to lower virus levels	Knowles & Samuel, (2003)
Antigen ELISA	Y	N	Y		Can lack sensitivity and serotype specificity, needs reagents from rabbits and guinea-pigs	Roeder & Le Blanc Smith, (1987)
PCR	Y	Y/N	N	More sensitive than virus isolation or genotyping		Callahan et al., (2002); Reid et al., (2000)
Serotype specific PCR	Y	Y/N	Y	Potentially very useful where sequencing not available	Pending full validation	Bachanek-Bankowska et al., (2014)
Commercial Non structural protein ELISA (3ABC blocking)	Y/N	Y	N	Sensitive and specific for diagnosing previous infections and easy to use		Brocchi et al., (2006); Bronsvoort et al., (2006b)
Solid Phase Competition ELISA	Y/N	Y	Y		Can lack serotype specificity, needs reagents from rabbits and guinea-pigs and lengthy antigen production process	Li et al., (2012); Mackay et al., (2001); Paiba et al., (2004)
Liquid Phase Blocking ELISA	Y/N	Y	Y			Hamblin et al., (1986)
Virus Neutralisation Testing	Y/N	Y	Y		Time consuming and needs cell culture and high expertise	OIE, (2012)
Commercial SP kits - serotype A and O	Y/N	Y	Y	Easy to use	Can lack serotype specificity	Chenard et al., (2003)
Commercial SP kits - SAT 1 and SAT2	Y/N	Y	Y	Easy to use	Not widely available	Brocchi, (2012b)

1.9.2 Retrospective diagnosis of FMDV infection

Outbreaks are more likely to be reported in areas with better infrastructure (Picado *et al.*, 2011), and this may cause bias due to under-reporting in remote areas. These challenges can be overcome by intensive surveillance and prospective studies, but this work is demanding in absence of a strong veterinary infrastructure. Retrospective diagnostic aids (I.e. diagnosing FMD infection history) can empower the epidemiologist to design structured cross-sectional studies with randomised sampling strategies (Dahoo, 2009).

It is possible to detect FMDV and its genome from oropharyngeal (probang) samples from persistently infected animals, and this constitutes the OIE "FMD carrier" definition, as explained in Section 1.4 (page 4). However, field conditions, the necessity of a cold chain, and low virus loads (Namatovu *et al.*, 2015), can mean that detection of FMDV through virus isolation from probang samples is challenging. Techniques using PCR are more sensitive (Reid *et al.*), and the advent of serotype-specific PCR may increase the information yielded by these samples (Bachanek-Bankowska *et al.*, 2014). However, due to a gap in our understanding of the epidemiology of persistent FMD infection at animal level (Juleff *et al.*, 2012; Parida, 2010; Thomson, 1996), and potential lack in diagnostic sensitivity due inherent variability in virus loads and sampling techniques, it is difficult to incorporate results from oropharyngeal samples into epidemiological risk-factor studies.

In contrast, serological surveys are widely used for risk factor analysis. Serological testing for antibodies against a pan-serotypic FMDV antigen (the 3ABC non-structural protein) has proven to be sensitive and specific (Brocchi *et al.*, 2006; Bronsvoort *et al.*, 2004; 2006b; 2008) (Table 1.5). The wide availability of commercial ready-to-use kits based on monoclonal antibodies against a well-conserved 3B peptide and recombinant 3ABC antigen is a major advantage of this approach (Chung *et al.*, 2002; Sorensen *et al.*, 2005).

Table 1.5: Performance characteristics of the foot-and-mouth disease non-structural protein ELISA kit used in the present study. (VNT = Virus neutralisation test, SAT = South African Territories serotype). Kit is PrioCHECK®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33, Lelystad, Netherlands.

Test sera	Analyses	Sensitivity	Specificity	Reference
Negative sera from European animals (N=675), positive sera from experimentally infected non-vaccinated animals up until 100 days after infection (N = 58).	ELISA results compared to experimental infection history as a "gold standard"	100%	98.10%	Brocchi *et al.*, (2006)
Zebu cattle sera from herds in Cameroon (FMD endemic area) (N = 1620)	Sera tested with two different NSP ELISA kits. Results were compared to VNT results as "gold standard"	71%	90.00%	Bronsvoort *et al.*, (2004)
Zebu cattle sera from herds in Cameroon (FMD endemic area) (N = 1375)	Sera tested with three different NSP ELISA kits and latent class analysis used for estimation of sensitivity and specificity of each kit	96.9%	90.9%	Bronsvoort *et al.*, (2006b)
Wildlife sera from East and Central Africa (N = 731)	Sera tested with one NSP kit and with SAT1, SAT2 and SAT3 VNTs . Latent class analysis used for estimation of sensitivity and specificity of ELISA kit	87.70%	87.30%	Bronsvoort *et al.*, (2008)

The diagnosis of exposure to specific serotypes of FMDV in endemic countries is more challenging compared to diagnosis of previous infection with any serotype. Differentiating infection from vaccination (Ludi *et al.*, 2014b), serial infections with different serotypes (Bronsvoort *et al.*, 2006a) and potential immunological cross-reaction (Hedger *et al.*, 1982; Namatovu *et al.*, 2013a; Di Nardo *et al.*, 2015) are obstacles to reconstruction of an animal's FMDV infection history from serological data.

Serotype-specific assays based on the structural proteins of FMDV include ELISAs (Hamblin *et al.*, 1986a; Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004) and virus neutralisation testing (VNT (Golding *et al.*, 1976; OIE, 2012a)) (Table 1.4). Whilst VNT is considered the "Gold Standard" serological diagnostic aid for FMD (OIE, 2012a), cross-neutralisation has been reported in serially infected cattle (Cottral & Gailunas, 1971). It was also suspected in sera from African buffalo where antibodies that neutralised serotypes A, O, C and Asia1 were detected despite only SAT serotypes ever being isolated from buffalo (Anderson *et al.*, 1979; Hedger *et al.*, 1982). Issues with interpreting ELISA data due to cross-reaction between serotypes are also reported in multiple studies attempting to utilise these assays for serotype specific FMD diagnosis in Africa (Namatovu *et al.*, 2013a;

Di Nardo *et al.*, 2015). Further studies are necessary to disentangle the drivers of this cross-reaction and cross-neutralisation in serially infected animals.

As well as diagnosis of infection with specific serotypes, another issue with serological testing is lack of understanding of how long ago an animal with a positive serological result was infected. A recent study showed that sera from six out of seven cattle with clinical signs of FMD three years previously still produced positive results with a commercial 3ABC non-structural protein ELISA (Elnekave *et al.*, 2015). Very little recent longitudinal serological FMD data are available from endemic countries, and this deficiency much be addressed if more information is to be extracted from serological results.

In experimental studies, the longevity of antibodies against FMDV structural proteins has been investigated through serum neutralisation and post infection protection studies. There is evidence for protection against the same (homologous) virus type a year after initial infection, and in one of three cattle 4.5 years after infection (Clunliffe, 1964). This protection is likely to correspond to the presence of neutralising antibodies against FMDV structural proteins, but further work to understand this association is required (Doel, 1996, 2005). Another early study reported neutralising antibodies in sera from cattle 5.5 years after infection (Garland, 1974). Where cattle were serially infected with different serotypes, cross-protection and cross-neutralisation against different (heterologous) serotypes has been described (Cottral & Gailunas, 1971). The duration of protection after vaccination rather than infection has been reported to be far shorter; antibodies detected by a structural protein ELISA were taken as a proxy for immunity and their half life was estimated to be 43 days (Woolhouse *et al.*, 1996).

As well as presenting challenges in the interpretation of serological results, these findings in previous studies highlight the gap in our understanding of long-term FMDV dynamics and of the epidemiology of serial FMDV infections in an endemic, multi-serotype environment.

1.10 Impacts of FMD

FMD is considered one of the most economically devastating diseases of animals globally (Sumption *et al.*, 2012). The economic impacts of FMD include direct effects of the disease on livestock productivity and the indirect effects of costly control measures and revenue forgone from loss of market access or the use of less productive livestock (Rushton, 2009) (Figure 1.9).

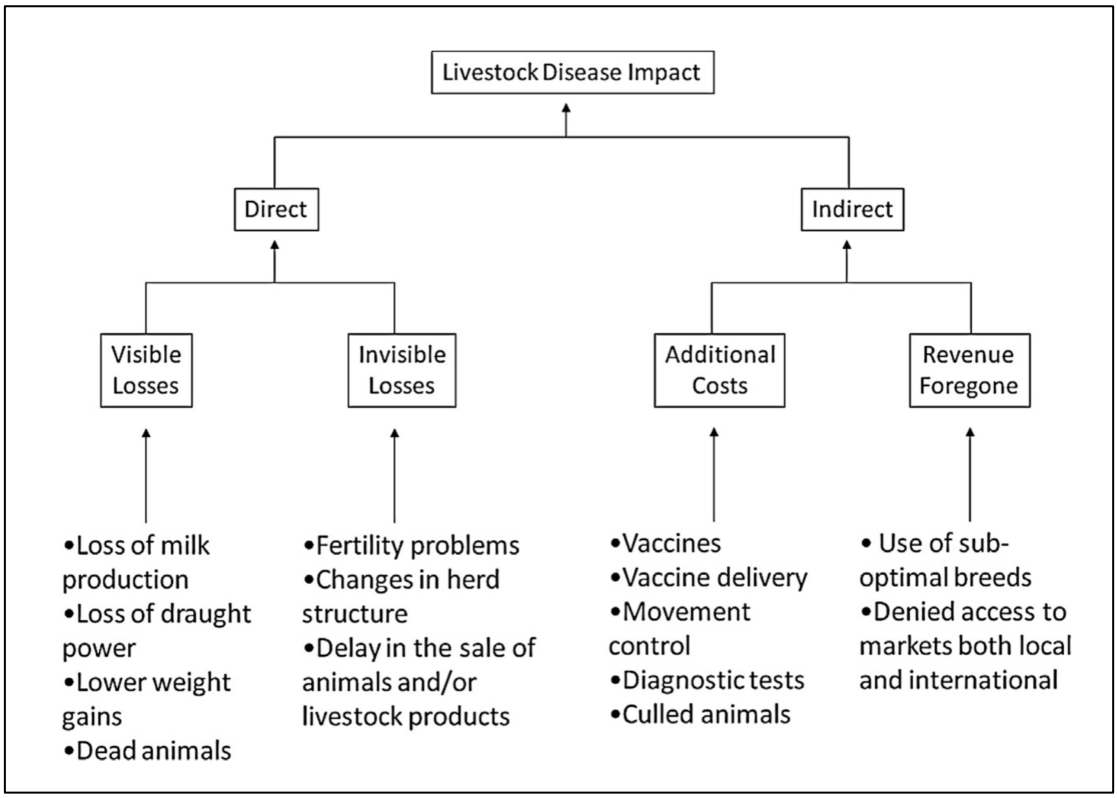

Figure 1.9: The impacts of livestock disease (Rushton, 2009).

A large proportion of FMD impacts in more developed countries are related to loss of access to international markets for livestock products. Animal product import policies are often based on the Terrestrial Animal Health Code of the OIE (OIE, 2015a). Countries, zones[2] or compartments[3] may be classified according to OIE measures of FMD freedom as follows:

1. FMD free country or zone where vaccination is not practiced
2. FMD free country or zone where vaccination is practiced
3. FMD free compartment
4. FMD infected country or zone

Many countries will only accept animal products from countries or zones with FMD freedom. The introduction of FMD to normally FMD free countries means that they are excluded from lucrative international markets for animal products for at least three months after the last case of FMD (OIE, 2015a). There are enormous costs involved in disease control measures and in regaining FMD free status to satisfy international trade requirements. For example, total direct costs to industry and government due to the 2001 UK FMD outbreak were estimated to be over £8 billion (National Audit Office, 2002). These incursions into FMD free countries occur periodically. For example, there have been outbreaks in the Republic of Korea and in Japan over the past six years (OIE, 2016). Total compensation expenses for culled animals in the 2010 Japanese outbreak amounted to approximately $550 million (Muroga *et al.*, 2012). Based on press reports, the 2010-11 Korean outbreak generated direct costs of over $2780 Million (reviewed by Knight-Jones & Rushton, (2013). South Korea has suffered further FMD outbreaks in 2014 and 2016 (OIE, 2016).

Ongoing control efforts in regions working to finalise and maintain FMD free status place an enormous demand on veterinary resources. For example, South America's intensive

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[2] **OIE disease free zone: A zone in which the absence of the disease under consideration has been demonstrated by the requirements specified in the Terrestrial Code for free status being met. Within the zone and at its borders, appropriate official veterinary control is effectively applied for animals and animal products, and their transportation (OIE, 2015a)**

[3] **OIE disease free compartment: Animal subpopulation contained in one or more establishments under a common biosecurity management system with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade. (OIE, 2015a)**

vaccination programme is estimated to cost $0.7 billion annually (Knight-Jones & Rushton, 2013). However, these efforts are economically justified in terms of improved animal productivity and access to international markets (Naranjo & Cosivi, 2013).

In contrast to South America where there are no wildlife reservoirs of FMD for livestock (Karesh, 2012), the achievement of OIE FMD free status in Africa is confounded by the presence of FMD in the African buffalo. Southern African countries, for example South Africa, apply geographic zones of FMD freedom for trading purposes. The economic impacts of FMD here are associated with rules for access to international markets and ongoing efforts to control disease. Until the most recent OIE code update (OIE, 2015a), southern African countries had to apply stringent controls based upon extensive veterinary cordon fencing to separate wildlife and livestock if they wanted to access international beef markets. As well as the expense of fencing, there are considerable impacts due to fences preventing the movement of wildlife and pastoral livestock that need to reach grazing and water (Ferguson & Hanks, 2010; Mbaiwa & Mbaiwa, 2006; McGahey, 2010; Wildlife Conservation Society, 2012). Given the high value of wildlife tourism to many African countries (Booth, 2010), interference with wildlife migration can translate into economic impacts. The trans-frontier conservation programmes to allow better connectivity between conservation areas in southern Africa are potentially in conflict with many of the measures supported by conventional OIE policy (AHEAD.GLTFCA, 2008; Ferguson *et al.*, 2013). For example, under the old guidelines, the presence of persistently infected buffalo in a geographic zone precluded access of animal products to international markets (OIE, 2011), encouraging measures to completely separate livestock and wildlife and discouraging free movement of wildlife across international borders. The update to the OIE code in 2015 (OIE, 2015a), addresses some of these issues. It means that African countries with FMD in wildlife but not normally in livestock are more likely to be able to trade animal products based on systematic reduction of FMD risk at every point in the livestock value chain (Thomson & Penrith, 2015; Thomson *et al.*, 2009). Measures include vaccination, surveillance and destruction of any possible FMDV in beef through a deboning process. As well as heralding potential economic benefits for livestock producers in South Africa and reduced interference by veterinary fences with conservation objectives, this change in the OIE code may also be beneficial for other African countries with buffalo in the future if they can establish acceptable FMD control standards in the animal product value chain.

Despite developing countries in Africa and Asia suffering the largest burden of FMD (Figure 1.4), less is known about the impacts of endemic FMD on the poor compared to knowledge of its impacts in more developed economies (Knight-Jones & Rushton, 2013). For the rural poor, direct impacts of FMD on livestock productivity are likely to be most immediately relevant, and trade rules for access to European, American or far Eastern markets are perhaps a more distant concern. In contrast to accessing remote international markets, intra-regional trading opportunities are accessible to rural livestock keepers, and represent an empowering source of revenue (Little, 2009).

The impact of livestock diseases on poverty has been assessed on the basis of treatment costs, reduced productivity of animals, loss of draught power for tillage and transport, disruption of access to markets, the cost of risk management, limitation of land usage in areas with high disease risk, and risk adversity to embracing advances in animal management (Perry et al. 2002a). Based on weighted analysis of socio-economic criteria and national impacts that also affect the poor, FMD was ranked third (after gastro-intestinal helminths and neonatal mortality syndrome) amongst animal diseases having greatest impact on overall poverty (Perry et al. 2002b).

FMD is prevalent in East African livestock (Bayissa *et al.*, 2011; Genchwere *et al.*, 2014; Mkama *et al.*, 2014; Namatovu *et al.*, 2013a; Wekesa *et al.*, 2015), and rural livestock owners rank it highly amongst the diseases affecting their herds (Bedelian *et al.*, 2007; Cleaveland *et al.*, 2001; Jost *et al.*, 2010; Ohaga *et al.*, 2007). These studies suggest that FMD has a major impact on rural communities across East Africa. However, these impacts have not been fully quantified. Impacts of FMD, and hence demands and incentives for control, are likely to differ across settings, production systems and segments of society (Perry & Rich, 2007). A better understanding of these differences could inform control policies targeted to benefit those whom FMD affects the most.

1.11 Global efforts to tackle endemic FMD

FMD is often preventable, and control is considered a public good (Sumption *et al.*, 2012). European countries successfully ended endemic FMD circulation in the region through a widespread and coordinated vaccination campaign, biosecurity measures and culling policies for infected animals (Sumption *et al.*, 2012). South America has achieved a high

degree of FMD control through a coordinated regional control programme, aiming to eliminate FMD from the continent by 2020 (Naranjo & Cosivi, 2013).

The success of the current South American control programme is believed to be based on regional international coordination, strengthening of veterinary infrastructure, buy in from the private sector and multiple institutions, the provision of permanent technical, laboratory and administrative support services and good training of staff and stakeholders involved in FMD control (Naranjo & Cosivi, 2013).

Although FMD elimination is not likely to be feasible in Africa due to wildlife reservoirs, a similar holistic, ecosystem-based approach for FMD control in Africa has been advocated (Maree *et al.*, 2014). The identification of primary endemic areas, animal husbandry practices, climate, and animal movement were highlighted as key considerations. Experiences with rinderpest eradication in Africa have shown that human behaviour and the engagement of rural communities with disease control efforts are vital for success (Mariner *et al.*, 2002, 2012). The need for continental disease control programmes to take into account contrasts in development and the different veterinary infrastructure in different countries is also highly relevant (Maree *et al.*, 2014; Naranjo & Cosivi, 2013).

In light of the recent successful eradication of rinderpest, ever increasing globalisation and subsequent FMD threats to free countries, and the recognition of the global impacts of FMD, the FAO and OIE have developed a pathway to structure and support FMD control efforts (Sumption *et al.*, 2012)(OIE & FAO, 2012) (Figure 1.10). The aim of the Progressive Control Pathway for FMD (PCP-FMD) is to reduce the impact and load of FMD globally. Progress to stage 5 (maintaining zero FMD circulation and incursions and withdraw vaccination) may not be possible given livestock management practices and FMD infected wildlife in many African countries. However, progressing through the earlier stages of FMD control could herald great benefits for poverty alleviation and food-security for subsistence farmers, and facilitates governments focussing on FMD control options (Ferguson *et al.*, 2013). With the advent of this international drive to control FMD, and the high importance of fighting poverty and promoting food security amongst current global Sustainable Development Goals (United Nations, 2016a), an improved understanding of FMD impacts and epidemiology in East Africa is a highly relevant.

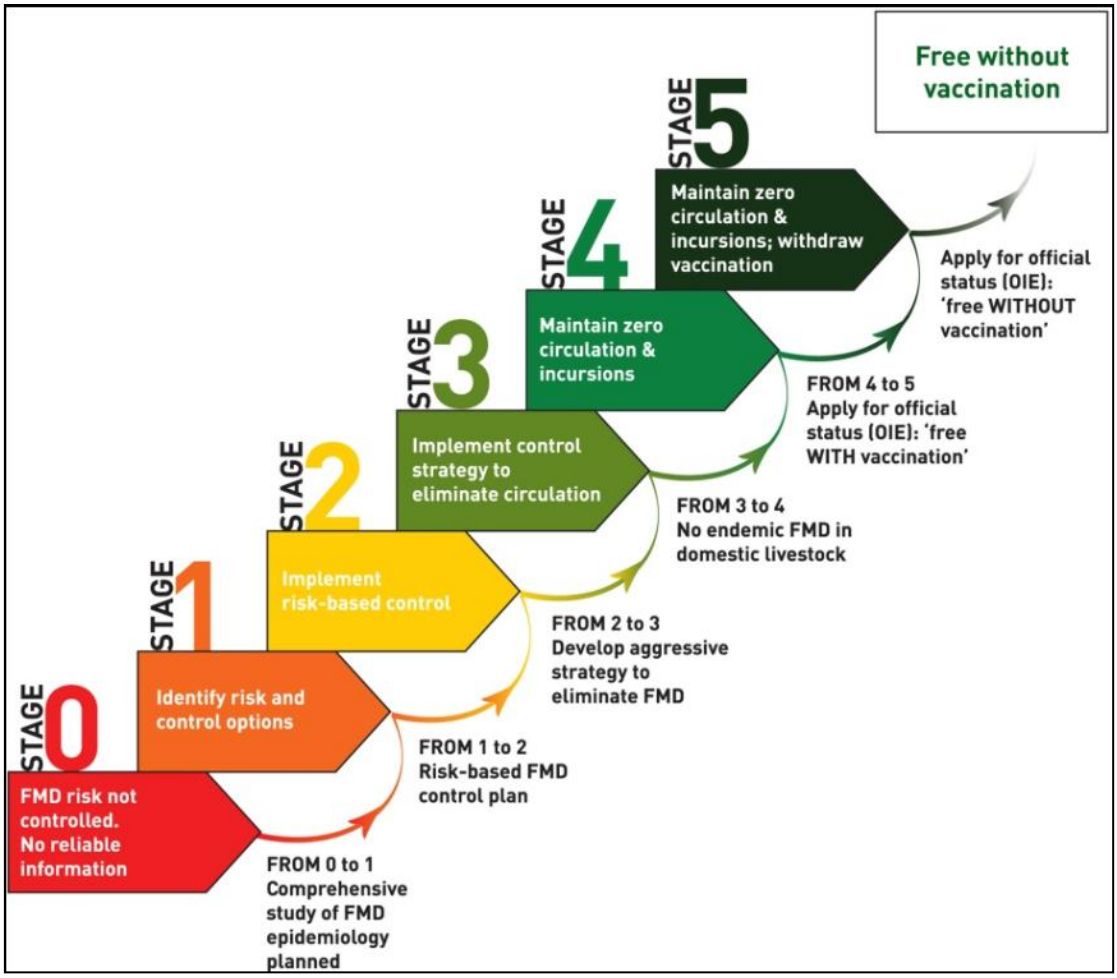

Figure 1.10: The Progressive Control Pathway for Foot-and-mouth disease (Sumption et al., 2012).

1.12 Aims

The review of the literature has highlighted the importance of FMD globally and the strong international motivation to reduce its burden and impact in endemic countries. However gaps in in our understanding of FMD epidemiology are evident, especially in regions where FMD is endemic in both livestock and buffalo, and where multiple FMDV serotypes are circulating. Furthermore, for greater prioritisation of FMD control as a public good in poorer communities, a better understanding of the nature and drivers of impacts on rural households in different livestock production systems is critical.

To address these knowledge gaps, the aims of this study were:

1. To determine the prevalence of FMD and evaluate its impact on rural livelihoods in East Africa.
2. To improve understanding of risk factors for FMD in East Africa and the role of wildlife in its epidemiology.
3. To characterise serotype specific FMD circulation patterns over space and time in East Africa.

1.13 Thesis outline

This project investigated the impact and epidemiology of FMD at the wildlife-livestock interface in northern Tanzania. The pursuit of these aims necessitated the optimisation of a serotype specific laboratory assay for East Africa and the development of a statistical tool to interpret serology results in a multi-serotype environment.

The thesis begins with an overview of the study area in northern Tanzania, field study design, laboratory methods and the project timeline. The optimisation of serological assays for East African purposes is also described (Chapter 2). This is followed by investigations into the prevalence of FMD and its impact on rural livelihoods (Chapter3) and into risk factors for FMD infection and outbreaks (Chapter 4). Chapter 5 addresses issues with inference of serotype-specific infection history from serology results through the development of a novel Bayesian methodology. In Chapter 6, this methodology is capitalised upon and combined with longitudinal virus isolation results and data on FMD infections in buffalo to elucidate patterns of FMDV infection over time in the study region.

The thesis concludes with a discussion of these findings and the opportunities that they highlight for future FMD control in East Africa. Figure 1.11 links the PhD chapters to the aims of the thesis.

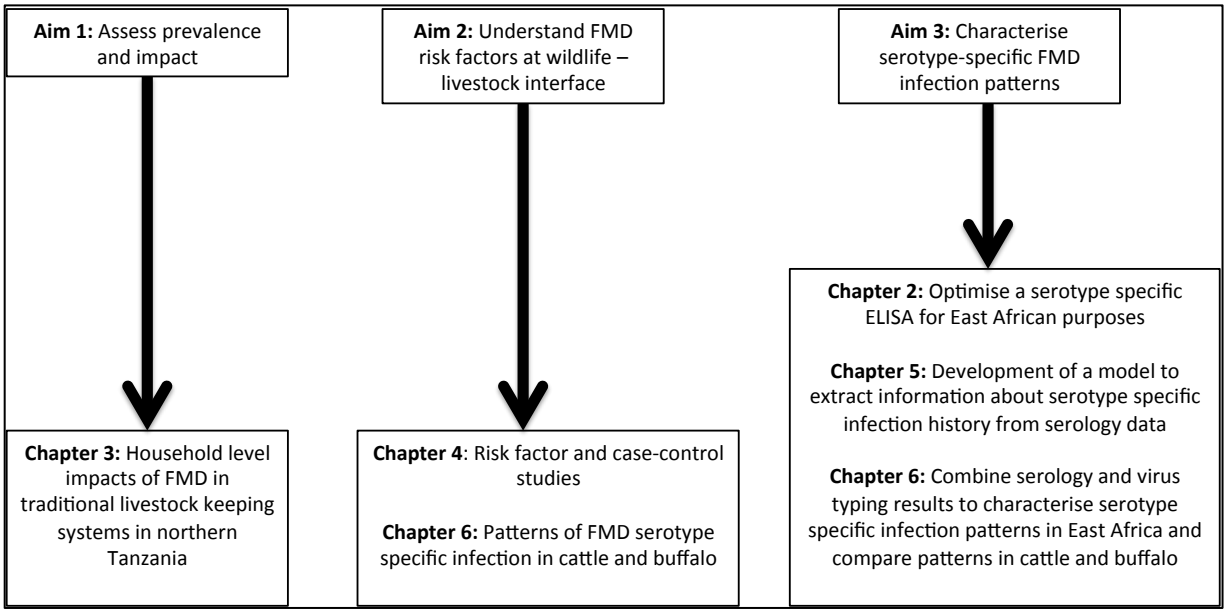

Figure 1.11: Linking the PhD chapters to the aims for the thesis.

Chapter 2: Background to study area and overview of field and laboratory methodology

2.1 Chapter overview

This chapter provides background information about the study area, field study design, diagnostic sampling and laboratory methodology.

2.2 Study area

The study area was in Northern Tanzania (Latitude 1-5 degrees south, Longitude 33 – 38 degrees east, Figure 2.1). The study period was from the beginning of 2011 to the end of 2014. Study areas included:

1. **Strictly protected wildlife areas:** Arusha, Kilimanjaro, Lake Manyara, Serengeti and Tarangire National Parks (NP).
2. **Areas shared by wildlife and livestock:** Loliondo Game Controlled Area (LGCA), Monduli Forest Park, Ngorongoro Conservation Area (NCA), conservation areas in Simanjiro and Monduli East and North of Tarangire NP and some game reserves to the west of Serengeti NP.
3. **Predominantly livestock areas:** Serengeti and Bunda districts, Arusha peri-urban area, and parts of Simanjiro. These areas are not wildlife-protected, but there are very few fences separating livestock and wildlife.

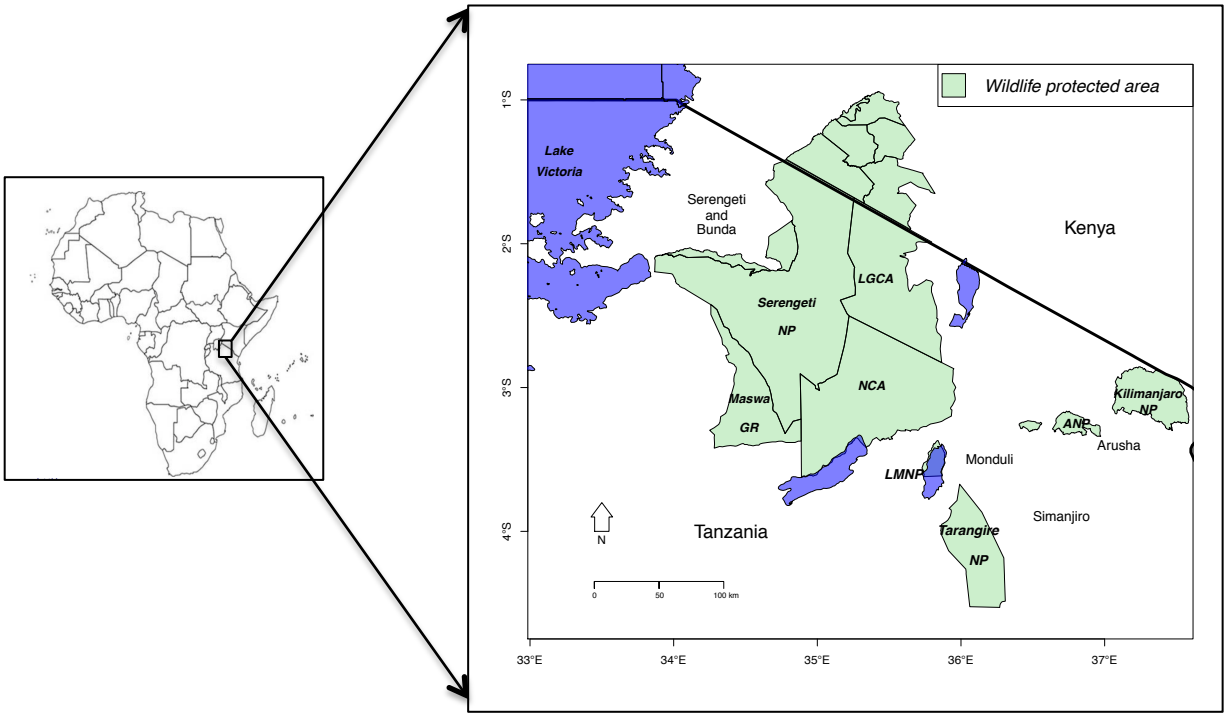

Figure 2.1: A map of the study area in Northern Tanzania. ANP = Arusha National Park, GR = Game Reserve, LGCA = Loliondo Game Controlled Area, LMNP = Lake Manyara National Park, NCA = Ngorongoro Conservation Area, NP = National Park.

2.2.1 Precipitation

Mean annual precipitation in the study area between January 2009 and December 2014 varied from less than 300 mm in the southeast to above 1000 mm in the northwest to (Figure 2.2).

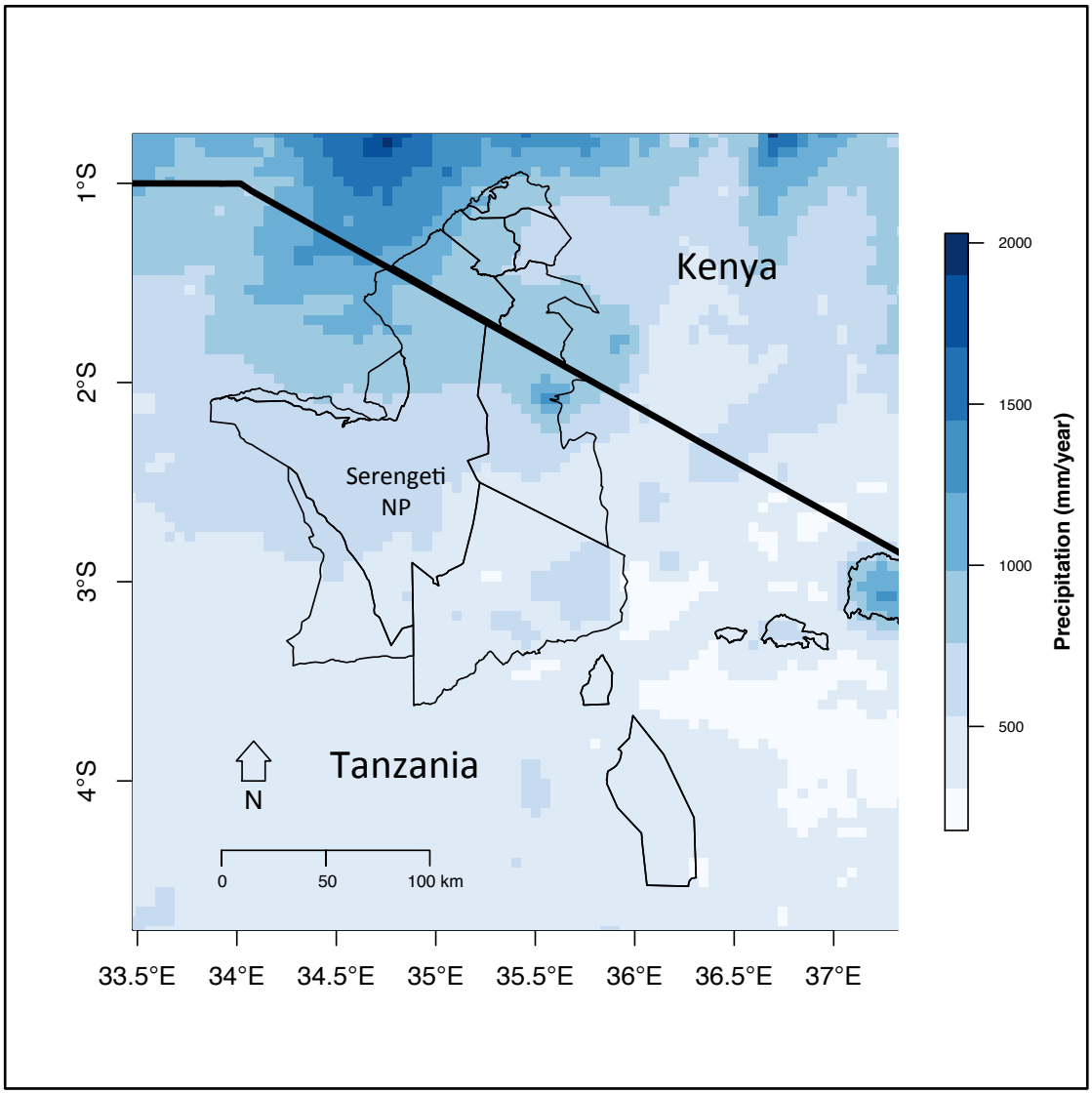

Figure 2.2: Average annual precipitation in the study area (Latitude 1-5 degrees south, Longitude 33 – 38 degrees east) between January 2009 and December 2014, expressed in average mm per year. NP = National Park. The wildlife areas in the study area as well as the border with Kenya are shown on the map as black lines. Data for the map came from the Climate Hazards Group Infra Red Precipitation with Station data (CHIRPS) (Funk *et al.*, 2014) Website: http://chg.geog.ucsb.edu/data/chirps/, and the map was made in the R Statistical environment (R development core team, 2008).

In East Africa there are wet and dry seasons. In the study area, rainfall varied throughout the year with peaks in November and December ("the short rains") and from March to May ("the long rains"). The dry season was from June to September (Figure 2.3).

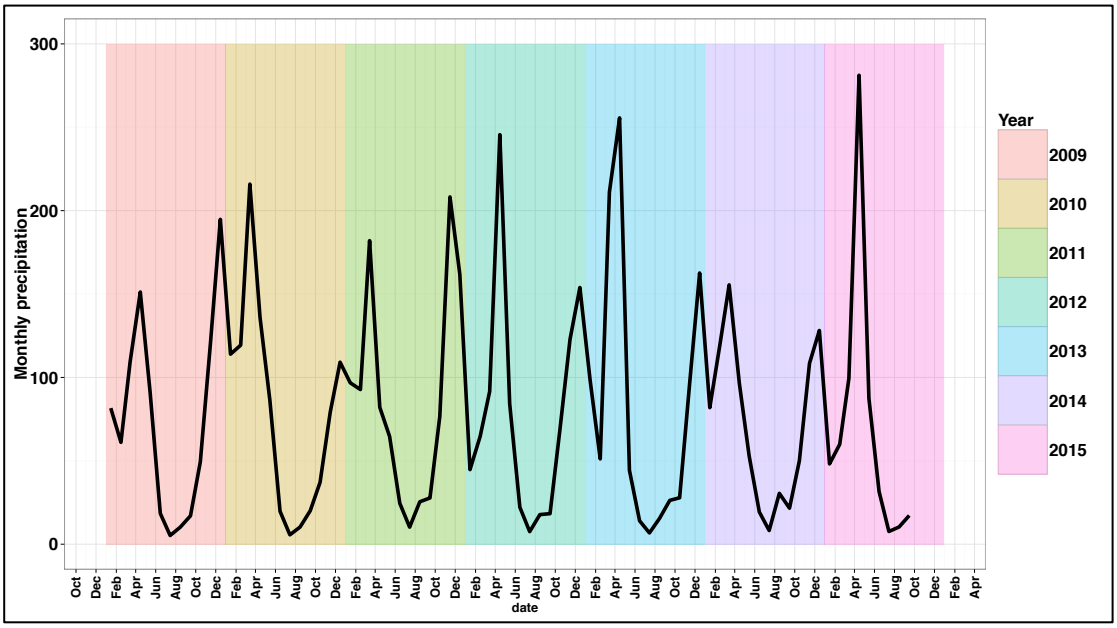

Figure 2.3: Average monthly precipitation in the study area (Latitude 1-5 degrees south, Longitude 33 – 38 degrees east) between January 2009 and October 2015, expressed in average mm per month. Data to make the plot came from Climate Hazards Group Infra Red Precipitation with Station data (CHIRPS) (Funk *et al.*, 2014) Website: http://chg.geog.ucsb.edu/data/chirps/

2.2.2 Human population

Tanzania has approximately 52.4 million inhabitants (Worldpop, 2015). In the study area for this project, human population density was highest around Arusha and Moshi urban centres and in the agropastoral areas to the west of Serengeti National Park (Tanzania National Bureau of Statistics, 2012). The areas to the east of Serengeti National Park and Tarangire National Park had lower human populations, consisting predominantly of pastoralists. Figure 2.4 shows human population density in the study area.

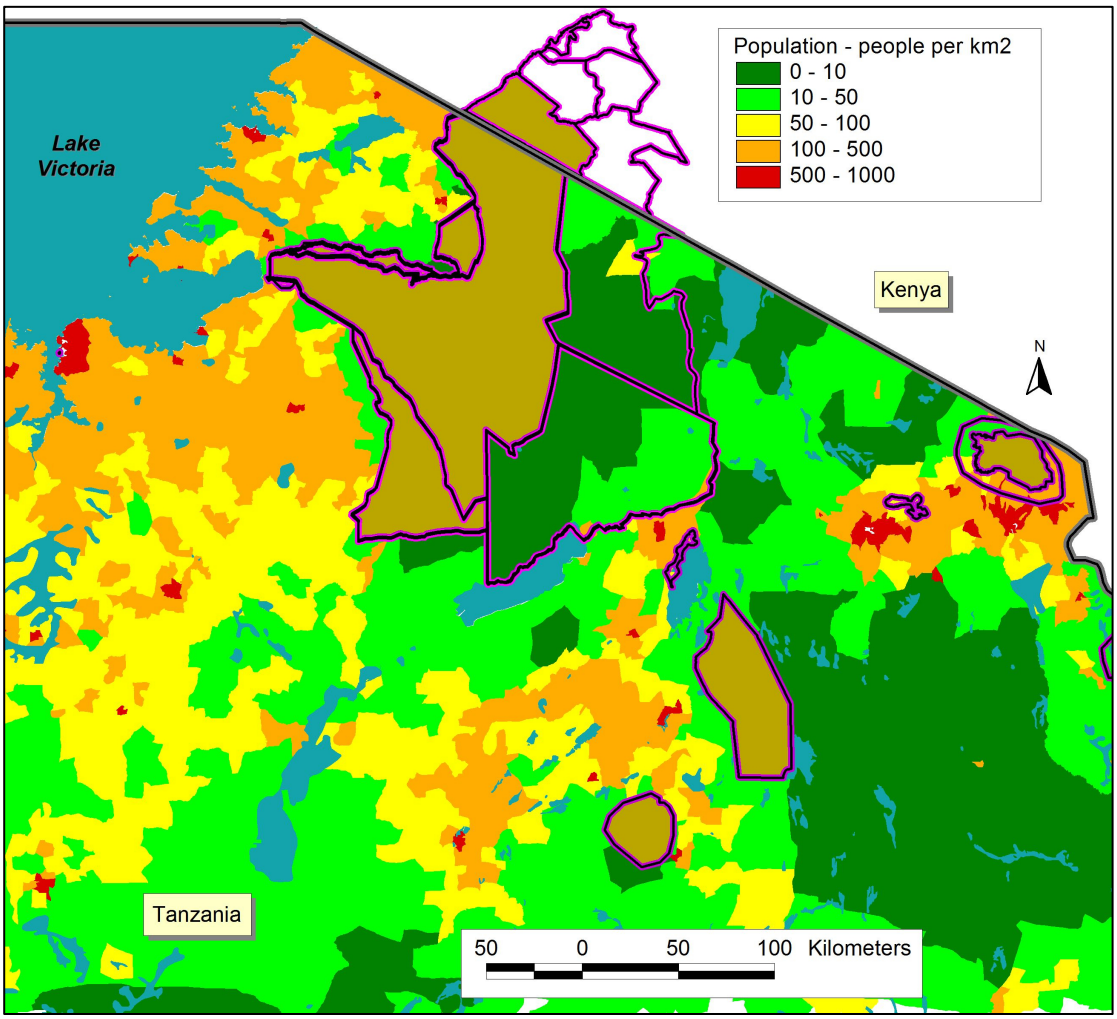

Figure 2.4: Human demographics in the study area from the 2012 national census. The data were available from the Tanzania National Bureau of Statistics (2012) and Dr. Mike Shand of the University of Glasgow. Mr. Guy McGrath, University College Dublin, using ArcView software (ESRI, 2011), generated the map. The purple outlines represent wildlife-protected areas. The brown fill within the protected areas means that no livestock are allowed in those areas and very few people live there.

2.2.3 Population of FMD susceptible livestock

Tanzania has the third largest cattle population in Africa (estimated to be over 21 million head in 2008) (FAO, 2013b; Tanzanian Ministry of Agriculture, 2012; Robinson *et al.*, 2007). The 2008 Tanzanian livestock census estimated that over 15 million goats and over 5.7 million sheep were also kept on mainland Tanzania, but that pig populations were low (1.58 million) compared to cattle, sheep and goats with very few pigs in the study area (Tanzanian Ministry of Agriculture, 2012). Of 182 households that were interviewed for the current study, only two reported owning pigs. Where domestic pigs are present, in parts of Kenya for example, a sero-prevalence of 48% has been reported (71 of 149 randomly sampled unvaccinated pigs seropositive). In contrast to domestic pigs, lower FMD seroprevalence (0-14%) has been reported for African wild pigs species such as wart hogs (*Phacochoerus africanus* and *P. aethiopicus*) (Bronsvoort *et al.*, 2008; Di Nardo *et al.*, 2015). Districts in the northern half of the country have higher cattle, sheep and goat populations compared to those in the south (Ministry-of-Agriculture-Tanzania, 2012). In the study area, livestock density was highest in the agropastoral areas west of Serengeti NP (Robinson *et al.*, 2007) (Figures 2.5-2.7).

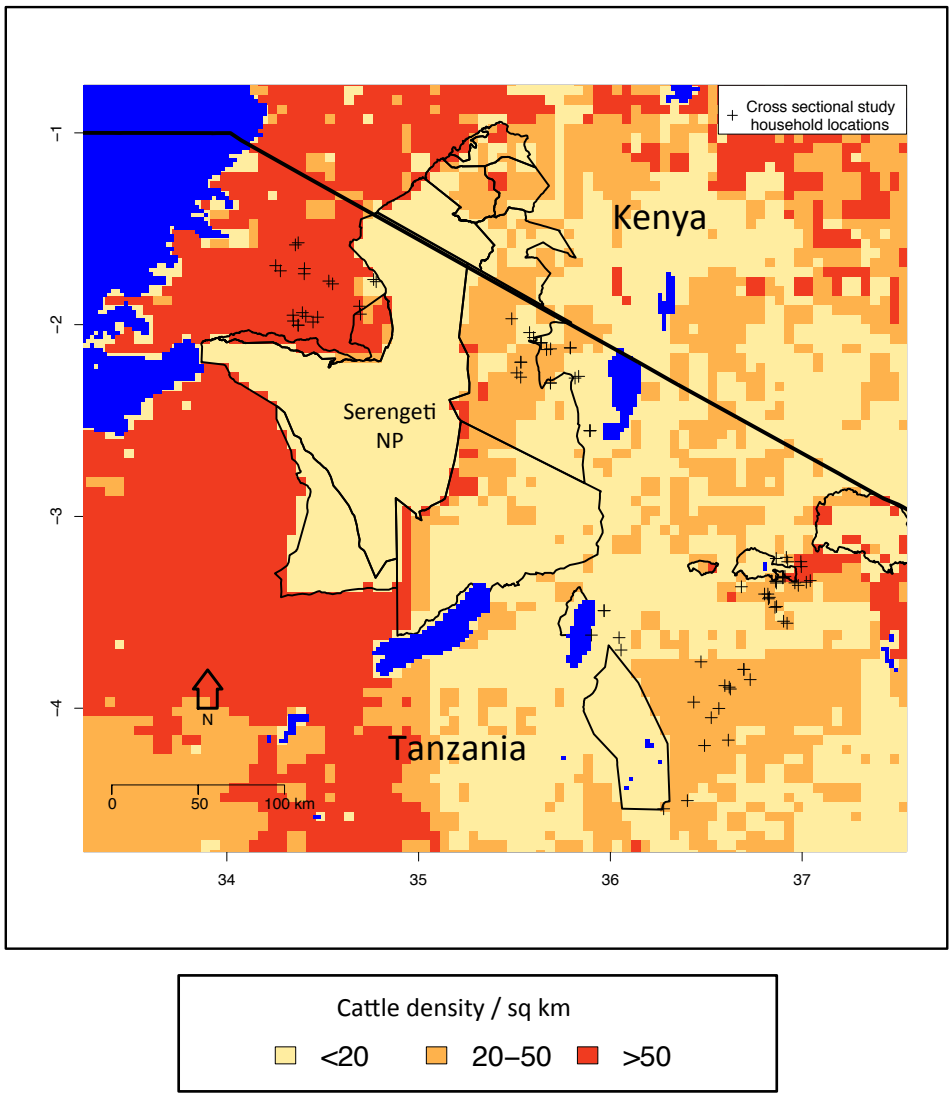

Figure 2.5: Estimated cattle density in the study area.
Data were taken from the Food and Agriculture Organisation's "Gridded Livestock of the World" (Robinson et al., 2007) and the map was generated in the R statistical environment (R development core team, 2008). The wildlife protected areas and the Kenyan border are shown as black lines. The crosses represent study household locations. NP = National Park.

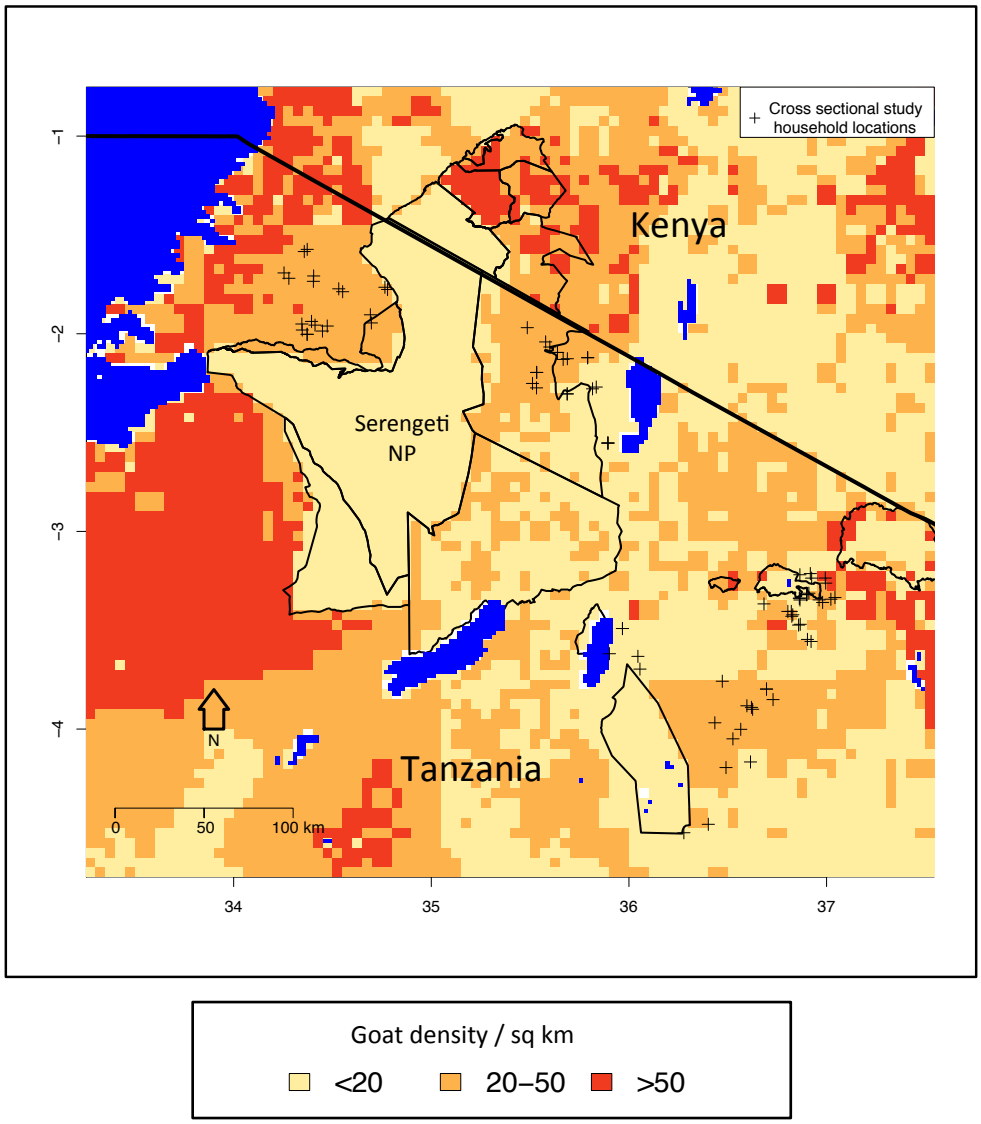

Figure 2.6: Estimated goat density in the study area.
Data were taken from the Food and Agriculture Organization's "Gridded Livestock of the World" (Robinson et al., 2007) and the map was generated in the R statistical environment (R development core team, 2008). The wildlife protected areas and the Kenyan border are shown as black lines. The crosses represent study household locations. NP = National Park.

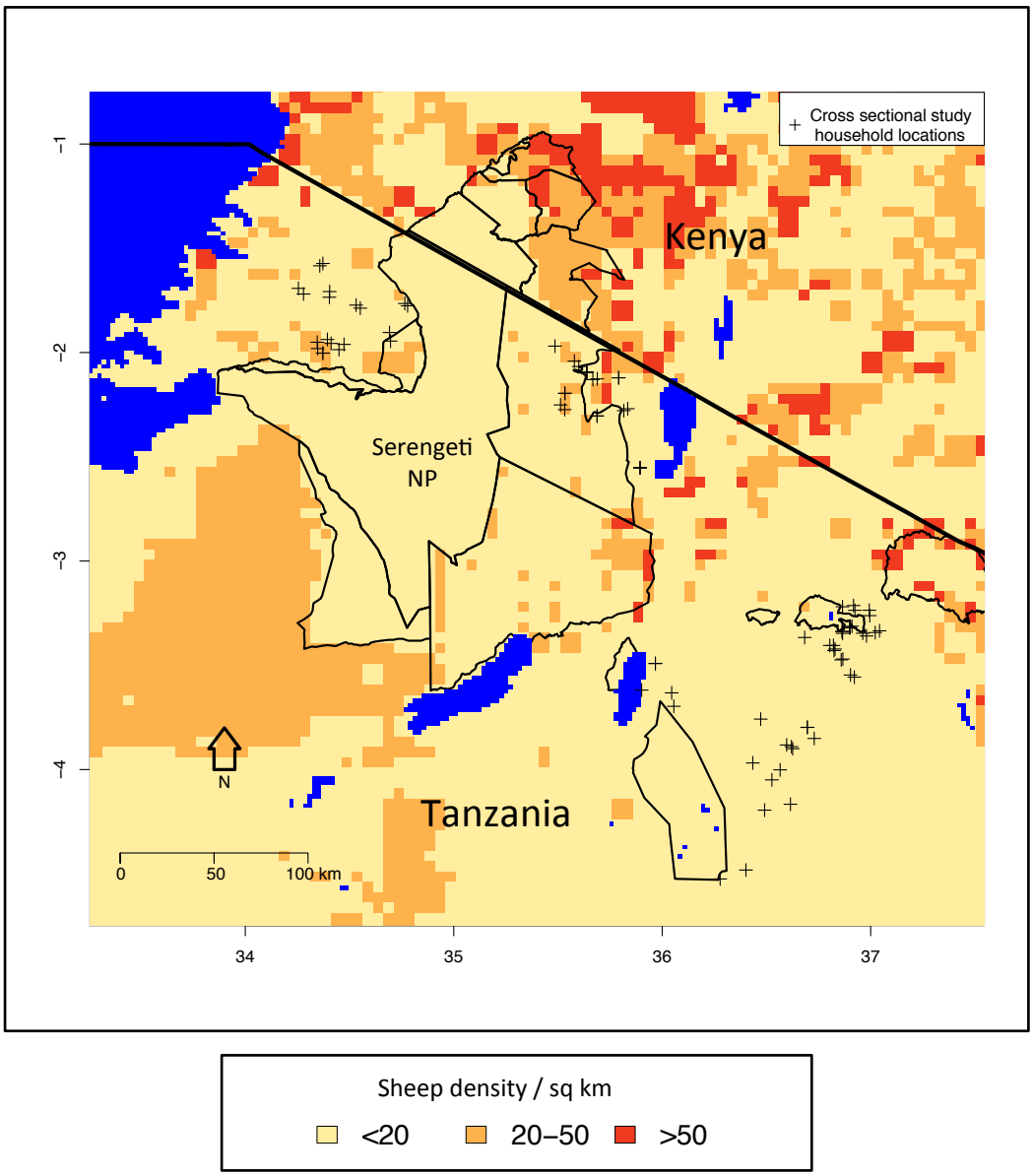

Figure 2.7: Estimated sheep density in the study area.
Data were taken from the Food and Agriculture Organisation's "Gridded Livestock of the World" (Robinson *et al.*, 2007) and the map was generated in the R statistical environment (R development core team, 2008). The wildlife protected areas and the Kenyan border are shown as black lines. The crosses represent study household locations. NP = National Park.

2.2.4 Populations of African buffalo

Any even-toed ungulate is potentially susceptible to FMD. Northern Tanzania hosts a range of these species amongst its wildlife, including a wide variety of antelope, wild pig species and buffalo (*Syncerus caffer caffer*). Of all potentially susceptible wild animals, only buffalo have been consistently shown to have high levels of FMD infection (Bronsvoort *et al.*, 2008; Karesh, 2012).

Tanzania has the highest African buffalo population (estimated to be >342,450 head in 1998 and reported to be increasing in the 2014 Tanzanian wildlife census) (East, 1999; TAWIRI, 2014). The 2014 wet season buffalo total count recorded 55,411 buffalo in Serengeti ecosystem. The majority of these (88%) were counted in Serengeti NP and contiguous reserves to the west. Almost 5% were counted in NCA. No buffalo were counted in LGCA and 3% were counted outside of protected areas. There are fewer recent data available on buffalo populations in other wildlife areas in Northern Tanzania. A 1999 survey estimated over 14,000 buffalo in Tarangire NP its surrounding conservation areas (East, 1999), but that number is likely to have risen since then.

There were no recent estimates available for buffalo numbers for Arusha, Lake Manyara and Tarangire NP. Therefore opinions on buffalo numbers in these areas were requested from experts on wildlife ecology in northern Tanzania. Key informants included Dr Abel Mtui, Dr. Julius Keyyu, Dr. Grant Hopcraft, Dr. Tom Morrison and Professor Tony Sinclair. Further information was about the buffalo in Lake Manyara NP retrieved from a PhD thesis (Prins, 1987). Figure 2.8 shows Serengeti ecosystem buffalo abundance based on (Hopcraft *et al.*, 2012) and estimated buffalo abundance based on key informant interviews in the other NP in the study area. For Serengeti ecosystem, the buffalo abundance index was calculated from 1985 – 2006 censuses as shown in Equation 2.1 (Hopcraft *et al.*, 2012).

$$Abundance\ index = Log\ \frac{Sum\ densities\ across\ all\ years^2}{SD\ densities\ across\ all\ years + 1} \qquad \textbf{Equation 2.1}$$

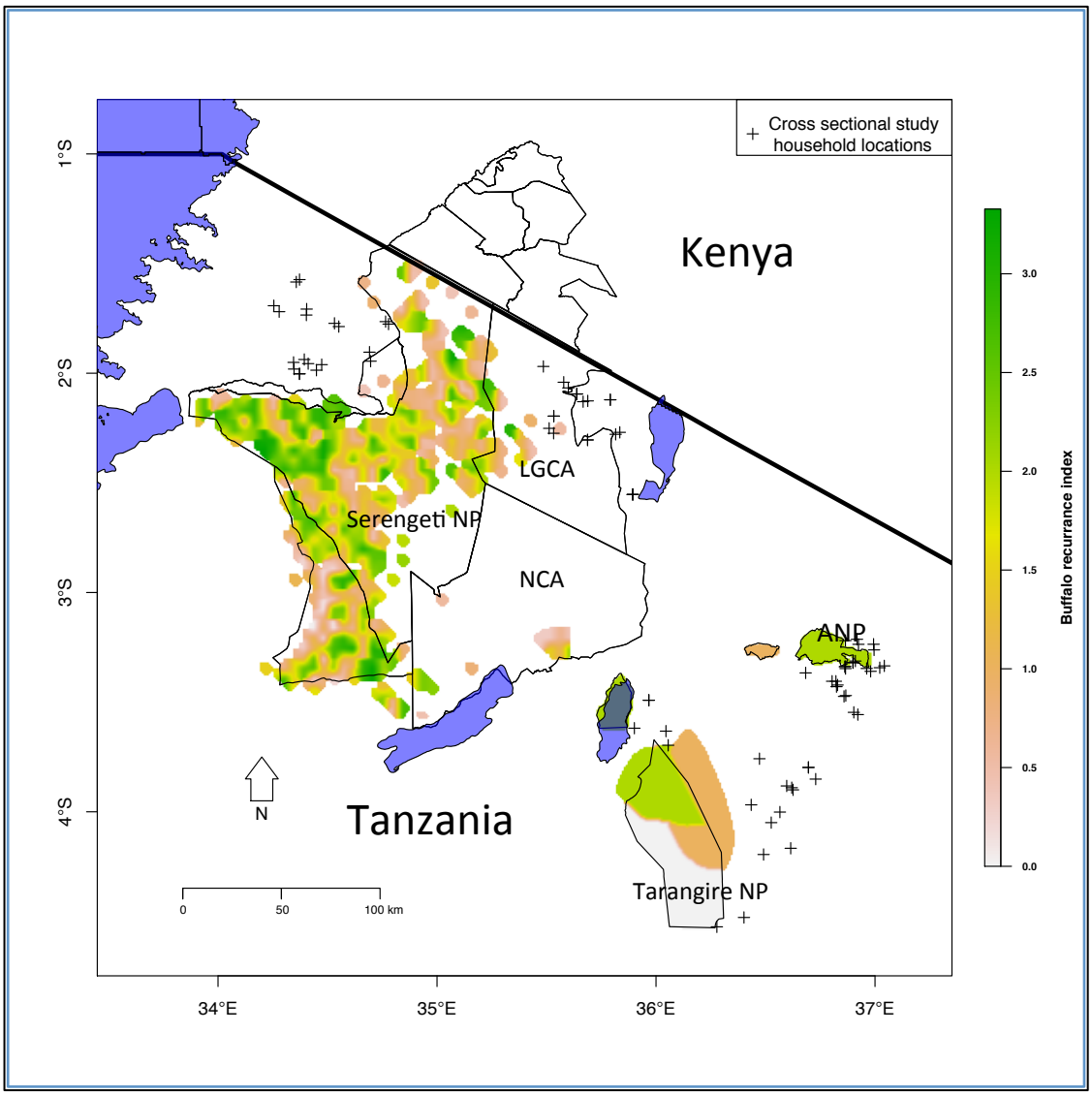

Figure 2.8: A map showing the areas where buffalo are likely to be present in the study area.
Study household locations (shown as crosses) and wildlife reserve area boundaries (black lines) were added to the map. The Serengeti National Park and Ngorongoro Conservation Area data came from (Hopcraft *et al.*, 2012). The estimates for the other wildlife areas came from discussions with key informants as no recent data were available for these areas. ANP = Arusha National Park. LGCA = Loliondo Game Controlled Area. NCA = Ngorongoro Conservation Area. NP = National Park.

2.2.5 Livestock production systems in the study area

The study comprised agropastoral, pastoral and smallholder livestock management systems. Pastoralist households are commonly defined as households that obtain greater than 50% of total gross income from mobile livestock reared on unimproved, communal

pastures. Households which obtain greater than 25% but less than 50% from cropping activities are defined as agro-pastoral (Swift, 1988).

Agropastoralist systems included Serengeti and Bunda districts located to the west of Serengeti NP (Figure 2.1). Figure 2.9 shows examples of agropastoral production.

Figure 2.9: Agropastoral crop and livestock production in Serengeti district west of Serengeti National Park.
Photo credit: Dr. Tiziana Lembo

Study sites also included pastoral communities in LGCA area, as well as those in Simanjiro and Monduli areas east and north of Tarangire NP (Figure 2.1). Pastoralists move their cattle to obtain sufficient grazing and water, especially in the dry season. Cattle are also moved to avoid wildebeest calving locations on the short-grass plains (and associated malignant catarrhal fever in their cattle) between February and May. Figure 2.10 shows examples of pastoral production systems.

(A)

(B)

Figure 2.10: Pastoral livestock management.
Cattle are walked farther for grass and water in the dry season compared to other management systems (Image A). Image B shows the Simanjiro plains, originally a completely pastoral area but with the increasing practice of land lease to immigrants for cultivation purposes (Kshatriya *et al.*, 2006).
Photo credit: Dr. Tiziana Lembo and Dr. Ahmed Lugelo

Finally the study included rural smallholders adjacent to Arusha urban centre (Figure 2.1) characterised by smaller numbers of livestock, less movement of livestock, and relatively more emphasis on crop production and other sources of incomes compared to the agropastoralists and pastoralists. Livestock also tend to be fed by their owners or graze in close proximity to households.

Figure 2.11: Smallholder livestock adjacent to Arusha urban area.

To highlight differences in livestock movements and herd size (respectively) between the management systems included in this study, Figures 2.12 and 2.13 summarise data collected from this study (fully described in Section 2.3.2 in Chapter 4). Pastoral and agropastoral livestock are moved farthest and smallholder livestock are moved least (Figure 2.12). Agropastoral and pastoral households have larger herds than rural smallholders (Figure 2.13)

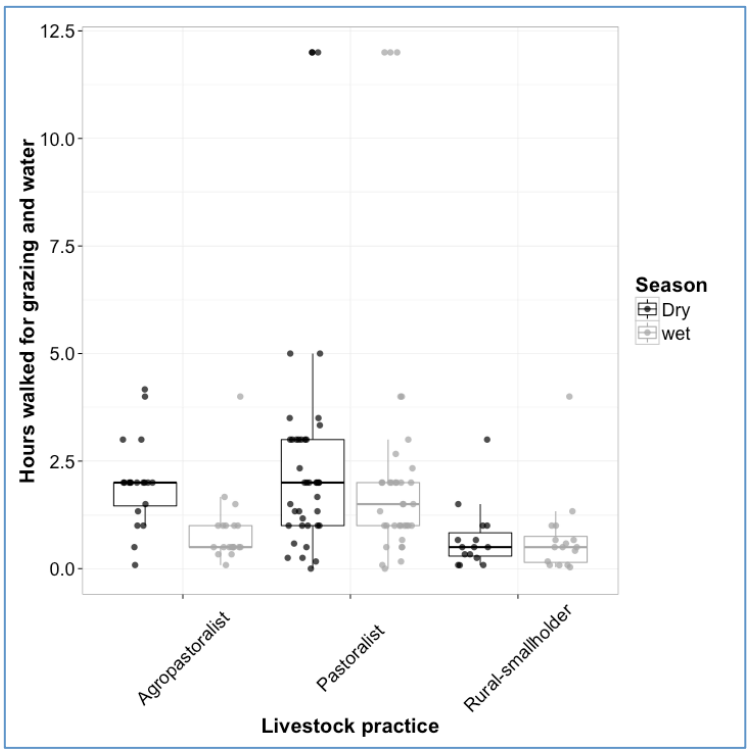

Figure 2.12: Box and scatter plots showing maximum hours walked in the wet and dry season for grazing and water in the three livestock management practices in the study area as reported by livestock owners in cross-sectional surveys (N = 84).
The data in this plot are from this study (Described in Section 2.3.2, and Chapter 4).

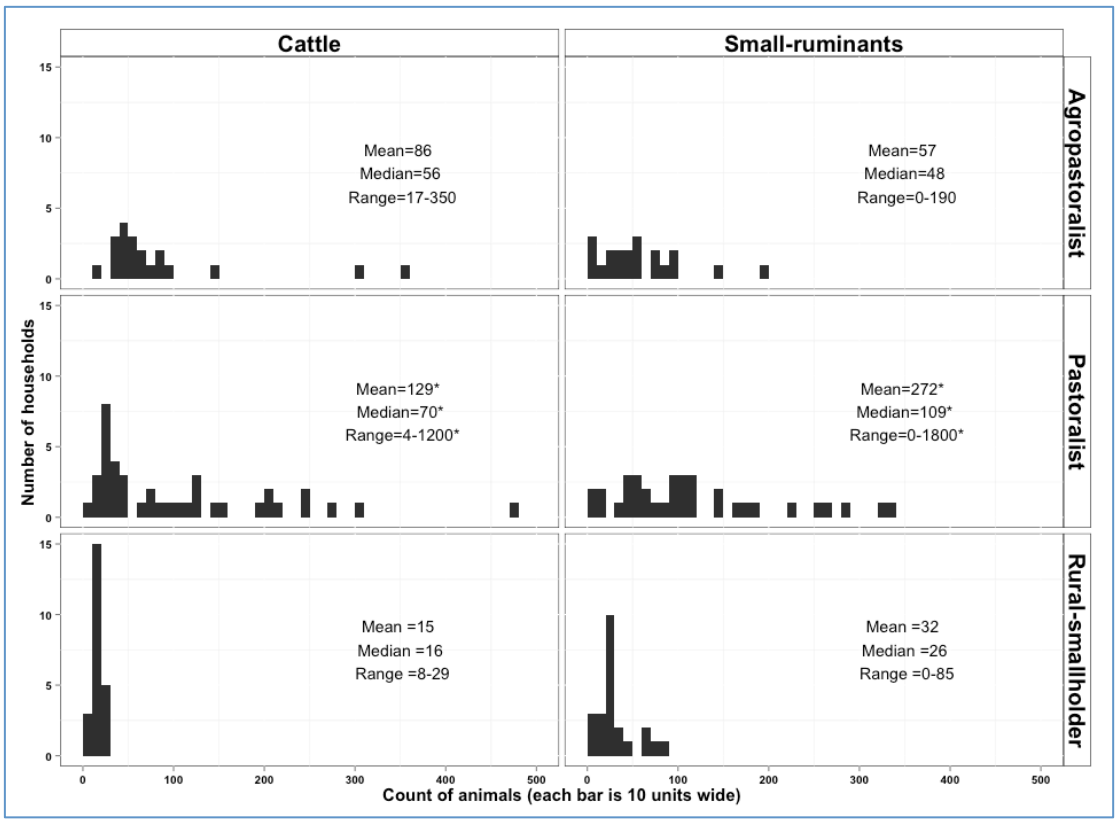

Figure 2.13: A bar-plot showing counts of cattle and small ruminants reported in the 84 agropastoral, pastoral and rural-small holder households that answered this question in the cross-sectional questionnaire.
*** Six pastoral households reported livestock counts above 500 that are not shown on the plots above. Five of the households reported small ruminant counts of 618, 646, 1135, 1350 and 1550. One further pastoral household reported a cattle count of 1200 and a small ruminant count of 1800. The data in the plot came from this study (described fully in Section x and Chapter 4).**

2.2.6 Livestock movements in the study area

In terms of North-South livestock movements, there are close cultural and trading connections between northern Tanzania and southern Kenya which could lead to FMDV trans-boundary movements. Northern Tanzanian cattle are taken to Nairobi and other Kenyan urban areas to generate better prices at market (FAO, 2013a; GFRA, 2013; Gertel & Le Heron, 2011; Di Nardo *et al.*, 2011) (Figure 2.14). Better grazing in northern Tanzania may motivate Kenyan cattle owners to bring their cattle southwards (Prof. Sarah Cleaveland, personal communication).

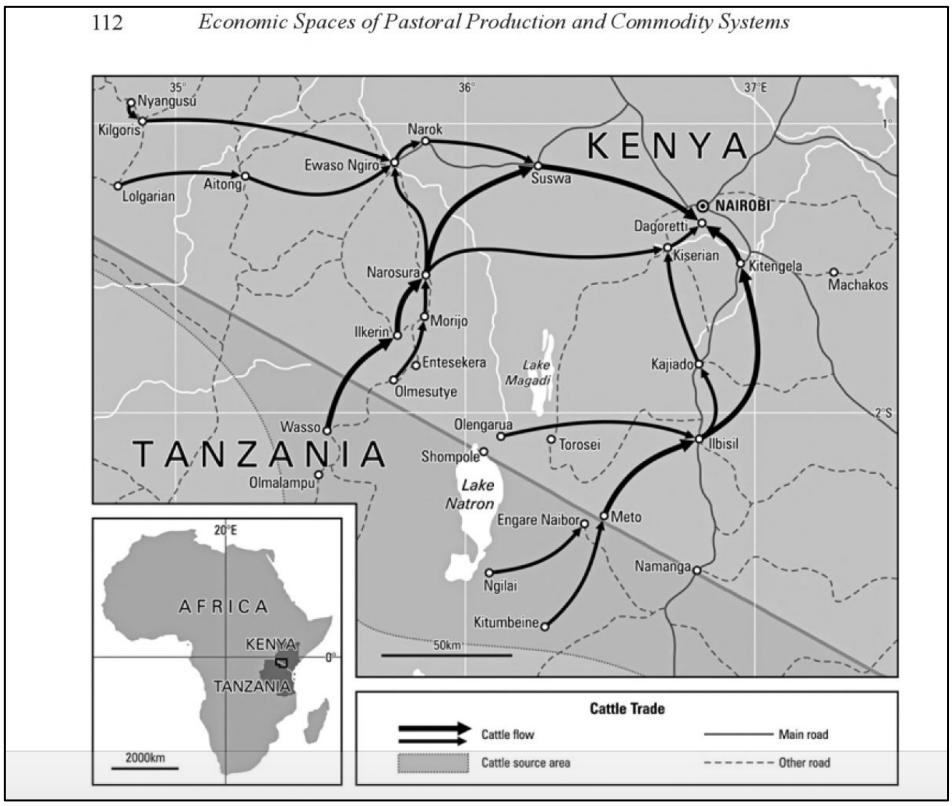

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Figure 2.14: Flows of animals from Tanzania through Kajiado and Narok districts in Kenya towards Nairobi for market purposes.
From (Gertel & Le Heron, 2011).

There is a dearth of information in the literature describing East-West livestock movements in northern Tanzania. From informal interviews with key informants (Mr. Raphael Mahemba, Dr. Tito Kibona, Dr Ahmed Lugelo), the following impressions have emerged.

- Livestock in the West of Serengeti NP go either north or west to markets or grazing.
- If there is a drought, livestock food and water sources to the East of Serengeti NP are diminished earlier compared to the West. Therefore, the livestock price difference between the East and West increases, as the pastoralists to the East cannot feed their livestock whereas the West there may still be some grazing and water. Cattle may be moved from East to West in this situation.
- There is some illegal movement of livestock from East to West through the north part of Serengeti NP, from Loliondo to Serengeti district. The route through the national park is much shorter than the route around the park. There is more

incentive for this illegal movement in times of drought where people from the East wish to sell their cattle in the West.

- Cattle in the East (pastoralist) go north to Kenya for markets, and South sometimes as far as Morogoro for grass.
- Cattle in the east converge on high areas (for example hills near Endulen and hills north of Simanjiro) during wildebeest calving season to avoid MCF.

2.3 Study design

2.3.1 Ethical approval and household consent

Permission for this study was obtained from the Tanzanian Wildlife Research Institute (TAWIRI), Tanzania National Parks and the Tanzania Commission for Science and Technology (COSTECH permit numbers 2010-385-ER-90-15 and 2012-182-ER-90-15). At district level, permission was obtained from the district veterinary officer. In each household surveyed, the aims of the study were explained to the head of household and written consent for questionnaires and livestock sampling was obtained (consent form available in Appendix 1).

2.3.2 Study types

The study was part of a larger project funded by the "Combating Infectious Diseases of Livestock for International Development" programme of Biotechnology and Biological Sciences Research Council and the British Department of International Development (http://r4d.dfid.gov.uk/Project/60672/). Project partners included the University of Glasgow, the Pirbright Institute, the Tanzanian Wildlife Research Institute, the Tanzanian Ministry of Livestock and Fisheries Development, Sokoine University of Agriculture, the Nelson Mandela African Institution of Science and Technology and Washington State University.

The project generated data to investigate seroprevalence, socioeconomic impacts, risk factors and drivers of FMDV circulation in the study area. Contributions of individual partners and myself in data generation are highlighted in Table 2.1. I performed part of the

lab-work and all of the analyses presented (Table 2.1). Data from the following studies were used for the thesis.

A. A cross-sectional study of livestock-owning households in the proximity of wildlife-protected areas was designed to obtain information on the seroprevalence of FMD in the study area, its socioeconomic impacts at households level, risk factors for FMD seropositivity and patterns of infection with specific FMDV serotypes.
B. A cross-section of buffalo in wildlife-protected areas was sampled to obtain information about FMD seroprevalence and serotype-specific circulation patters in this species.
C. An outbreak tracking study, based on active surveillance, collected lesion material from outbreaks with the objective of identifying serotypes responsible for outbreaks, patterns of FMDV circulation and of measuring morbidity and mortality associated with outbreaks.
D. A longitudinal outbreak follow up study was performed to assess the frequency and economic impacts of FMD outbreaks and to identify the FMDV variants causing serial outbreaks in the same herds.
E. A case-control study was designed with the aim of identifying risk factors for FMD outbreaks.
F. A prospective longitudinal study sampled a research herd of cattle tracked through serial FMD outbreaks with the objective of characterising the serological response to FMD infections.

The different types of study design, and the data analyses and laboratory work associated with them, are summarised in Table 2.1.

Table 2.1: Summary of data used for PhD thesis. ML = Tanzanian Ministry of Livestock and Fisheries Development, UG = University of Glasgow field team or academic staff; TPI = Pirbright Institute laboratory staff, World Reference Laboratory staff or academic staff, TAWIRI = Tanzanian Wildlife Research, WSU = Washington State University Institute, MC = Miriam Casey, PhD applicant. The Nelson Mandela African Institution of Science and Technology provided logistical support, especially in relation to sample storage and shipment to TPI.

Study type and dates	Data type	Data management and analyses	Samples analysed	Laboratory analyses
A. Livestock cross-sectional (2011 -2012)	Questionnaire data generated through interviews of 20 agropastoral, 36 pastoral and 22 rural smallholder farmers including: assets, herd size, herd management, wildlife interactions, outbreak frequency and clinical characteristics, perceived importance of FMD compared to other livestock diseases and impact of FMD (e.g. in terms of morbidity, mortality, changes in herd management practices, etc.).Serological data. Contributors: UG, SUA, ML, WSU	Created an SQL database and performed summary statistics and a generalised mixed linear model for risk factor analysis Contributors: MC	1300 cattle, 816 goat, 418 sheep sera	Commercial pan-serotypic ELISA kit for antibodies against FMDV non-structural proteins (all samples). Virus neutralisation testing of a subset of sera (128 cattle) Contributors: TPI, UG, MC
B. Buffalo cross-sectional (2010 -2012)	Information on buffalo age, sex, location and herd size. Serological data. Contributors: TAWIRI, SUA and UG	Collated and analysed buffalo serological and field data. Contributors: MC	199 buffalo sera	Commercial pan-serotypic ELISA kit for antibodies against FMDV non-structural proteins (N=199) and VNT (N = 55) Contributors: TPI, UG, MC
C. Outbreak investigations 2011-2014	Questionnaire data generated through interviews of 43 agropastoral and 29 pastoral herd visits at the time of an outbreak covering: clinical signs, outbreak morbidity and mortality, herd management practices and FMD history in herd. Detailed questionnaires like (A) in 17 herds. Clinical and virus isolation data. Contributors: UG, SUA, ML	Created an SQL database and performed summary statistics Contributors: MC	159 lesion samples from acutely infected cattle from 62 outbreak investigations. Virus isolation successful in 110 samples from 53 outbreaks. Serotypes identified: Serotype A (n=26), Serotype O (n=11), Serotype SAT1 (n=50) Serotype SAT2 (n=23)	Virus isolation, PCR, typing by antigen ELISA and sequencing of part of the genome encoding the VP1 capsid protein Contributors: TPI
D. Longitudinal monitoring of outbreak herds: 2011 - 2014	Follow up with 26 agropastoral herds (visits 6 weeks and 6 months after each outbreak) Contributors: UG, SUA	Created an SQL database and performed summary statistics. Serial outbreaks tracked in 15 herds, 8 with virus isolation from serial outbreaks. Contributors: MC	64 lesion samples collected from 27 outbreaks (subset of outbreak study). Virus isolation successful from 51 samples from all 27 outbreaks.	As for outbreak study. Contributors: TPI
E. Case-control : 2012	Questionnaires close to the time of an outbreak covering outbreak characteristics, management and FMD history in herd in 69 agropastoral households (36 where an outbreak occurred during the risk period and 33 where no outbreak was reported). Clinical data to confirm outbreaks. Contributors: UG	Created an SQL database, performed summary statistics and risk-factor analyses Contributors: MC		Cases confirmed through standard diagnostic techniques (see C) Contributors: TPI

Study type and dates	Data type	Data management and analyses	Samples analysed	Laboratory analyses
F. Intensive monitoring of a study herd 2011 - 2014	A herd suffering four serial FMD outbreaks over three years was monitored more intensively to parameterise a Bayesian model developed to infer animals' infection history from serological data. Clinical information was also recorded for animals displaying FMD signs. Contributors: UG	Description of serology results, outbreak times and clinical signs. Bayesian statistical model relating serology results to animals' infection histories developed. Contributors: MC	Sera obtained from 100 cattle for each of 20 time-points over three years. Lesion samples (N=10) from at least two acutely infected animals in three of the four outbreaks were available for laboratory confirmation	Commercial pan-serotypic ELISA kit for antibodies against FMDV non-structural proteins. Contributors: MC Optimised solid phase competition ELISA for antibodies against serotype- specific structural proteins. Contributors: MC Virus neutralisation testing for serotype-specific neutralising antibodies of a subset of samples (ten animals over 13 time-points). Contributors: TPI (MC) Virus isolation as for outbreak study. Contributors: TPI

(A) Livestock cross-sectional study

The cross-sectional study was conducted throughout 2011. It had a stratified random sampling design. In each study area (rural smallholders near Arusha urban area, pastoralists in Simanjiro/Monduli, Loliondo and agropastoralists in Serengeti) the field team aimed to randomly select five villages within 5km of protected area boundary and five villages more than 20km from protected area boundary. This was so that proximity to wildlife areas could be explored as a potential risk factor for FMD. For each village, the field team randomly selected two subvillages, and one balozi (ten household unit) for each subvillage. For each balozi, at least one household was randomly selected, with a total of 40 villages and 85 households (Figure 2.15).

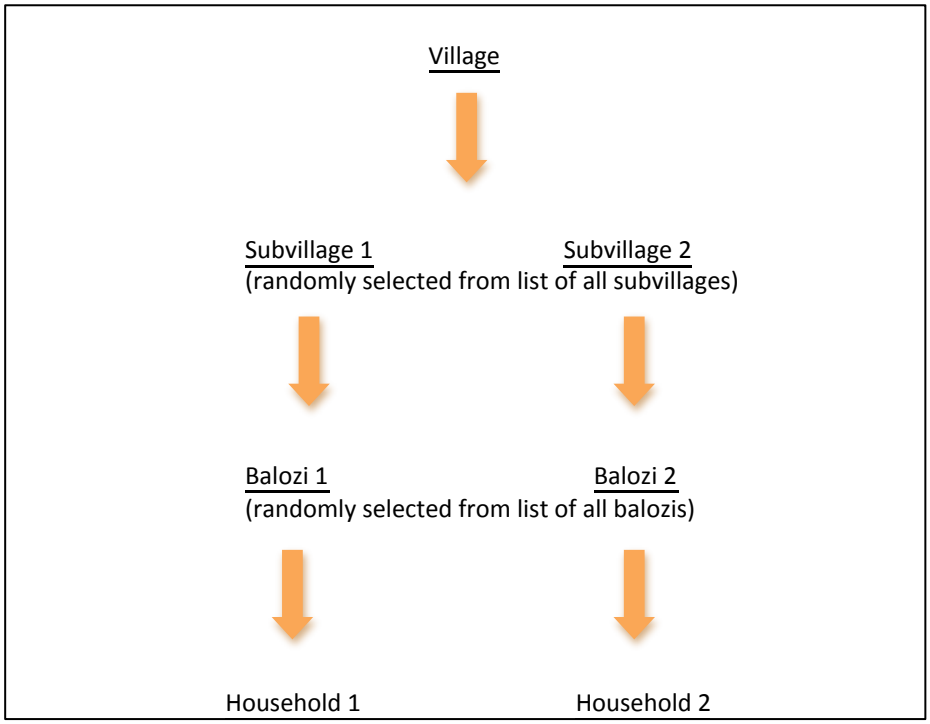

Figure 2.15: Semi-stratified randomised sampling design for the cross-sectional study.

Questionnaires and livestock sampling were conducted in two randomly selected households from two different sub-villages per village and locations of the households were recorded using a global positioning system (GPS). Cross-sectional household locations are shown in Figure 2.16 and their distances from wildlife-protected areas are shown on Figure 2.17.

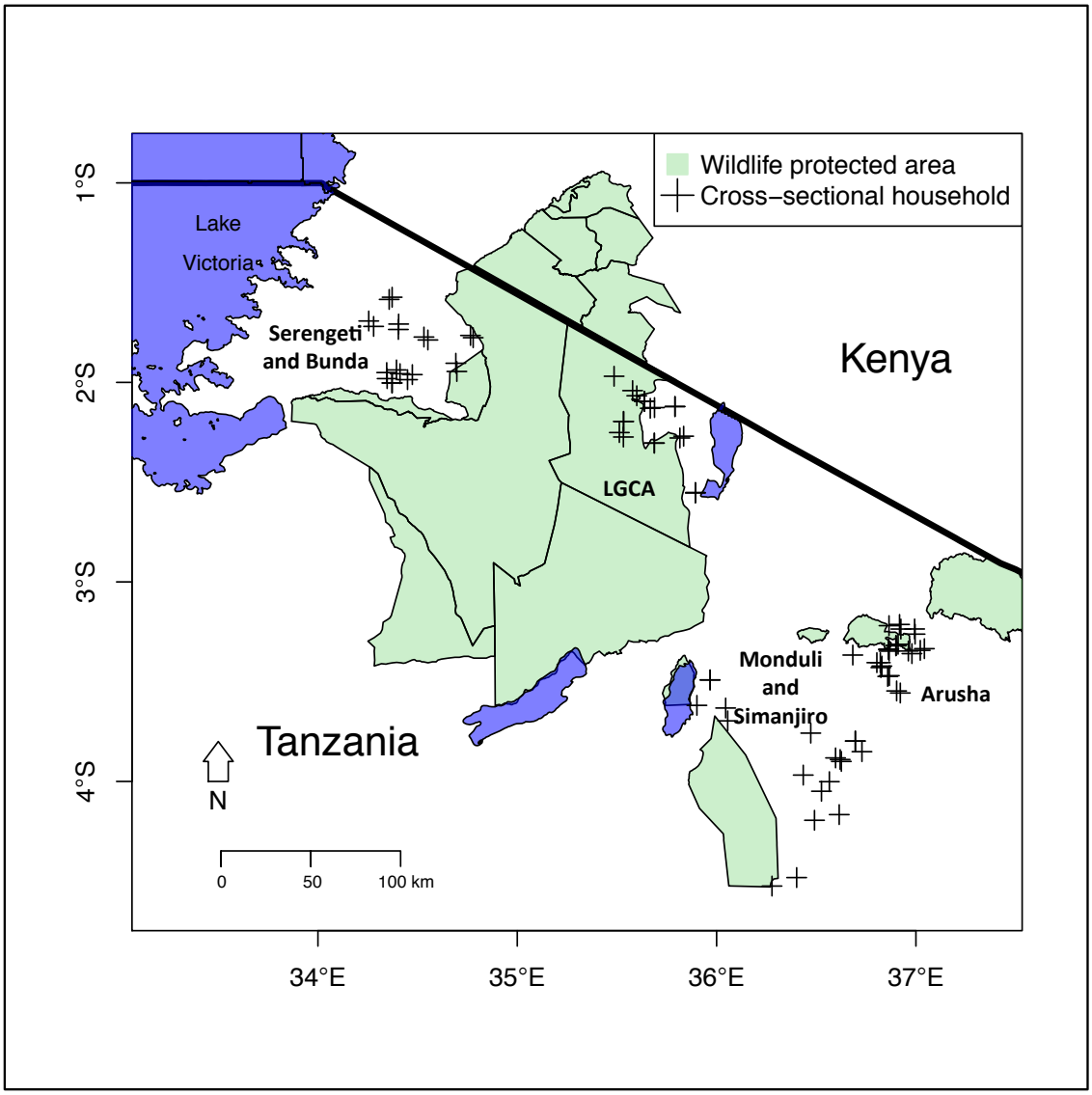

Figure 2.16: Cross-sectional study household locations.
The crosses represent household locations. LGCA = Loliondo Game Controlled Area.

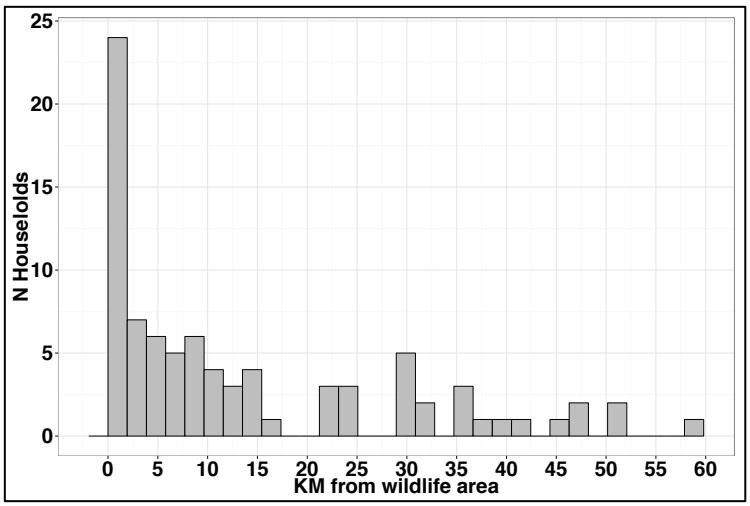

Figure 2.17: A histogram summarising the distances of cross-sectional study households from the boundaries of wildlife areas.
Where the household was within a wildlife area, such as in LGCA, the distance was zero KM.
N = number of households, KM = Kilometre.

Questionnaires to investigate socioeconomic impacts of FMD and risk factors for infection were conducted in each household. The full questionnaire is available in Appendix 2. The questionnaire respondents were the heads of the households who owned and managed the livestock. Serum samples were taken from 40 livestock per household (or all of the livestock if there were fewer than 40 in the household). A range of age classes of livestock was selected for sampling. The field team estimated livestock ages from dentition and aimed to sample the following as randomly as possible in each herd:

Cattle: 5 animals aged 6 months – 1 year
5 - 10 animals aged 1 – 3 years
5 animals aged >3years

Sheep and goats: 5 animals aged 6 months – 1 year
5 - 10 animals aged 1 year – 2 years
5 animals aged >2 years

Figure 2.18 shows the age distribution of the livestock sampled in the cross-sectional study. These included 1410 cattle, 877 goats and 451 sheep. If the total number of livestock sampled for the two households in the village was less than 40, then one more sub-village was randomly selected, and then one more Balozi and one more household.

This approach was continued until the total number of livestock sampled per village was 60 – 80.

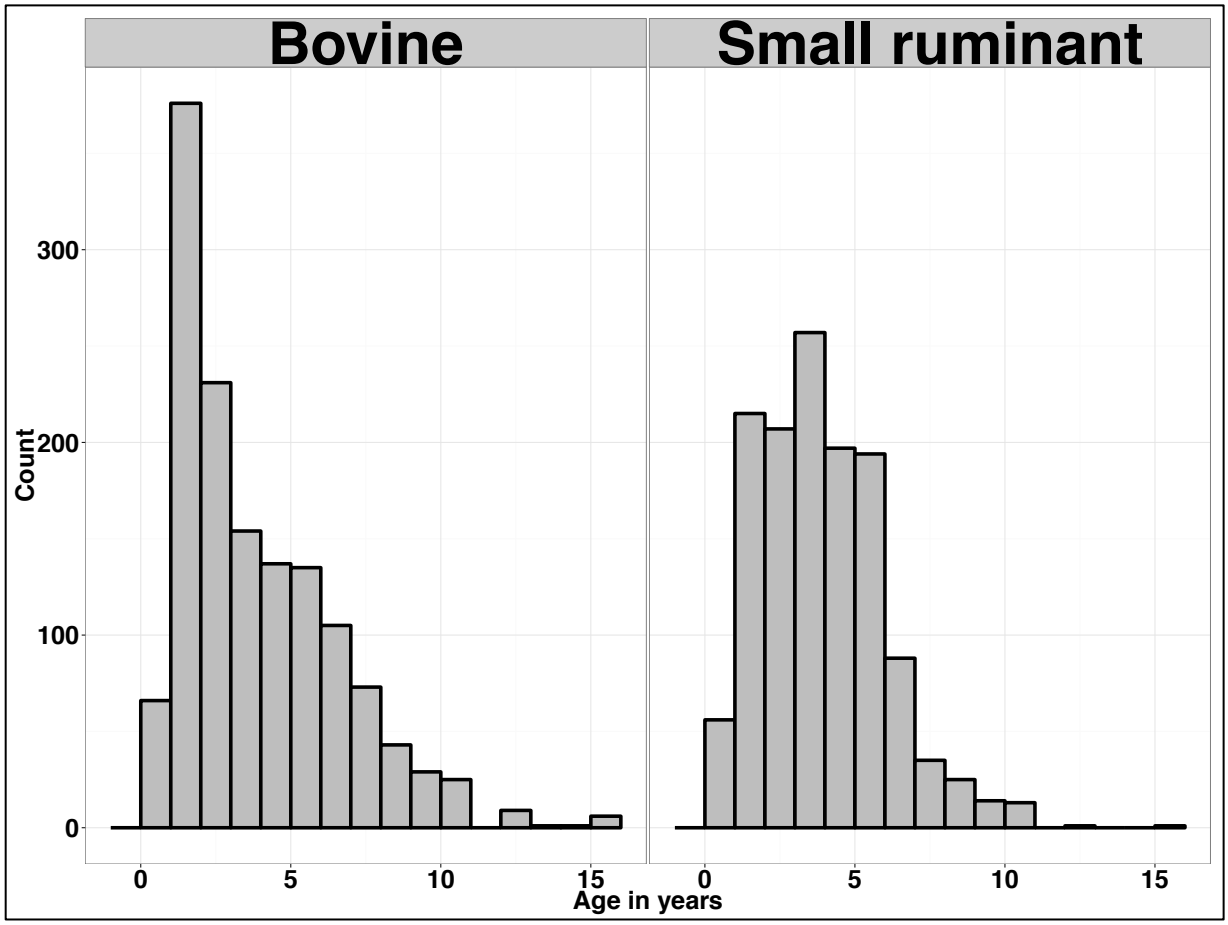

Figure 2.18: Histograms showing the ages of livestock that were serum sampled in the cross-sectional study.

(B) Buffalo cross-sectional study

Buffalo were sampled in Arusha, Serengeti and Tarangire NPs and NCA. The Tanzanian Wildlife Research Institute and Tanzania National Parks strictly regulate wildlife immobilisations. Therefore, the field team was permitted a quota of up to 25 buffalo per ecosystem. In NCA and Serengeti NP, additional buffalo sera were available as part of additional serological surveillance operations performed by TAWIRI veterinary teams. The buffalo in this study were sampled between July 2010 and April 2012. Buffalo were anaesthetised for sampling, their age was estimated from their dentition and horn size (Sinclair, 1977). Their sex, location (GPS coordinates) and the size of the group that they were with were also recorded. Serum samples were collected from buffalo (N = 199) in

four different ecosystems in the cross-sectional study; Arusha NP (23), NCA (N=116), Serengeti NP (N=36) and Tarangire NP (N=24). Figure 2.19 shows the buffalo sampling locations and Figure 2.20 shows the estimated ages of the buffalo that were sampled.

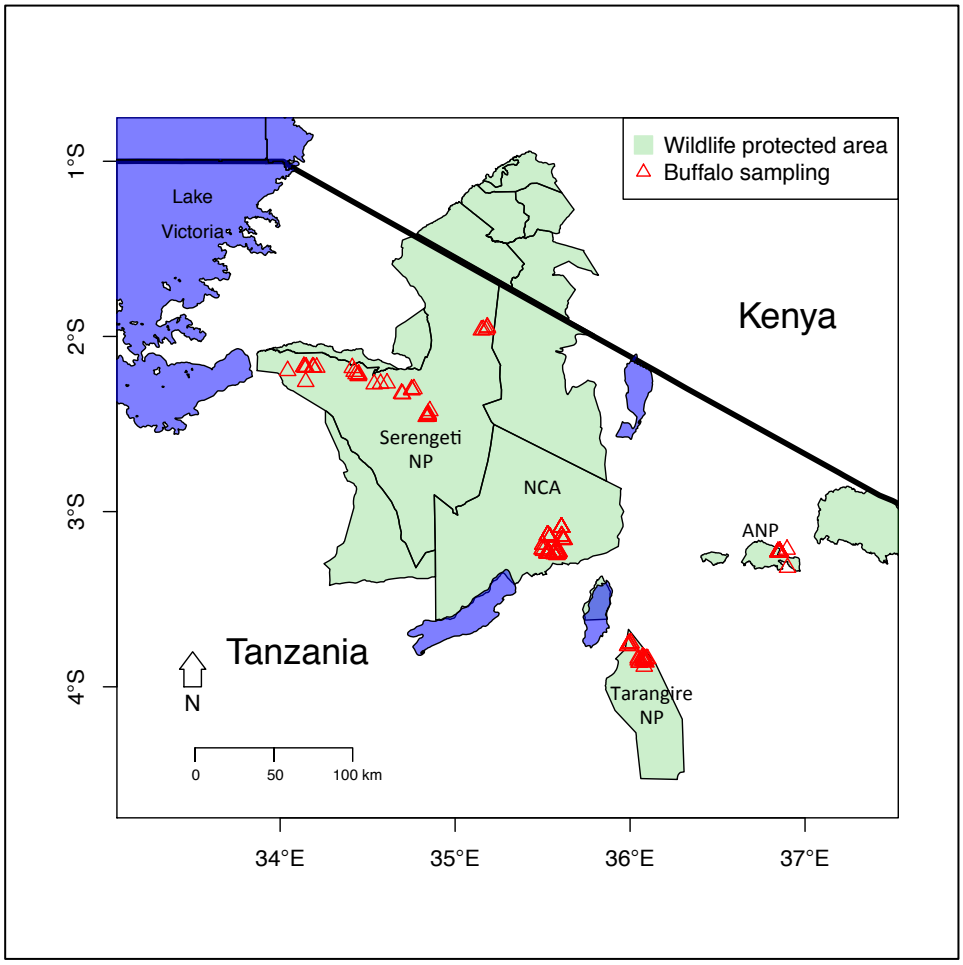

Figure 2.19: Buffalo sampling locations.
ANP = Arusha National Park. NCA = Ngorongoro Conservation Area. NP= National Park.

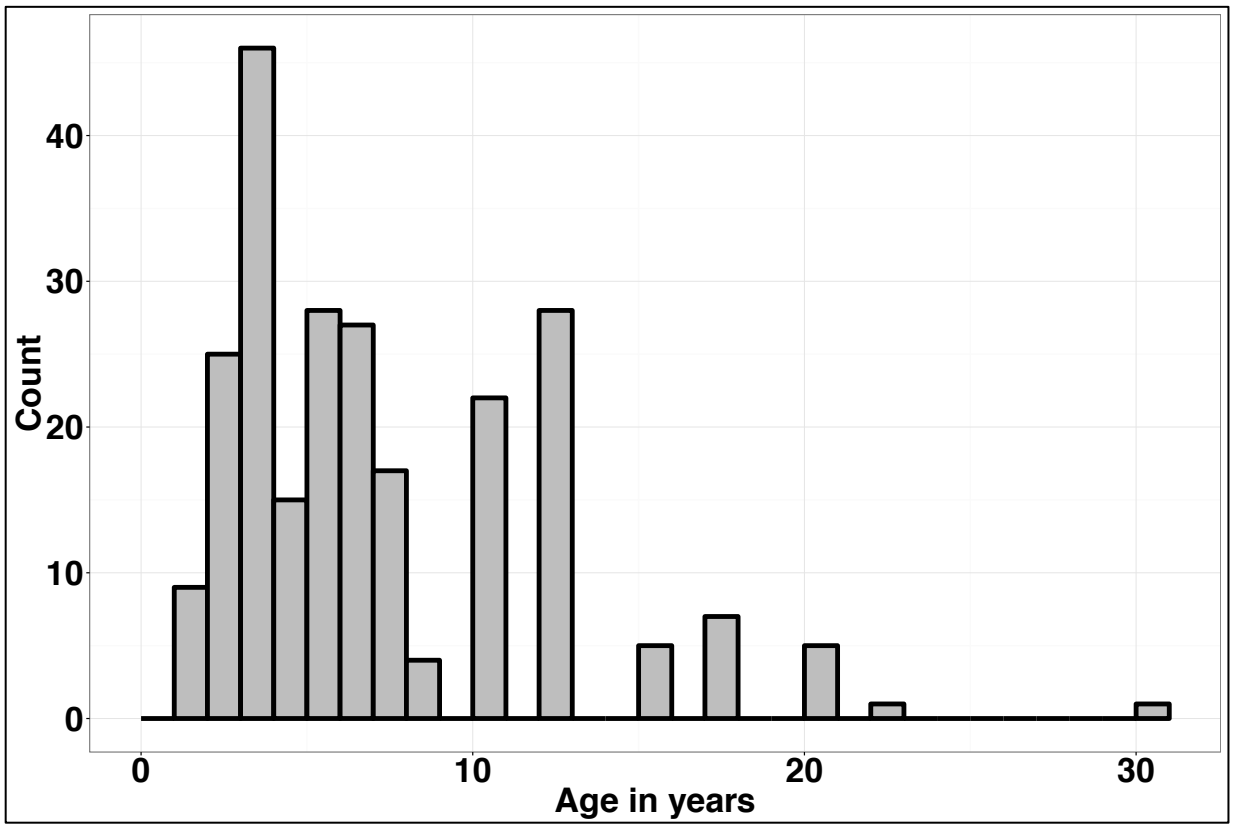

Figure 2.20: A histogram showing the ages of buffalo that were sampled in the cross-sectional study.

(C) Outbreak tracking study and (D) longitudinal follow up study

An active surveillance platform was established to receive reports of outbreaks as they occurred in selected FMD high-incidence areas (Figure 2.21). A dedicated field team made regular contact with village leaders and livestock owners to obtain information about FMD outbreak occurrence. Outbreak investigations occurred between 2011 and 2015. After June 2012, outbreak investigations were confined to Serengeti and Bunda districts, where the research team set up a permanent base to be able to reach outbreaks in a timely fashion and recruit the affected herds into a longitudinal study. If an FMD outbreak was reported, the field team visited the village and conducted outbreak investigations, aiming to sample at least two affected herds from each village. These investigations included livestock sampling and a questionnaire to quantify total livestock numbers and animals with FMD lesions and outbreak history in the herd, village and neighbouring villages. Detailed examinations were conducted on ten livestock with lesions from the herd. Epithelium and vesicular fluid were obtained from lesions from two clinically affected animals per household. These were sent to the World Reference Laboratory for FMD (WRL-FMD) in

Pirbright UK for confirmatory testing by virus isolation and characterisation. Follow up visits were conducted four to eight weeks after the outbreak and affected animal estimates were updated.

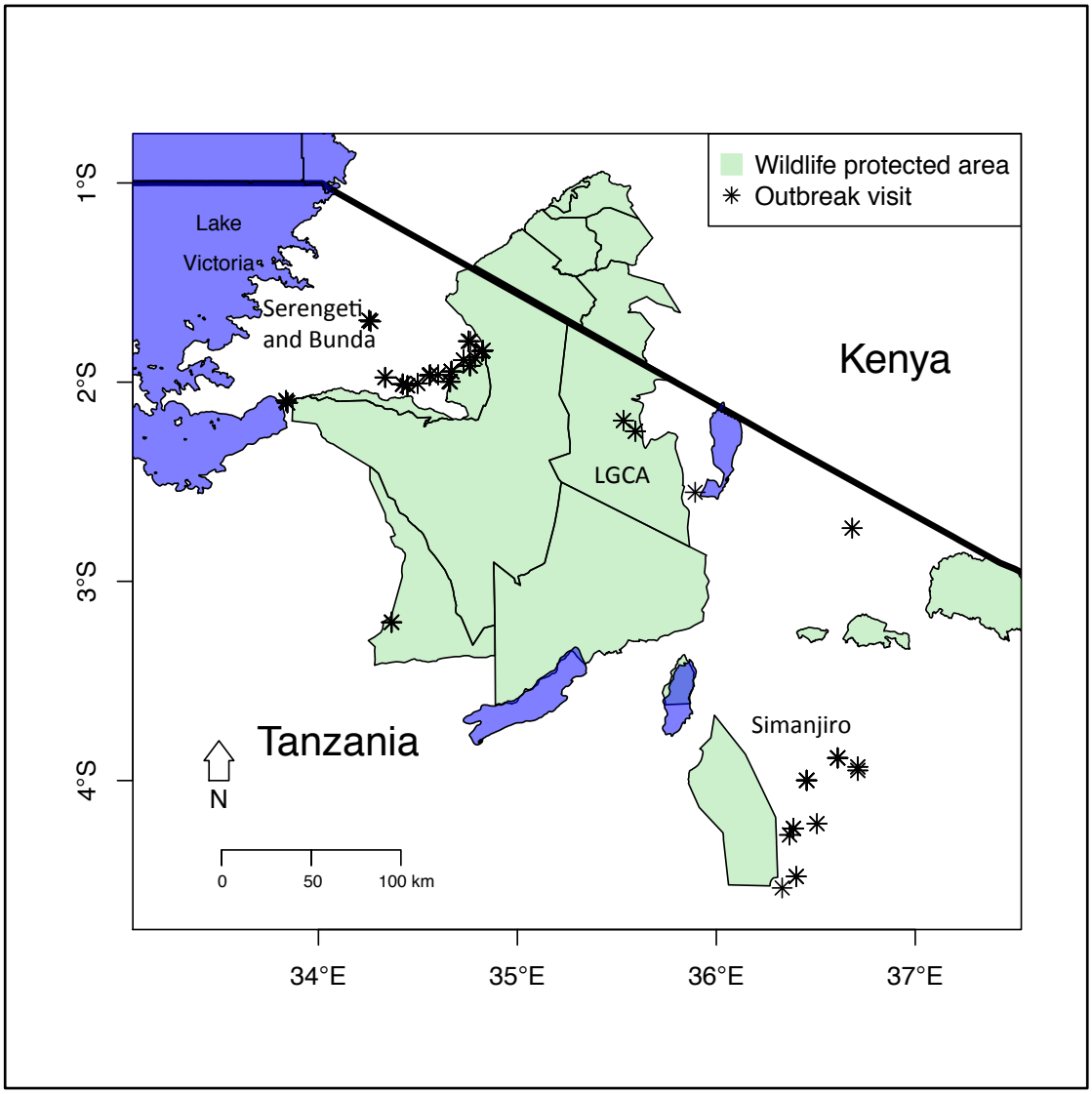

Figure 2.21: The locations of the foot-and-mouth disease outbreak investigations for this project that were conducted between 2011 and 2015.
LGCA = Loliondo Game Controlled Area.

A subset of 17 households in Serengeti district that participated in outbreak investigations were tracked longitudinally. These herds were visited six months unless they suffered another FMD outbreak, in which case an outbreak investigation was conducted.

(E) Case – control study

To investigate risk factors for individual outbreaks, a case-control study was implemented

in seven of the agropastoral villages in Serengeti district covered by outbreak visits. In total 70 households participated in the case-control study. Five herds suffering FMD outbreaks and five herds that did not suffer an outbreak were selected randomly from the list of all affected and unaffected herds in each of the seven villages during the risk-period. The herds that suffered outbreaks were cases and the herds that did not were controls. Information about potential risk factors for FMD outbreaks including herd size, livestock movements and contacts with other livestock, people or wildlife was collected from cases and controls using a household questionnaire (Appendix 3).

The five affected case herds included the two herds where an outbreak investigation had been conducted to ensure that diagnostic results for the outbreak were available. Control herds were revisited after six weeks to check that the animals had not shown clinical signs of FMD since the initial visit. If a control herd had an FMD outbreak within six weeks from the initial visit, it was excluded from the study. The case-control study design is summarised in Table 2.2.

Table 2.2: Summary of the case-control study design.

Source population	Livestock owning households in Serengeti district living in villages where the village leader reported an FMD outbreak during active surveillance by the FMD project field team
Risk period	One month prior to the first case observed in the village associated with the reported outbreak
Matching criteria	Matching was done at village level – five cases and five controls per village. Questionnaires of cases and controls at village level were conducted within a short time-span (one week maximum) and covered the same risk period.
Case definition	A household that reported livestock in their herd with FMD lesions in the village outbreak
Control definition	A household in the same village as a case that reported that no livestock in their herd had FMD lesions or clinical signs in the village outbreak and reported that no FMD lesions were observed in their livestock in the six weeks after the initial questionnaire visit.
Case validation	Two of the five cases per village had their livestock clinically examined and FMD lesion material sampled and sent for virus isolation and typing at the WRL

During the risk-period, thirty-seven case households and 33 control households were recruited into the study from the seven villages in Serengeti district (Figure 2.22). Two villages had six cases and four controls, as few herds in the villages were unaffected by FMD outbreaks. One of the case herds was visited at a different time compared to the other cases and controls in the same village and was therefore excluded from analyses, leaving 69 herds in total (36 cases and 33 controls).

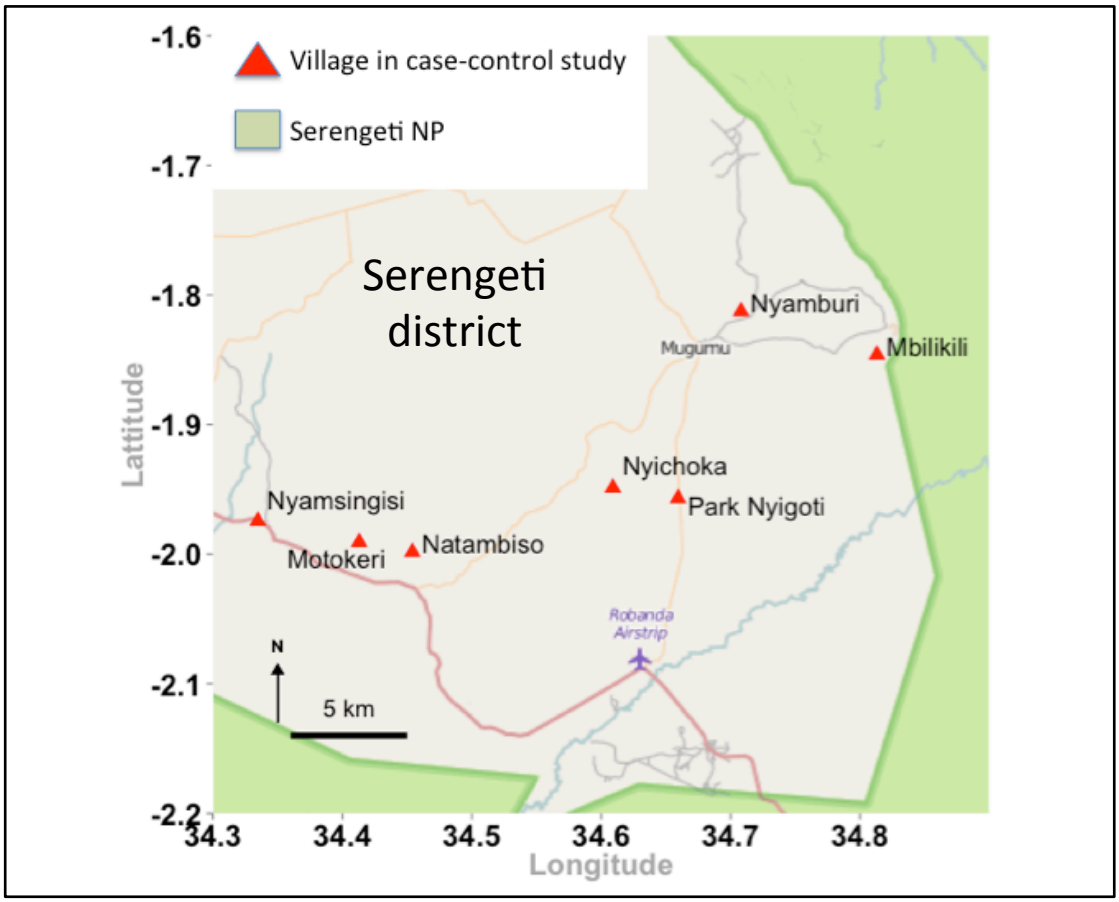

Figure 2.22: Large-scale map of the case-control study village locations in Serengeti district.
The red triangles indicate the centroid of all ten household positions in each of the seven villages. The green shading shows Serengeti National Park. The blue lines indicate rivers and the grey, yellow and red lines indicate roads. Mugumu, the largest town in the area where the FMD research base is located is also shown. The background map was sourced from Open Street Map (https://www.openstreetmap.org).

(F) Prospective longitudinal study with study herd

Two herds of 100 young cattle were purchased, clinically monitored and serum sampled regularly for the purposes of a separate study – a field vaccine effectiveness trial for Malignant Catarrhal Fever (MCF). Lankester *et al.* (2015a,b) provide details of the MCF study design. These herds were managed on the Simanjiro plains, east of Tarangire NP (Figure 2.1). Throughout a three-year study period, these herds suffered serial FMD outbreaks and were therefore recruited into a longitudinal study. Herd managers reported FMD outbreaks, the FMD field team visited and conducted outbreak investigations and FMD lesion material was shipped to the WRL-FMD for FMDV isolation and

characterisation. Both for the purposes of the MCF vaccine trial and to characterise FMD ELISA reactivity over time in cattle suffering serial FMD infections, sera were collected at intervals between two weeks and five months. A longitudinal serological dataset was generated from the sera and the ELISA reactivity patterns associated with serial FMD outbreaks were characterised. Serological results and FMD outbreak data from the herds were used and as training and validation data for a Bayesian model to infer FMD infection history from ELISA results.

2.4 Sample management

Sera were stored at minus 20 degrees and inactivated at 57 C for 2 hours before shipment to the WRL. Immediately after collection, FMD lesion material was stored in liquid nitrogen and then transferred to a minus 80-degree freezer prior to shipment to the WRL. Sera and lesion samples were maintained at -20 and -70 degrees, respectively, at the WRL.

2.5 Laboratory methods

All laboratory work was conducted in TPI. Staff in the WRL-FMD performed virus isolation, genotyping and antigenic typing on lesion material from the outbreak study. Sera from the study herds and the cross-sectional study were tested by non-structural protein ELISA (NSP ELISA), solid phase competition ELISA (SPCE) and virus neutralisation testing (VNT). MC, UG staff and Ms. Krupali Parekh of TPI undertook serological sample organisation and NSP ELISA testing. MC optimised the SPCE with Tanzanian antigen obtained in this study and used it for serum testing. MC was trained in VNT in the WRL but, as VNT results are liable to vary widely with different operators (Hingley & Pay, 1987), Pip Hamblin of the WRL-FMD staff did the majority of VNT testing to ensure consistent results from a single experienced operator.

2.5.1 Virus isolation and typing

Virus isolation and genotyping was performed in the WRL-FMD using OIE manual methods (OIE, 2012a). Genotyping was based on sequencing of the 1D gene encoding the VP1 viral protein, a major part of the FMDV capsid (Knowles & Samuel, 2003). Antigen

typing with an antigen detection sandwich ELISA was also conducted (OIE, 2012a; Roeder & Le Blanc Smith, 1987).

2.5.2 Non Structural Protein antibody ELISA

A commercial blocking ELISA based on monoclonal antibodies and recombinant antigen was used to detect antibodies against FMDV 3ABC non-structural proteins (NSP ELISA) (PrioCHECK FMDV NS[4]) (Chung *et al.*, 2002; Sorensen *et al.*, 2005). Non-structural proteins are only exposed to an animal's immune system during FMDV replication (or due to vaccine contamination). In a Northern Tanzanian setting where FMD vaccination is uncommon, a positive NSP-ELISA result will therefore reflect previous infection with FMDV. The 3B peptide that the monoclonal antibodies in commercial kit are based upon appears to be well conserved in different serotypes of FMD, meaning that the kit is likely to diagnose previous infection with any serotype of FMDV (Prof. Satya Parida, personal communication). The kits include plates lined with FMDV 3ABC NSP antigen. The test was performed according to the manufacturer's guidelines. In brief, the test sera were incubated overnight with the antigen and then the sera were washed away. Any test sera antibodies that remained bound to the antigen blocked binding of horseradish-peroxidase (HRP) conjugated marker antibodies (monoclonal antibodies against a FMDV 2B peptide). The optical density from a HRP- tetramethylbenzidine chromagen reaction (measured at 450 nm wavelength) reflected what proportion of marker antibody binding had been blocked by test antibodies (Figure 2.23).

[4] PrioCHECK®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33 Lelystad, Netherlands.

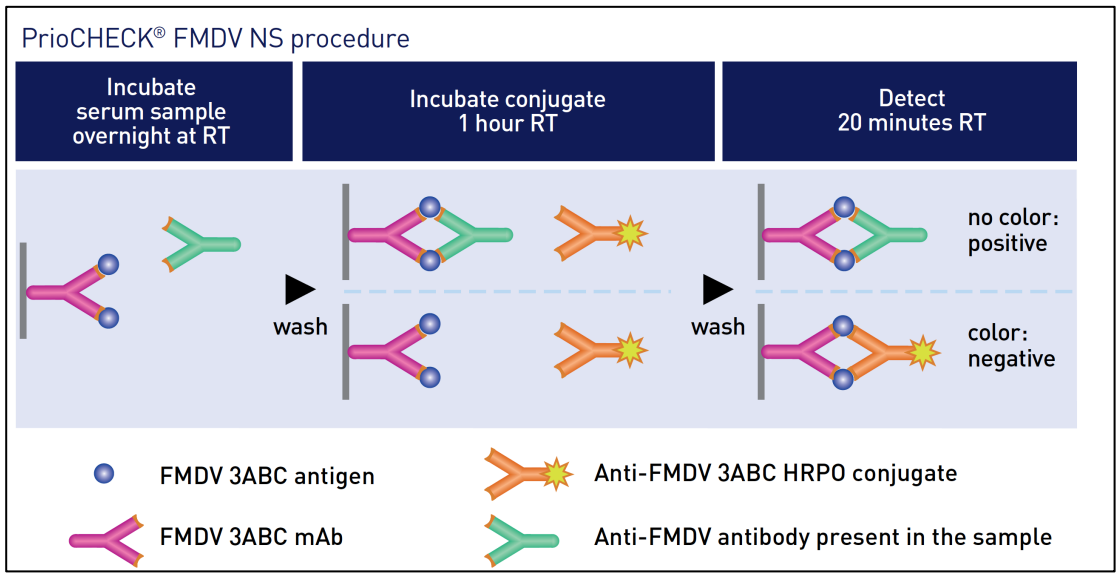

Figure 2.23: An overview of the non-structural protein ELISA mechanism from the manufacturer's (Prionics) website[5].
RT =Room temperature. mAb = Monoclonal antibody. HRPO = Horse-radish-peroxidase.

The NSP ELISA results were expressed in terms of percentage inhibition (PI), a measure of the optical density (OD) in the test sample ELISA well compared to the maximum OD in an ELISA well with nothing blocking the HRP conjugated antibody binding (Equation 2.2).

$$PI = 100 - \frac{OD_{450}\ test\ sample}{OD_{450}\ max} \times 100 \qquad \textbf{Equation 2.2}$$

A positive NSP ELISA result was defined as one with a percentage inhibition of 50% or greater, as per the manufacturers' recommendations. Figure 2.24 shows the distribution of NSP ELISA PIs generated from 2694 livestock in the cross-sectional study.

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[5] PrioCHECK®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33 Lelystad, Netherlands. Website: https://tools.thermofisher.com/content/sfs/brochures/animalhealth_flyer_priocheck_fmdv_ns_C0121102.pdf

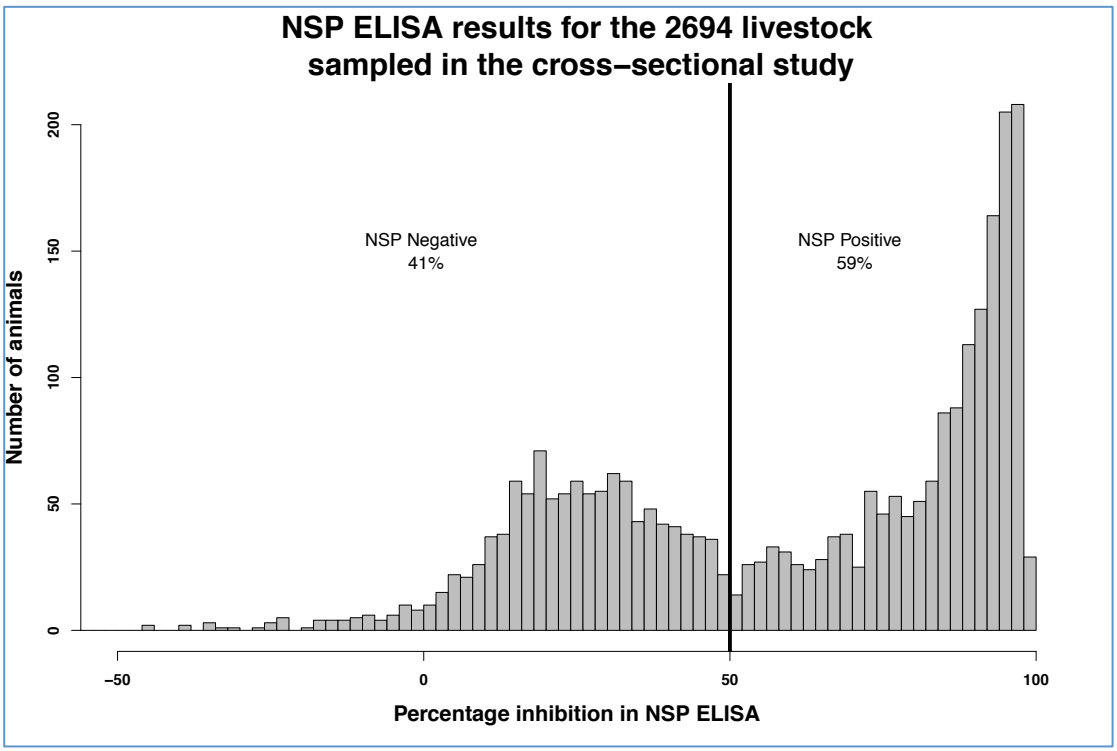


Figure 2.24: A histogram summarising the results of non-structural protein (NSP) ELISA testing of sera from the cross-sectional study livestock. The vertical line represents the manufacturer recommended cut-off between results considered positive and negative.

2.5.3 Solid Phase Competition ELISA

An assay based on the structural proteins of FMDV was necessary for measurement of antibodies against specific serotypes. The Solid Phase Competition ELISA (SPCE) structural protein assay developed by Mackay *et al.* (2001) and validated by Paiba *et al.* (2004) and Li *et al.* (2012) was optimised for use with East African sera. The SPCE was chosen over the liquid phase blocking ELISA (LPBE) (Hamblin *et al.*, 1986a) as the SPCE was shown to be more specific than the LPBE during validation (Mackay *et al.*, 2001). Also, the SPCE required lower volumes of reagents.

An "in house" SPCE assay was used. This test required the generation of antigen, the availability of rabbit and guinea-pig polyclonal antisera, commercial anti-guinea-pig conjugate, chromagen, and positive and negative control sera from experimental animals. Optimisation to find which rabbit and guinea-pig antibodies would bind to the Tanzanian antigen used in the test, titration to calculate the appropriate concentrations of each reagent

and testing of the assay with experimental sera were necessary before any field samples could be tested.

Antigen production

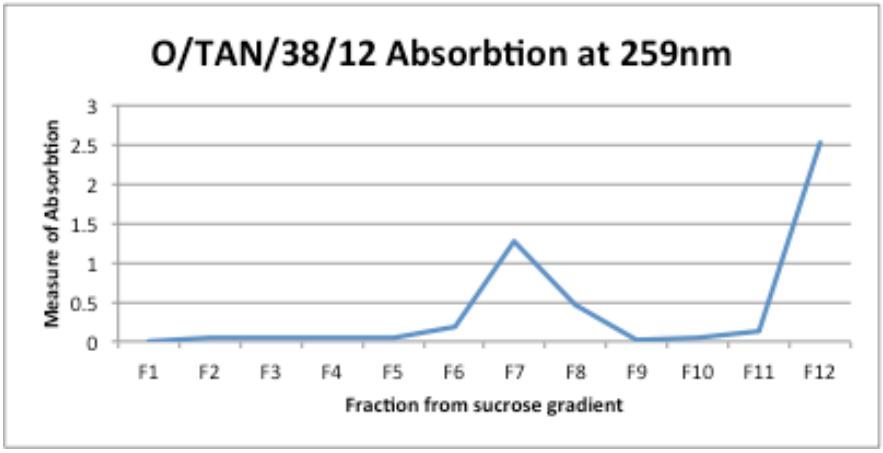
To optimise the SPCE for use with Tanzanian sera, FMDV isolated from the outbreak study in Northern Tanzania were used to generate antigen (Table 2.3).

Table 2.3: Viruses and control sera used for solid phase competition ELISA and virus neutralisation testing of sera from the study. (*Anti-A rabbit and guinea-pig reagents bound poorly to Tanzanian antigen and were in short supply, therefore the serotype A SPCE was not taken forward). SPCE = Solid phase competition ELISA. VNT = Virus neutralisation test.

SPCE /VNT serotype	Virus	Origin	WRL-FMD SPCE Rabbit, Guinea-pig sera supplied	WRL-FMD reference control serum from vaccine experiments
O	O/TAN/38/12	Loliondo, Tanzania, 2012	Anti O1 Manisa	Anti O1 Manisa (ref UM72), O Uganda 2001, O Kenya 98
A	A/TAN/40/12	Loliondo, Tanzania, 2012	Anti A22 Mahamatli,* Anti A 22 Iraq	Anti A22 Iraq (ref US53)
SAT1	SAT1/TAN/22/12	Simanjiro, Tanzania 2012	Anti SAT1 105	Anti SAT1 105 (ref VP80), Serum from known SAT1 infected from Tanzanian outbreak (SAT1/TAN/45/12)
SAT2	SAT2/TAN/8/11	Simanjiro Tanzania 2011	Anti SAT2 Eritrea	Anti SAT2 Eritrea (ref VL86)

The Tanzanian viruses were adapted to cell-culture and amplified over at least four passages through "Baby Hamster Kidney" (BHK) cells. Virus inactivation was achieved with binary ethylenimine (Bahnemann, 1975) and the method of Ferris *et al.* (1984) was used to purify FMDV capsid proteins ("146S particles") based on their sedimentation coefficients (Figure 2.25A). Lipids were removed, proteins were precipitated, concentrated and re-suspended, and sucrose gradient centrifugation was used to separate out proteins with a 146S sedimentation coefficient. The presence of VP1, 2 and 3 proteins was verified by polyacrylamide gel electrophoresis (Figure 2.25B).

(A)



(B)

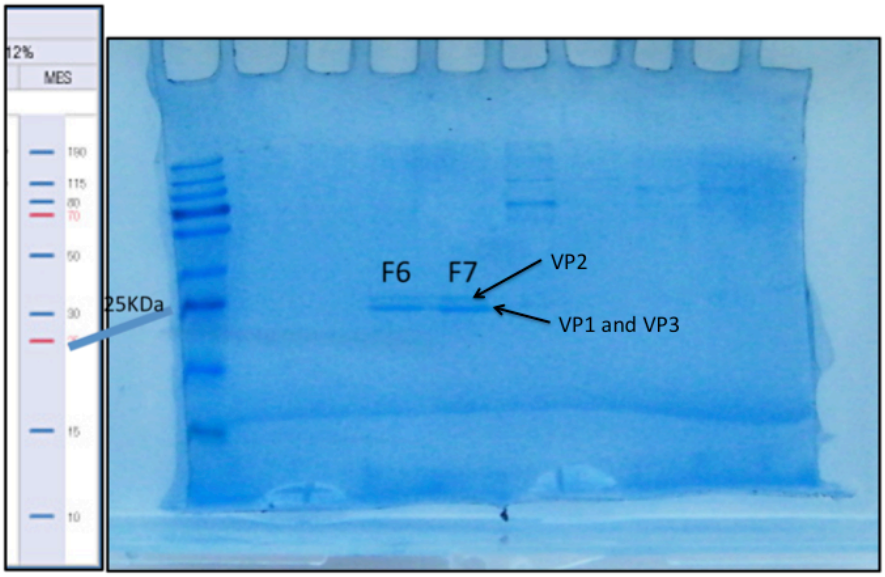


Figure 2.25: An example of the results of the FMDV antigen purification procedure. After concentrating the antigen, centrifugation through a sucrose gradient was used to separate out proteins with different sedimentation coefficients. Figure 2.25 (A) shows spectrophotometry results for 12 one ml fractions of the centrifuged sucrose gradient with the separated proteins showing which fractions are protein rich. Intact FMD virus proteins have a sedimentation coefficient of 146S and generally migrate to fractions 6-8 of the gradient during centrifugation. The gel photo in Figure 2.25 (B) shows how the presence of purified FMDV in these fractions is confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, where the proteins in fractions 6 and 7 migrate under influence of an electric current to positions on the gel which are consistent with the 23-27kiloDalton (kDa) size of the three FMDV capsid proteins (VP1, VP2 and VP3).

Rabbit and guinea-pig antisera

Rabbit and guinea-pig polyclonal antibodies against specific serotypes of FMDV were produced at TPI through vaccinating laboratory animals with inactivated FMDV antigen and harvesting their sera (Ferris & Donaldson, 1984; Ferris, 1988). Due to the closure of facilities for the production of these antisera, it was not possible to generate antisera against the Tanzanian antigens. Therefore standard WRL rabbit and guinea-pig sera were used as described in Table 2.3. Dr. Nigel Ferris and Dr. John Bashiruiddin produced the reagents used several years prior to the current study. Rabbit sera were diluted at 1/5 prior to use. Guinea-pig sera were blocked to avoid non-specific binding and diluted at 1/10. In the latter part of the study a shortage of these reagents became apparent and fewer sera were tested with the SPCE than originally planned.

Solid phase competition ELISA protocol

The SPCE protocol was performed as described by Mackay *et al.* (2001). Rabbit trapping antibodies were allowed to adhere to ELISA plates overnight, then incubated with antigen. Using a blocking buffer with commercial sera from FMDV naïve rabbits and cattle prevented nonspecific antibody binding. Test antibodies and guinea-pig antibodies specific for the antigen were set into competition for binding sites on the antigen. Finally, after washing, the amount of guinea-pig antibody that bound to the antigen was measured by applying HRP conjugated anti-guinea-pig antibody[6] and measuring the OD at 290 nm wavelength generated by its reaction with o-phenylenediamine chromagen[7] and hydrogen peroxide. This protocol is summarised in Figure 2.26.

[6] Commercial rabbit anti guinea-pig polyclonal antibodies conjugated to horseradish peroxidase. Dako product number PO141, Agilent Technologies Dako Denmark. Antibodies were pre blocked in house with an equal volume of bovine serum and then diluted 1/5 with phosphate buffered saline.

[7] O-phenylenediamine (OPD) (Sigma product number P8412), Sigma-Aldrich Ltd, Dorset, UK

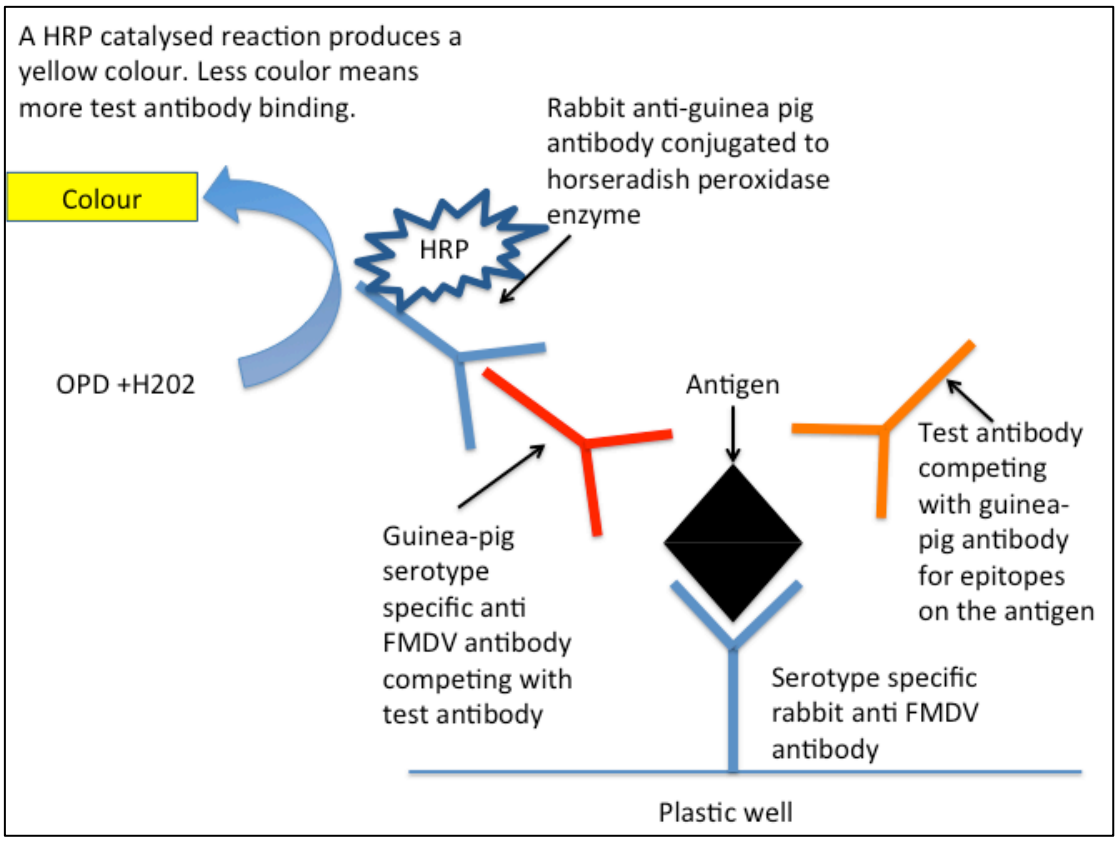


Figure 2.26: An overview of the solid phase competition ELISA. HRP = Horseradish peroxidase. OPD = O-phenylenediamine chromagen. H2O2 = Hydrogen Peroxide

Similarly to the NSP ELISA, the results of the SPCE were expressed in terms of PI (Equation 2.3).

$$\mathbf{PI} = \mathbf{100} - \frac{\mathbf{OD_{490}\ test\ sample} - \mathbf{OD_{490}\ background}}{\mathbf{OD_{490}}\ \boldsymbol{max} - \mathbf{OD_{490}\ background}} \times \mathbf{100} \qquad \text{Equation 2.3}$$

Previously published cut-off values for the Pirbright SPCE were used (Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004). For serotype O and A SPCEs, a PI of ≥50% represented a positive result. For the SAT SPCEs a PI ≥40% was positive.

Titration of Solid Phase Competition ELISA reagents for use with Tanzanian antigens

Before field sera were tested with the optimised SPCE, repeated, systematic titrations of antigen, rabbit and guinea-pig sera were conducted until acceptable OD and repeatable results were achieved with WRL-FMD standard control sera for each serotype. The SPCE was optimised for serotypes O, SAT1 and SAT2. Serotype A rabbit and guinea-pig reagents (Table 2.3) were required in very high concentrations to produce acceptable optical density (OD) values (0.4 – 1.40 at 490 nm wavelength) with the Tanzanian serotype A antigen. These serotype A reagents were in short supply and therefore the serotype A SPCE was not taken forward. Instead, a commercial serotype A ELISA was used as described in section 2.5.4.

For the serotype O, SAT1 and SAT2 SPCE, a concentration of 1μg per ml of purified antigen was trapped with a dilution of 1/1000 of the pre-diluted rabbit polyclonal antibodies shown on Table 2.3. The concentration of pre-diluted guinea-pig antibodies required to produce an acceptable OD value varied for the different serotypes. For the serotype O and SAT2 assays a dilution of 1/100 guinea-gig serum gave acceptable results, but for the SAT1 assay, a dilution of 1/5 was requited. The reagent dilutions required vary with batches of reagents and therefore any further work with new batches of reagents would require repeat titrations. A consistent dilution (1/200) of commercially available rabbit anti guinea-pig HRP conjugated antibodies was used in all of the SPCEs. The chromogen was used as per manufacturers instructions.

Testing of the Solid Phase Competition ELISA

The serotype O, SAT1 and SAT2 SPCEs were tested with experimental control sera. The control sera listed in Table 2.3 were trialled. Sera from experimental cattle 36 days after vaccination and challenged with the SAT2 Eritrea vaccine strain gave a strong positive response on the SPCE.

In contrast to the strong reaction between Tanzanian SAT2 antigen and SAT2 Eritrea anti-sera, sera from cattle 21 days after vaccination with SAT 105 (Rho/12/78) produced weak positive rather than strong positive reactions on SAT1 SPCE (Table 2.4). Consistent weak positive reactions were evident with serial batches of SAT105 antisera. As no alternative

experimental SAT1 strong positive control sera were available, serum was used from an animal in the study herd in Tanzania a SAT1 FMDV infection confirmed by WRL-FMD virus isolation. The serum used was collected six weeks after the animal had FMD lesions due to a SAT1/Tan/45/2012 infection. This serum produced consistently strong positive results on the SAT1 SPCE (Table 2.4).

For the O SPCE, an initial batch of anti O_1 Manisa sera produced only weak positive results. Therefore alternative control anti-sera were sought. Strong positive results were achieved with sera from experimental cattle 21 days after vaccination against FMDV O Uganda 2002, and from those 21 days after vaccination against FMDV O Kenya 78. Dr. Mana Mahapatra of TPI provided these experimental sera for serotype O. Later in the study, further batches of anti O_1 Manisa control sera became available and, in contrast to the first batch, these produced strong positive reactions on the serotype O SPCE (Table 2.4).

Table 2.4: Results with negative and strong positive control sera in the solid-phase competition ELISAs optimised for Tanzanian usage.
N = Number of, PI = Percentage Inhibition, IQR = Inter quartile range, SD = Standard deviation

Serotype	Control	N Controls tested	Mean PI	SD PI	Median PI (IQR)
O	Negative	77	0.12	0.17	0.15 (0.05-0.21)
O	Strong Positive O_1 Manisa	35	0.89	0.07	0.91 (0.83-0.94)
O	Strong Positive O Uga 2002/ Ken 1978	94	0.95	0.03	0.95 (0.93-0.97)
SAT1	Negative	87	0.15	0.22	0.17 (0.03-0.31)
SAT1	Strong Positive SAT105	96	0.72	0.2	0.74 (0.6-0.89)
SAT1	Strong Positive SAT1/TAN/45/12	26	0.9	0.25	0.96 (0.92-0.97)
SAT2	Negative	132	0.08	0.18	0.07 (-0.01-0.17)
SAT2	Strong Positive SAT2 Eritrea	103	0.93	0.03	0.94 (0.92-0.95)

After testing the O, SAT1 and SAT2 SPCEs with control sera, a limited number of control sera were tested in SPCE of heterogeneous serotypes to investigate cross-reaction between experimental sera and SPCE antigens of different serotypes. From the sera tested, there was no evidence in the samples available for high levels of cross-reaction (Table 2.5).

Table 2.5: Results from testing heterologous experimental control sera on the solid-phase-competition ELISAs that were optimised with Tanzanian antigen.

Serotype of SPCE antigen	Serotype of experimental post vaccination / challenge serum	N sera tested	Mean	SD	Median (IQR)
O	A	4	0.5	0.33	0.48 (0.22-0.77)
O	SAT1	4	0.29	0.18	0.29 (0.16-0.42)
O	SAT2	2	0.3	0.18	0.3 (0.24-0.36)
O	SAT3	2	0.27	0.07	0.27 (0.24-0.29)
SAT1	A	4	0.16	0.18	0.15 (0-0.31)
SAT1	O	4	0.44	0.11	0.42 (0.36-0.5)
SAT1	SAT2	6	0.42	0.25	0.45 (0.2-0.63)
SAT2	A	4	0.28	0.1	0.26 (0.2-0.35)
SAT2	C	4	0.23	0.07	0.2 (0.19-0.25)
SAT2	O	8	0.34	0.08	0.37 (0.25-0.4)
SAT2	SAT1	12	0.06	0.22	-0.03 (-0.12-0.28)

2.5.4 Serotype A ELISA

As an alternative to the SPCE, A serotype specific commercial blocking ELISA[8] was used to detect antibodies against the structural proteins of FMDV serotype A. The commercial A ELISA was a blocking ELISA that worked in the same way was the NSP ELISA (Figure 2.23). However, the test sera were incubated with the antigen for one hour rather than overnight. The results were expressed as PI with a PI ≥ 50% considered positive.

2.5.5 Virus Neutralisation Testing

Virus neutralization testing (VNT) measures the ability of a serum to neutralise a fixed dose of virus and prevent the appearance of a readily observable cytopathic effect in susceptible cells grown in culture. The output variable of the test is the titre, or the lowest concentration of serum that neutralizes virus in 50% of test cell culture wells (Karber, 1931). Sera from the cross-sectional study were tested by VNT according to the OIE manual protocol (OIE, 2012a).

Whilst VNT is considered the "Gold standard" for serotype specific diagnosis (Mackay *et al.*, 2001), it is time consuming and requires cell culture and live virus (OIE, 2012a). For

[8] **Priocheck FMDV Type A PrioCHECK ®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33 Lelystad, Netherlands**

this reason, limited sera (128 from cross-sectional livestock, 55 from buffalo and 83 from the longitudinal study in the research herd) were tested against serotypes A, O, SAT1 and SAT2. The 55 buffalo sera were also tested against SAT3 (SAT309, a standard WRL VNT FMDV strain).

The same northern Tanzanian viruses as were used for production of SPCE antigen were used as VNT test viruses (Table 2.3). These viruses were titrated by serially diluting them and applying them to immortalised pig kidney cell (IBRS) cultures. A concentration of one 50% Tissue Culture Infective dose ($TCID_{50}$) of virus per µl was used for serum titration and testing. Reference sera from the WRL were repeatedly titrated with the viruses to generate expected titre ranges. Finally the test sera were tested in duplicate at eight serial dilutions (from 1/16 to 1/1024). Three days were allowed for cytopathic effects to develop and the titre of each test serum was calculated (Karber, 1931).

The VNT cut-off values for positive and inconclusive results recommended by the OIE and WRL-FMD were used. Titres between 16 and 32 were considered inconclusive and titres of above 32 were taken as positive (OIE, 2012a). Low volumes of serum for same samples precluded titres lower than 16 being measured. Therefore reciprocal serum neutralising titres of less than 16 were considered negative instead the negative cut-off of titres less than 11 that is used when higher volumes of serum are available.

2.5.6 Comparison between VNT and SPCE

When VNT and serotype specific ELISA results were generated the same sera from cattle in the cross-sectional study (N=96) and the longitudinal study herd (N = 83), the results of the two different assays were compared. Table 2.6 and Figure 2.27 summarise the results of these comparisons. When binary results were compared, Kappa statistics (Cohen, 1960) suggested moderate to substantial agreement (Dahoo, 2009) between the ELISAs and VNTs (Table 5). A linear regression model was used to investigate ELISA PI as an explanatory variable for logged VNT titre. The model fit was poorest for the serotype A VNT and ELISA, followed by the SAT1 assays, and best for the serotype O assays followed by the SAT2 assays. (Table 2.6, Figure 2.27). The commercial A kit antigen was different to the serotype A VNT virus, possibly explaining why agreement between the A assays was poorest. The SAT1 result possibly reflects the poor avidity between the

SAT105 rabbit and guinea-pig reagents and the Tanzanian antigen used in the solid phase competition ELISA. Further analyses of the ELISA results using a Bayesian methodology are presented in Chapter 5.

Table 2.6: Comparison between VNT and serotype specific ELISA results. Pearson's correlation coefficient (r) was used to measure correlation between ELISA PI and VNT titre. Kappa statistics (Cohen, 1960) were used to measure agreement between positive and negative (binary) ELISA and VNT results. Cutoff values for binary results were used as recommended by (Li *et al.*, 2012) for the serotype O, SAT1 and SAT2 solid phase competition ELISAs and by the manufacturers[9] for the commercial serotype A ELISA. For the VNT, reciprocal titres above 32 were considered positive and those below 16 were considered negative. Statistics from a linear model with ELISA PI as an explanatory variable for VNT log titre are reported. Finally, the diagnostic sensitivity and specificity for binary ELISA results, taking VNT results as the "gold standard" were calculated. * The data-points available for each serotype varied as ELISA assays were repeated variable numbers of times for each serotype, depending on reagent and serum availability. A model incorporating serum ID as a random effect was not used as a proportion of sera only had single ELISA data-points for each serotype and this caused model convergence problems. Further modeling of the ELISA results using Bayesian methodology is shown in Chapter 5.

Attribute measured	Statistic used	Serotype			
		A	O	SAT1	SAT2
Agreement between ELISA and VNT					
	Pearson's r	0.58	0.81	0.64	0.70
	Kappa	0.59	0.88	0.71	0.78
ELISA PI as an explanatory variable for VNT titre in a linear model					
	Adjusted R^2	0.34	0.66	0.40	0.55
	F statistic	76.95	460.20	198.00	324.20
	P-value	3.6×10^{-15}	2.2×10^{-16}	2.2×10^{-16}	2.2×10^{-16}
	Degrees of freedom*	150	240	291	267
Diagnostic sensitivity of ELISA (VNT = "gold standard")		0.91	0.98	0.98	0.98
Diagnostic specificity of ELISA (VNT = "gold standard")		0.70	0.90	0.59	0.84

[9] **Priocheck FMDV Type A PrioCHECK ®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33 Lelystad, Netherlands**

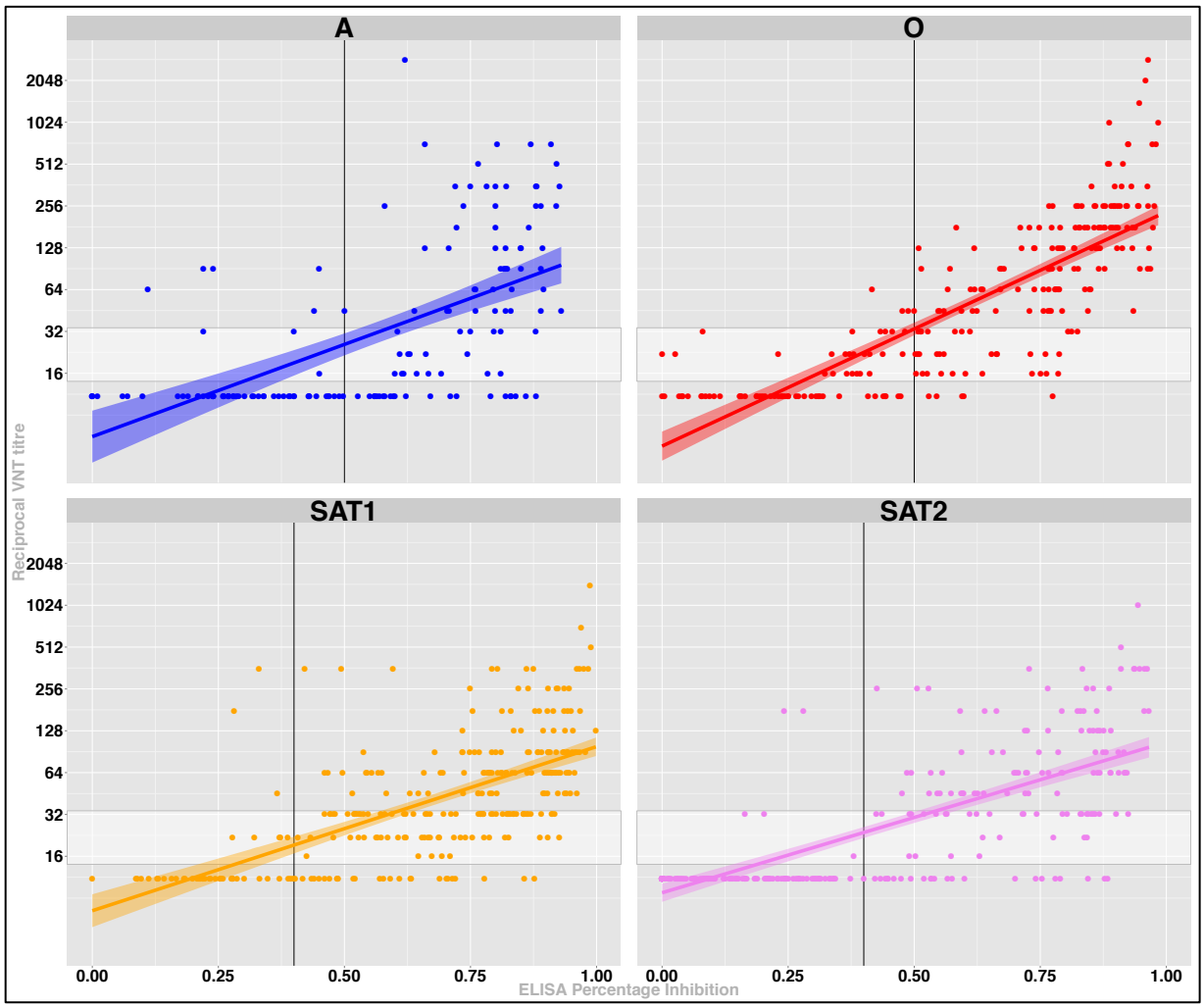


Figure 2.27: Scatterplots with ELISA percentage-inhibition (PI) on the y-axis and virus neutralisation test (VNT) titre on the x-axis (with a logged scale). A regression line from a simple linear model with VNT titre as the response variable and ELISA PI as the explanatory variable is drawn onto the scatter-plot. The coloured shaded areas adjacent to the regression line represent 95% confidence intervals. The vertical black lines indicate the recommended ELISA cut-off values for positive and negative results (0.4 for serotype SAT1 and SAT2 ELISAs, 0.5 for serotype A and O ELISAs). For the VNTs, a reciprocal titre of 16 or less was considered negative and a reciprocal titre of above 32 was considered positive. Horizontal black lines highlight these cut-off titres. Reciprocal titres between and including 16 and 32 were considered inconclusive and correspond to the lighter grey background on the plot.

2.6 Timeline of PhD

Table 2.7 summarises the activities of the PhD project.

Table 2.7: PhD project timeline.

Date	Location	Activity
June 2012	Glasgow	Start of PhD
June 2012 - August 2012	Glasgow	Initial organisation and cleaning of field data. Learnt R and SQL languages and built an SQL database for cross-sectional field data Literature review, learning to use GIS programmes and production of a map showing livestock and buffalo populations in Africa
August 2012	Pirbright	Initial biosecurity training Negotiations for laboratory resource with collaborators at Pirbright NSP ELISA testing of cross-sectional sera Sample organisation
September 2012	Glasgow	Initial FMD risk-factor analysis using cross-sectional database and NSP ELISA results
October 2012	Pirbright/Spain	Induction, biosecurity training and negotiations for project activities at the Pirbright Institute. First year report EuFMD international foot-and-mouth disease meeting
November 2012	Glasgow	Statistical modelling courses Preparation of Elsevier book chapter based on literature review
December 2012	Pirbright	Initial amplification of Tanzanian viruses Introduction to virus isolation, antigen ELISA and antigen purification techniques
January and February 2013	Glasgow	Programming and disease transmission modelling courses Submission and acceptance for publication of book chapter
March 2013	Tanzania	Field visit and planning for longitudinal herd study Organisation and shipping of field samples Entry and organisation of paper questionnaire data Visits to wildlife reserves Discussions with livestock and wildlife managers
April 2013	Pirbright	Negotiating access to and organising field samples Amplifying virus and purifying antigen Generation of NSP antibody ELISA results. European Wildlife Disease Association Conference in Annecy, France.
May – July 2013	Pirbright	Sample organisation Virus amplification Antigen purification Reagent optimisation, Trouble-shooting for O, A and SAT SPCEs Negotiations for fresh reagents Generation of SAT2 and SAT1 SPCE results Writing second year report
August - September 2013	Glasgow	Learnt Bayesian statistics and JAGS language Entered and organised longitudinal serological data from study herd Initiated building Bayesian model to infer infection history from serological data Outbreak study data entry Preparation of initial model output for presentation
October 2013	Tanzania	Presented Bayesian model at GFRA meeting in Arusha, Tanzania Assisted with stakeholder workshop about FMD control in Tanzania Organised field samples for shipment from Tanzania Collected information and field data from field team
November - December 2013	Glasgow	Entered final cross-sectional field data and outbreak study data Collated and organised results Initial analyses of longitudinal virus isolation data Developed skills in spatial analysis and mapping in the R statistical environment Generated preliminary maps of buffalo and livestock density in study area Developed Bayesian model

January - May 2014	Pirbright	Negotiating access to freezer storage space and organisation of Tanzanian field samples Discovery of a SPCE reagent shortage in Pirbright and making alternative plans for generating serotype specific data from sera Epidemiology conference (SEVPM) in Dublin, Ireland VNT training in WRL and testing of initial an batch of sera SPCE testing of sera from study herd for Bayesian modelling purposes Development of an algorithm to select most useful cross-sectional samples to submit for VNT in WRL-FMD. Production and purification of antigen for SPCE Development of an automated system for uploading lab results and merging them with animal data
May - September 2014	Glasgow	Data entry, cleaning and analyses from lab and for case-control, longitudinal and outbreak studies Development and validation of Bayesian model for serology interpretation Analyses of VNT and virus isolation results Initial analyses of FMD impact on rural livelihoods Case - control study analysis Completion of distance measurement from study household locations to buffalo areas
October 2014	Pirbright / Glasgow	Finalisation of lab work and sample archiving Preparation of preliminary economic impact results for presentation at the EuFMD conference
November 2014	Croatia/Glasgow	Presentation of economic impact results at EuFMD conference Drafting of Risk factor and case-control chapter (Chapter 4)
December 2014 - May 2015	Glasgow	Finalisation of risk-factor/case-control chapter Spatial statistics course Drafting of Bayesian modelling chapter (Chapter 5) and validation of model Analyses of VNT and virus isolation results
May - December 2015 (part-time)	Glasgow / Ireland	Final analyses of VNT and VI data with comparison to Bayesian model output Review of East African virus isolation results and drafting of Serotype specific patterns chapter (Chapter 6) Drafting and revisions of Bayesian (5), impact (3) and serotype (6) chapters as well as introduction (1), methods (2) and discussion (7)

Chapter 3: Household level impacts of foot-and-mouth disease on traditional livestock keeping systems of northern Tanzania

3.1 Summary

Livestock have great potential to contribute to the livelihoods of the poor, particularly in developing countries where people are heavily dependent on livestock. FMD ranks highly amongst diseases constraining pro-poor growth in these settings. Impacts, hence demands and incentives for control, are likely to differ across settings, production systems and segments of the society. Such heterogeneities are poorly characterised, hence well-informed control policies benefiting those mostly affected by the disease are lacking.

In order to investigate such impacts, household questionnaire data (n = 182) were generated across three production systems of northern Tanzania (pastoralist / agro-pastoralist / rural smallholder), including: (1) income sources; (2) uses of livestock and their products; (3) frequency of FMD outbreaks; (4) morbidity and mortality due to FMD outbreaks; (5) outbreak impacts on herd production and performance; and (6) perceived importance of FMD compared to other livestock diseases, which are prevalent in the area. Household reports of morbidity and outbreak frequency were compared to seroprevalence data (n = 2738 livestock from 84 herds) and longitudinal field observations (n = 15 herds).

Livestock sales were the most frequently reported income source across the three production systems, followed by crop- and milk-related income. In 81.8% [95% CI: 64.5-93.0%] of pastoral and 80.0% [95% CI: 56.3-94.2%] of agro-pastoral households, respectively, at least one FMD outbreak was reported in the past year. Of the herd owners reporting outbreaks in the past year, 39.5% [95% CI: 25.0-56.5%] suffered two or more outbreaks, and 25.6% [95% CI: 13.5-41.2%] three or more. Longitudinal field observations confirmed up to four serial FMD outbreaks in the same herds in less than three years. Relatively fewer rural small-holders reported outbreaks in the past year (30.0% [95% CI: 13.2 -52.9%]).

A high seroprevalence of FMD was detected in both northern Tanzanian cattle (69%, [95% CI: 66.5 - 71.4%]) and small ruminants (48.5% [95% CI: 45.7% - 51.3%]). Reported herd level morbidity during FMD outbreaks was very variable (median: 42.9%, IQR: 21.9-68.8% for cattle; median: 10.2%, IQR: 0 -56.6% for small ruminants). Adult female cattle were especially affected (49.85% [95% CI: 46.04-53.66%]) and impacts on milk production were considerable: 90.0% [95% CI: 83.5-94.6%] and 66.0% [95% CI: 51.2-78.8%] of respondents reported decreased milk production in cattle and goats, respectively, while 63.9% [95% CI: 53.5-73.5%] stopped selling milk. A loss of traction capacity affected 66.1% [95% CI: 52.6-77.9%] of households. FMD was the disease of greatest concern to agro-pastoralists and was ranked second by pastoralists, but was of less concern to smallholder farmers. Herd FMD seroprevalence levels could be explained by the FMD outbreak frequency reported by the household, but not by reported morbidity levels, suggesting that levels of FMD infection were higher than animals detected with clinical signs.

This study provides evidence that FMD has important consequences for livestock-dependent communities in Tanzania. FMD control in these systems has the potential to reduce vulnerability through increased milk and crop production. Livestock owners in traditional livestock-keeping systems were familiar with FMD and their reports of frequent outbreaks were consistent with field observations and laboratory analyses. This barrage of serial FMD outbreaks causes durable attrition on livestock productivity and subsequently on livelihoods and food-security.

3.2 Introduction

Livestock have enormous potential to contribute to the livelihoods of the poor. In Sub-Saharan Africa (SSA), over 60% of the population and 32% of the Gross Domestic Product (GDP) depend on agriculture of which livestock make up a significant proportion (35% of agricultural GDP in SSA) (Otte & Chilonda, 2001; Upton, 2004). In Tanzania, 62% of rural households depend on livestock for their livelihoods (Longin, 2015). With rapid growth in the human population, the number of individuals dependent on livestock and their products is increasing every year (Upton, 2004).

Livestock productivity contributes to the food-security and livelihoods of rural communities, where the majority of SSA's poorest people live. Milk is a vital food source for pastoralists, and interference with milk supply due to drought or livestock disease can have serious consequences for human health (Barasa *et al.*, 2008; Seaman *et al.*, 1978). Increasing demand for milk products in urban areas represents a development opportunity for small-scale milk producers. Livestock also play an important role as draught animals in the production of crops for agropastoral and rural smallholder systems, as well as the increasing number of traditionally pastoralist households engaging in crop production (Bayissa *et al.*, 2011; FAO, 2015; Upton, 2004). Livestock embody savings, funds for education and an emergency reserve for times of hardship as well as being a keystone of cultural identity in East Africa. With the advent of mobile phone technology, a vibrant industry of intra-regional cross-border livestock trade is emerging in East Africa, opening up livelihood opportunities and routes out of poverty for rural households (Little, 2009).

Despite all of the opportunities, drought, land shortage and livestock disease constrain livestock productivity, pro-poor growth and threaten food-security amongst the most vulnerable. Threats to livestock generate risk adversity to embracing opportunities to improve productivity and limit time and energy for diversifying income sources (Perry *et al.*, 2002).

Foot-and-mouth disease (FMD) is highly ranked amongst livestock diseases constraining pro-poor growth in developing countries (Perry & Rich, 2007). Impacts on milk production and draught ability in traditional settings have been described (Barasa *et al.*, 2008; Bayissa *et al.*, 2011) as well as mortality in young animals and a chronic heat intolerance syndrome

that reduces productivity for the lifetime of the animal (Barasa *et al.*, 2008; Bayissa *et al.*, 2011; Catley *et al.*, 2004; Rufael *et al.*, 2008). These impacts of FMD on the poor have been described at aggregate level (Perry & Rich, 2007; Perry *et al.*, 2002), but there is a scarcity of studies on the household level impacts of FMD in traditional settings where FMD is endemic (Knight-Jones & Rushton, 2013). Studies in East Africa have described the impacts of FMD on Ethiopian agropastoral, pastoral and smallholder households (Bayissa *et al.*, 2011; Jemberu *et al.*, 2014; Rufael *et al.*, 2008), Sudanese pastoralists (Barasa *et al.*, 2008), and on a Kenyan diary farm (Lyons *et al.*, 2015a, b). However, a knowledge gap has been highlighted in terms of comparison of impacts between livestock management systems (Perry & Rich, 2007). There is a need for these differences to be clearly quantified, as incentives for control are likely to differ depending on the affected livestock system. Estimation of the socio-economic impacts of FMD on different stakeholders is also advocated by the Food and Agriculture Organisation as a key component of progressing to the first stage of the Progressive Control Pathway for FMD (FAO/OIE/EuFMD, 2012).

This study focuses on agropastoral, pastoral and smallholder households at the wildlife-livestock interface in northern Tanzania. Inhabitants in this area face the challenges of increasing human populations, land shortage and conservation concerns. They are highly dependent on their livestock (Upton, 2004) and vulnerable to threats from animal diseases. This study addresses the need for an improved understanding of the impacts of FMD (i) in endemic countries, (ii) at household level, (iii) in different livestock management systems and (iv) on the rural poor at an intensive wildlife-livestock interface.

The aims of this study were to investigate the contribution of livestock to rural livelihoods in northern Tanzania and the impact of FMD on this. With reference to pastoral, agropastoral and rural smallholder households in traditional livestock keeping systems, the objectives were:

1. To describe socioeconomic indicators and sources of income.
2. To describe household uses of livestock and their products.
3. To quantify the burden of FMD through measuring seroprevalence, reported and observed outbreak frequency.
4. To quantify reported morbidity and mortality associated with FMD outbreaks.
5. To describe the impact of FMD outbreaks on the productivity of livestock.

6. To describe the perceived importance of FMD relative to other prevalent livestock diseases in the region.

3.3 Methods

3.3.1 Sources of data

Table 3.1 summarises sources of data for each objective covered in this study. See Chapter 2, Section 2.3.2, Pages 49 – 62 for details of study designs.

Table 3.1: Data sources used in Chapter 3.
The study designs are described in Chapter 2 and the reference letters "A" – "F" in this table correspond with Chapter 2 (Table 2.1, Page 51). A = Cross-sectional study, C = Outbreak investigations, C* = Outbreak investigations with more detailed questionnaires, D = Longitudinal study tracking FMD outbreaks in the same herds, E = Herds with outbreaks from the case-control study, F = Research herd tracked through serial FMD outbreaks.

Study design reference on table 2.1 page 51, Chapter 2		A	C	C*	D	E	F
N Households/Herds		84	50	17	15	37	1
Objective							
1. Describe baseline socioeconomic indicators and sources of income		✔		✔			
2. To describe household uses of livestock and their products							
	Livestock numbers	✔	✔	✔		✔	
	Livestock uses in household	✔		✔			
	Livestock products consumed	✔		✔			
3. To quantify the burden of FMD							
	Seroprevalence	✔					
	Reported frequency of outbreaks	✔					
	Observed frequency of outbreaks				✔		✔
	Reported seasonality of outbreaks	✔	✔	✔			
4. To quantify reported morbidity and mortality		✔	✔	✔		✔	
5. To describe the impact of FMD outbreaks on the productivity of livestock							
	Milk produced	✔		✔			
	Milk sales	✔		✔		✔	
	Draught ability of oxen	✔		✔		✔	
	FMD impacts on herd management	✔		✔		✔	
	Duration of impacts			✔	✔		
6. To describe the perceived importance of FMD		✔		✔			

3.3.2 Overview of analyses

Questionnaire data were collated in a specially designed SQL database. Data were imported into the R statistical environment (R development core team, 2008) and summary statistics were generated for all variables relevant to household level impacts. As well as descriptive statistics, statistical models were used to investigate the following:

1. Can FMDV seroprevalence in a herd be explained by the FMD outbreaks reported by the herd owner?
2. Can FMD outbreak occurrence be explained by season (wet or dry)?
3. Can FMDV seroprevalence in a herd be explained by the morbidity during the most recent FMD outbreak reported by the herd owner?
4. Can morbidity levels in an FMD outbreak be explained by herd size, livestock practice, season or the serotype of FMDV causing the outbreak?
5. Can livestock practice explain the litres of milk produced per cow?
6. Can reported milk loses during an FMD outbreak be explained by herd size, reported morbidity, milk production levels or season?

Further, to quantify the perceived importance of FMD compared to other livestock diseases, a pairwise ranking algorithm was developed.

3.3.3 Socioeconomic indicators, income and livestock uses

Longevity, adult literacy rates, household size and standard of living are part of the human development index of the United Nations (United Nations, 2016b) and are recognised to reflect poverty levels in Tanzania (Tanzanian Government, 2005). To get a snapshot of the background economic landscape in our survey, indicators of these development parameters were quantified from questionnaire data. Adult education levels, household size, distance travelled to collect household drinking water and ages of household members were summarised for the 101 households in the study for which this information was available. Herd composition and size, income sources, uses of livestock and consumption of animal products were also summarised.

3.3.4 FMD burden in the study area

Seroprevalence and outbreak frequency

Seroprevalence from the cross-sectional study and reported outbreak frequency were summarised. Further, the frequency of outbreaks observed through the active surveillance platform in Serengeti district (agropastoral area) and in a longitudinally tracked herd in Simanjiro (pastoral area) was quantified.

Association between livestock owner reports of FMD and seroprevalence in their herds

The association between household reports of FMD outbreaks and serological results was examined using a generalised linear mixed model (GLMM) with a herd level random effect. Positive or negative serological results ($y_{a,j}$) from animal a in herd j were assumed to follow a Bernoulli distribution based on a probability of $p_{a,j}$ of being seropositive.

$$y_{a,j} \sim Bernoulli(p_{a,j})$$

A logit function was used to link $p_{a,j}$ to the GLMM as $\eta_{a,j}$.

$$\eta_{a,j} = \log\left(\frac{p_{a,j}}{1 - p_{a,j}}\right)$$

The GLMM included coefficients for animal age (β_1), species (α_s), reported cases of FMD in the herd ever, in the past year or in the past four months ("yes" or "no") (ω_h) and a herd level random effect (γ_j). The intercept was termed β_0. (Model 3.1)

$$\eta_{a,j} = \beta_0 + \beta_1 x_{a,1} + \alpha_s + \omega_h + \gamma_j \qquad \textbf{Model 3.1}$$

a = animal ID

j = herd ID

$x_{a,1}$ = animal age

s = bovine or small ruminant

h =reported FMD case in herd yes or no,

The herd level random effect was assumed to follow a normal distribution with a mean of 0.

$$\boldsymbol{\gamma_j \sim Normal(0, \sigma_\gamma^2)}$$

Likelihood ratio testing (LRT) (Neyman & Pearson, 1928) was used to interrogate the explanatory ability of the model with and without reported cases of FMD as an explanatory variable. The difference in the log likelihood (LL) of the data with the more complex (e.g. with reported cases of FMD as an explanatory variable) and the simpler model (e.g. without reported cases of FMD as an explanatory variable) was calculated.

$$\delta LL = LL(complex) - LL(simpler)$$

Twice this difference was assumed to be Chi-squared distributed with degrees of freedom (k) equalling the degrees of freedom taken by the coefficients by which the two models differed.

$$\boldsymbol{2 \times \delta LL \sim Chisquared(k)}$$

The cumulative probability (p) of $2 \times \delta LL$ on the Chi-squared distribution with k degrees of freedom was calculated. If 1-p was less than 0.05, the difference between the likelihood of the data with the complex model and the simple model was taken to be greater than what would happen by chance based on the difference in coefficients between the models, and therefore the retention of the extra explanatory variable in the more complex model was justified. If this was not the case, retention of the extra variable was not justified and it was dropped from the model. The difference to Akaike's Information Criterion (AIK) made by dropping each explanatory variable was also reported. The residual deviance was examined to assess model fit. This model assessment process was repeated for all models described below. Where model selection suggested significant explanatory variables, and biologically plausible conclusions could be drawn from the model outputs, predictions were compared to the data to further interrogate the explanatory ability of the model for the data.

Assessing the seasonality of reported FMD outbreaks

Respondents recalled the months and years of previous FMD outbreaks in their areas and these were summarised. To examine whether more outbreaks were reported in the wet or dry seasons, the opportunity for outbreak reporting in each month from November 2009 to November 2013 was quantified by counting the number of questionnaires with answers for the relevant questions that were conducted after that month. For each month, and for households in each livestock practice, the opportunities for reporting and the reported outbreaks were quantified. The effect of season on the likelihood of an outbreak being reported was explored with a generalized linear model (GLM). For each month (i) between November 2009 and November 2013, outbreak reports (y_i) were assumed to be binomially distributed based on n_i opportunities to report and a probability of p_i.

$$\boldsymbol{y_i \sim Binomial(n_i, p_i)}$$

A logit function was used to link p_i as η_i to the model. A GLM with the explanatory variables of season (θ_l), livestock practice (λ_k), and the interaction between them ($\theta_l * \lambda_k$) used to model outbreak reports (Model 3.2).

$$\eta_i = \beta_0 + \theta_l + \lambda_k + \theta_l * \lambda_k \qquad \textbf{Model 3.2}$$

$$i = \text{month}$$

$$l = wet\ season\ or\ dry\ season$$

$$k = agropastoral\ or\ pastoral$$

3.3.7 Morbidity and mortality

Calculating morbidity and mortality

Each questionnaire respondent provided information on livestock numbers in their herd and on how many animals of each species, sex and age category were observed to have clinical signs of FMD during the most recent FMD outbreak in the herd. Morbidity at species, livestock category and at herd level was calculated.

$$\boldsymbol{Morbidity = \frac{N\ livestock\ with\ clinical\ signs}{Total\ livestock}}$$

As was done for household FMD outbreak reports (Model 3.1), the association between FMD serological testing results and morbidity reported in the most recent outbreak was examined using a GLMM with a herd level random effect.

Respondents also reported on animal deaths during the most recent FMD outbreak in their herd. Mortality at species, livestock category and at herd level was calculated as for morbidity.

Modelling of drivers of morbidity

A GLMM was used to investigate whether livestock practice (agropastoral or pastoral), herd size season of the outbreak and the FMDV serotype isolated from the outbreak (A, SAT1 or SAT2) helped explain variation in herd level morbidity. There were 118 households with data available for morbidity in the most recent FMD outbreak to affect their livestock as well as livestock practice and herd size. A lower number of households (n=31) had extra information relating to virus typing and confirmed timing (wet or dry season) from outbreak investigations. Therefore, two approaches were used. The first, using the full 118 data points available, investigated only livestock practice and herd size as explanatory variables for morbidity. As well as these variables, the second approach, using the 31 data points with extra information available, investigated season of the outbreak and the FMDV serotype as explanations for morbidity.

Reports (y_j) of animals with clinical signs of FMD in each herd (j) with an FMD outbreak were assumed to be binomially distributed based on n_j animals in the herd and a probability of p_j for clinical signs in the animals.

$$\boldsymbol{y_j \sim Binomial(n_j, p_j)}$$

A logit function was used to link p_j as η_j to the model. A GLMM with coefficients for livestock practice (λ_k), herd size (β_1), season (θ_l) and FMD virus serotype isolated (ψ_q) was built, incorporating a herd level random effect (γ_j) (Model 3.3).

$$\eta_j = \beta_0 + \beta_1 x_1 + \lambda_k + + \theta_l + \psi_q + \gamma_j \qquad \text{Model 3.3}$$

j = herd ID

x_1=n livestock in herd

k = agropastoral or pastoral

l = dry season or wet season

q =serotype A or SAT1 or SAT2

Clinical signs of FMD and human illness during FMD outbreaks

The clinical signs reported at herd level in each household questionnaire and recorded at animal level during veterinary examinations were summarised. Reports of human illness during FMD outbreaks and perceptions as to whether it was possible for people to contract FMD from infected animals or their products were also collated.

3.3.8 Impacts on production

Quantitation of milk losses

Households estimated how much milk their cattle goats and sheep produced daily for household consumption and sales, and the number of producing animals for each species. Litres of milk produced per animal were estimated separately for each species.

$$Litres\ per\ animal = \frac{Litres\ of\ milk\ produced\ daily}{Number\ of\ animals\ producing\ milk}$$

Litres per animal produced during the FMD outbreaks were also quantified. Data about milk production with and without FMD were available from 55 herds for cattle, and five herds for goats. Milk production per animal during an FMD outbreak was expressed as a proportion of milk production per animal without FMD.

$$Proportion\ of\ \ milk\ production\ during\ FMD\ outbreak$$

$$= \frac{Milk\ per\ animal\ during\ FMD\ outbreak}{Milk\ per\ animal\ when\ no\ there\ is\ no\ FMD\ outbreak}$$

Litres per cow were compared in households in the three production systems using a GLM. In each herd (j), litres of milk per cow (l_j) was assumed to be normally distributed around a mean of μ_j with a variance of σ_ϵ.

$$l_j \sim Normal\ (\mu_j, \sigma_\epsilon)$$

A general linear model with a coefficient for livestock practice (λ_k) was used to explain the variation in litres of milk per cow in each household.

$$\mu_j = \beta_o + \lambda_k$$

j = herd ID

k = agropastoral or pastoral

Model 3.4

The difference between milk produced normally, and milk produced during outbreaks was examined using a paired t-test.

Modelling drivers of milk loss

Potential explanatory variables that were examined to explain variation in the proportion of normal milk production during an FMD outbreak included livestock practice, herd size and season. As virus isolation data were only available for five herds that provided milk loss data, the virus serotype causing the FMD outbreak could not be investigated as an explanatory variable.

Two separate approaches, a logit transform and an arc-sine square-root combination, were trialed to linearize the proportion of normal milk production during an FMD outbreak so that a linear model based on a Normal distribution could be used. However, both transformations of the response variable produced multimodal residual distributions and model diagnostics revealed violation of assumptions of normality of residuals. Therefore the proportion of normal milk production during an FMD outbreak (p_j) was instead assumed to be Beta distributed with shape parameters a_j and b_j.

$$\boldsymbol{p_j \sim Beta\ (a_j, b_j)}$$

Using the "betareg" package (Zeileis *et al.*, 2015) in the R statistical environment, the shape parameters were transformed to mean (μ_j) and precision (ϕ) parameters using a link function (Simas *et al.*, 2010). The precision parameter (ϕ) was assumed to be a constant. This approach resulted in more symmetric residuals compared to the conventional linearization.

$$\boldsymbol{\mu_j} = \frac{\boldsymbol{a_j}}{\boldsymbol{a_j} + \boldsymbol{b_j}}$$

$$\boldsymbol{\phi} = \boldsymbol{a_j} + \boldsymbol{b_j}$$

A GLM was built to investigate potential explanatory variables for the variation in milk produced during FMD outbreaks with coefficients for herd size (β_1), litres per animal per day (β_2), morbidity reported in the most recent FMD outbreak (β_3), livestock practice (λ_k) and season (θ_l) (Model 3.4). The final model was selected using LRT. Pseudo R^2 statistics (the difference between residual deviance and null deviance divided by null deviance) rather than raw deviance were available from the betareg package as a measure of model fit.

$$\boldsymbol{\mu_j} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 x_{1j}} + \boldsymbol{\beta_2 x_{2j}} + \boldsymbol{\beta_3 x_{3j}} + \boldsymbol{\lambda_k} + \boldsymbol{\theta_l} \qquad \text{Model 3.5}$$

j = herd ID

$\boldsymbol{x_1}$= litres of milk per animal when not affected by FMD

$\boldsymbol{x_2}$= number of cattle in the herd

$\boldsymbol{x_3}$= reported morbidity during most recent FMD outbreak

k = agropastoral, pastoral or small holder livestock practice

l = wet season or dry season

Reported impacts of FMD on rural livelihoods

Impacts of FMD on milk sales and consumption, on the ability of oxen to pull carts and ploughs and on livestock management and sales were summarised. In addition, for a subset of livestock owners in the longitudinal study, it was possible to quantify the duration of these impacts.

3.2.9 Pairwise comparisons for disease ranking

Households were asked to identify and rank seven livestock diseases known to be present in the area in order of importance. The diseases (in alphabetical order) were:

1. Anthrax and black quarter
2. Brucellosis
3. East coast fever (ECF)
4. FMD
5. Malignant catarrhal fever (MCF)
6. Tick borne diseases other than ECF (e.g. babesiosis and heartwater)
7. Trypanasomiasis

A pairwise ranking algorithm was developed to compare the perceived importance of each disease. Knowledge of and ranking of each of the seven diseases (τ_k) by livestock owners was compared to that for the other 6 diseases (τ_j). Pairwise ranking scores $(\tau_k P \tau_j)$ were produced for every possible pairwise combination of diseases for every household.

$$\tau_k P \tau_j = \begin{cases} \mathbf{1}\ if\ \tau_k\ ranked\ above\ \tau_j \\ \mathbf{1}\ if\ \tau_k\ known\ and\ \tau_j\ unknown \\ \mathbf{0}\ if\ \tau_j\ ranked\ above\ \tau_k \\ \mathbf{0}\ if\ \tau_j\ known\ and\ \tau_k\ unknown \\ \mathbf{0.5}\ if\ \tau_j\ and\ \tau_k\ ranked\ the\ same \\ NA\ if\ both\ \tau_j\ and\ \tau_k\ unknown \end{cases}$$

k = disease 1 … 7,

j = disease 1…6 that disease k is compared to

NA = "Not answered"

For agropastoral, pastoral and smallholder livestock practices, pairwise ranking scores for each disease against all the other diseases, $\tau_k P \tau_j$, were summed and divided by the number of pairwise comparisons (n_k) between that disease and the others to produce an average pairwise score per comparison (v_k).

$$\nu_k = \frac{\sum \tau_k P \tau_j}{n_k}$$

$$0 \leq \nu_k \leq 1$$

n_k = Number of pairwise comparisons between τ_k and τ_j

j = 1 …..6 diseases that disease k is compared to

Finally, for plotting purposes, the neutral pairwise comparison score of 0.5 was subtracted from ν_k for each disease to highlight whether the disease was ranked higher (positive value) or lower (negative value) than this.

3.4 Results

3.4.1 Socio-economic indicators in surveyed households

Pastoralists had the lowest adult education levels, tended to walk farther to collect drinking water and had the largest number of household members (Table 3.1). No differences in adult ages amongst the three management systems were apparent from the sampled households, with the upper quartile between 40 and 46 years old for all three management systems. Only 24.2% (CI: 16.0-34.1%) of households had savings accounts.

Table 3.1: Adult education levels, minutes walked to collect drinking water, household size and adult age in surveyed households.

Livestock management system	Adults with no education	Adults with primary education	Adults with secondary education	Adults with third level education	Minutes walked for household drinking water	Number of people in household aged less than 15 years old	Number of people in household aged 15 years or older	Adult female age (15 years are older)	Adult male age (15 years or older)
	Proportion (95% CI)	Proportion (95% CI)	Proportion (95% CI)	Proportion (95% CI)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Agropastoral	0.06 (0.03-0.1)	0.66 (0.59-0.73)	0.21 (0.15-0.27)	0.07 (0.04-0.12)	20 (11 - 53)	9 (2 - 16)	3 (2-6)	28 (21-37)	31 (24-40)
Pastoral	0.45 (0.39-0.51)	0.51 (0.45-0.57)	0.03 (0.01-0.06)	0.02 (0-0.04)	36 (24-113)	14 (8 - 22)	6 (4 -9)	29 (22-38)	30 (25-44)
Smallholder	0.04 (0.02-0.09)	0.56 (0.48-0.65)	0.27 (0.2-0.35)	0.12 (0.07-0.19)	24 (8 - 60)	7 (5 - 12)	5 (4 -7)	28 (20 - 44.5)	30 (23-46)

3.4.2 Livestock numbers, species and breeds

Across the study area, the surveyed households owned a median of 35 cattle (IQR: 17-100) and 36 small ruminants (IQR 12-80). Rural smallholder households had smaller numbers of cattle in their herds compared to the agropastoral and pastoral systems. Pastoralists reported the largest herds, in terms of both cattle and small ruminants. Agropastoralists owned relatively fewer small ruminants compared to cattle (Table 3.2).

Table 3.2: Livestock numbers in households in the three management systems.

Livestock practice	Total households that answered	Species	Mean	Median (IQR)
Agropastoral	97	Cattle	82	35 (17 - 80)
		Small ruminants	37	26 (7 - 51)
Pastoral	52	Cattle	166	107 (31.5 - 203.75)
		Small ruminants	272	113 (68.25 - 295.5)
Rural-smallholder	23	Cattle	15	16 (11 - 19)
		Small ruminants	32	26 (19.5 - 37)

Local breed livestock predominated in study households. Agropastoral livestock contained only 0.1% (CI: 0-0.8%) exotic breeds and pastoral livestock contained 0.4% (0.1-0.9%). A greater proportion of rural smallholder livestock consisted of foreign breeds (32.4%, CI: 28.9-36%).

3.4.3 Income sources

Sales of livestock, crops and milk were listed as the three main sources of income amongst the surveyed households (Table 3.3).

Table 3.3: Reported sources of income in survey households.

Income	Total answered out of 101	Percentage (95% CI)
Livestock sales	100	99 (94.6 - 100)
Crops	95	81.1 (71.7 - 88.4)
Milk sales	96	80.2 (70.8 - 87.6)
Food relief	87	63.2 (52.2 - 73.3)
Other livestock income	86	38.4 (28.1 - 49.5)
Honey	80	12.5 (6.2 - 21.8)
Wildlife	81	9.9 (4.4 - 18.5)
Off farm employment	79	7.6 (2.8 - 15.8)

Reported income sources were similar across the management systems. More rural smallholders (100% of households (CI: 84.6-100%)) reported milk sales compared to agropastoralists (77.5%, CI: 60.8 – 89.9%) and pastoralists (71.5%, CI: 54.1-84.6%). Smallholders also reported more wildlife-related income (25.0%, CI: 7.2-52.3%) compared to agropastoralists (2.7%, CI: 0 – 14.1%)) or pastoralists (10.7%, CI: 2.2 – 28.2%). Fewer agropastoralists (29.7%, CI: 15.9 – 50.0%) availed of food relief compared to smallholders (88.2%, CI: 63.6-98.5%) and pastoralists (87.9%, CI: 71.8 – 96.6%).

3.4.4 Uses of livestock

Cattle and small ruminant uses reported by households in the three management practices are summarised in Figures 3.1 and 3.2 and Table 3.4. The majority of respondents used their cattle and goats for milk and meat (Table 3.4). Outside of pastoral settings, sheep were less commonly used for milk, but were also used for meat (Figure 3.2). Very few agropastoralists reported using small-ruminants for milk (Figure 3.2). Rural smallholders used goats more commonly than sheep for milk and a high proportion of pastoralists reported using both goats and sheep for milk (Figure 3.2). A high proportion of respondents also used their cattle to pull ploughs and carts (Table 3.4). Sales of all species of livestock to release cash were reported by large proportions of households in all three livestock practices (Table 3.4). Other livestock uses included use of their skins and manure and as presents and offerings.

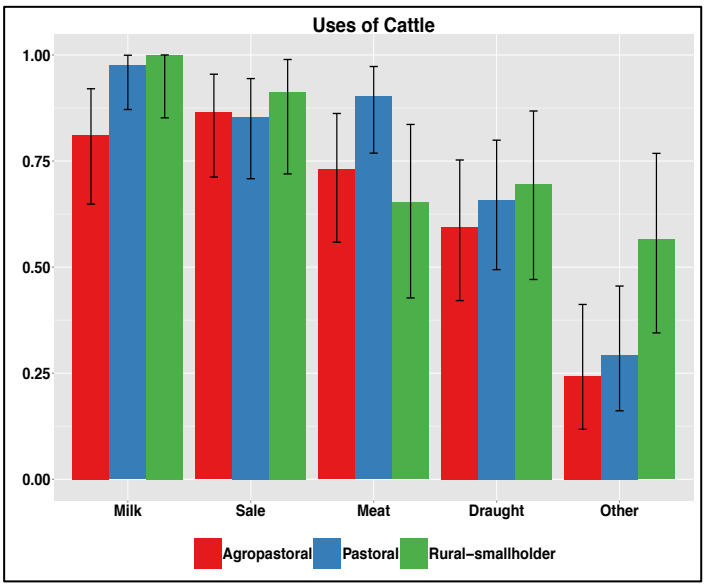


Figure 3.1: Comparison of the uses of cattle in the three management systems investigated.
The y-axis represents the proportion of respondents answering "yes" to the question of whether they used their cattle for the purpose shown on the x axis. Bars represent 95% confidence intervals.

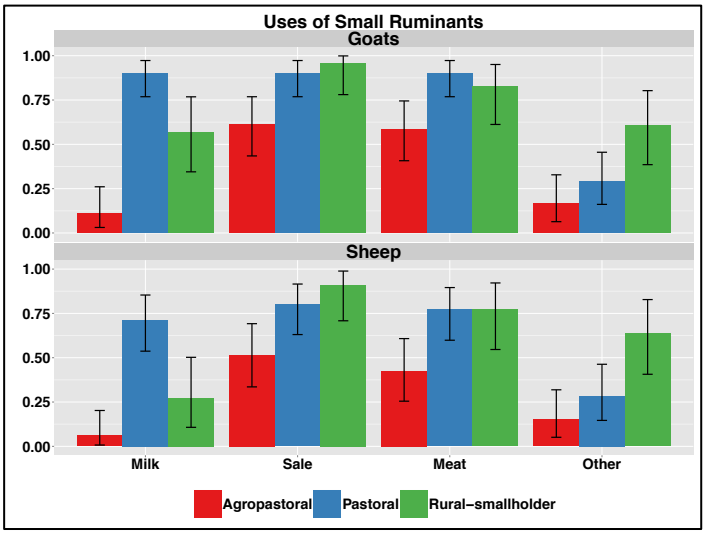


Figure 3.2: Comparison of the uses of small ruminants in the three management systems investigated.
The y-axis represents the proportion of respondents answering "yes" to the question of whether they used their goats or sheep for the purpose shown on the x axis. Bars represent 95% confidence intervals.

Table 3.4: Uses of livestock by households in the study area. These data are shown for the different livestock management systems in Figures 3.1 and 3.2.

	Total respondents	Livestock use	Percentage respondents (95% CI)
Cattle	101	Milk	92.1 (85 - 96.5)
		Sale	87.1 (79 - 93)
		Meat	78.2 (68.9 - 85.8)
		Draught	64.4 (54.2 - 73.6)
		Other	33.7 (24.6 - 43.8)
Goats	100	Milk	54 (43.7 - 64)
		Sale	81 (71.9 - 88.2)
		Meat	77 (67.5 - 84.8)
		Other	32 (23 - 42.1)
Sheep	90	Milk	36.7 (26.8 - 47.5)
		Sale	72.2 (61.8 - 81.1)
		Meat	64.4 (53.7 - 74.3)
		Other	32.2 (22.8 - 42.9)

3.4.5 Households' consumption of animal products

Respondents reported that most of the milk and eggs that they consumed were produced at home, and fuel for cooking was also gathered near home (Table 3.5). However, beef and other meats were purchased the majority of the time. Maize, rice and beans predominated in the "other food" category of purchased food on Table 3.5. Agropastoral households reported eating meat a median of once a week (IQR once to twice per week). Pastoralists in Simanjiro and Monduli also eat meat a median of once per week (IQR once to three times per week). However, no pastoral household in Loliondo reported eating meat. Their reported diet constituted of milk and maize. Rural smallholders eat meat a median of twice per week (IQR once to twice per week).

Table 3.5: Food consumption reported by questionnaire respondents.

Food	Number of respondents who answered (out of 101)	Mean percentage reported by respondents		
		Produced at home	Purchased	Not specified
Beef	51	28.9	69.5	1.6
Other Meat	17	40.6	59.4	0.0
Cow milk	40	90.5	9.5	0.0
Goat milk	12	100.0	0.0	0.0
Sheep milk	10	100.0	0.0	0.0
Eggs	18	82.2	12.2	5.6
Fuel for cooking	18	88.8	5.7	5.6
Other food	38	25.3	72.4	2.4

3.4.6 FMD in the study area

FMD seroprevalence

Of the 2738 sera from the cross-sectional study, 59.0% (CI: 57.1-66.1%) were seropositive for antibodies against FMDV NSP. A higher proportion of cattle (69.0%, CI: 66.5 – 71.4%) were seropositive compared to small ruminants (48.5%, CI: 45.7-51.3%). Higher proportions of livestock belonging to agro-pastoralists (67.2%, CI: 63.6-70.7%) and pastoralists (65.5%, 63.2-68.4%) were seropositive compared to livestock belonging to smallholders (37.1%, 33.5-40.9%). FMD seroprevalence by species in the three production systems is shown in Figure 3.3.

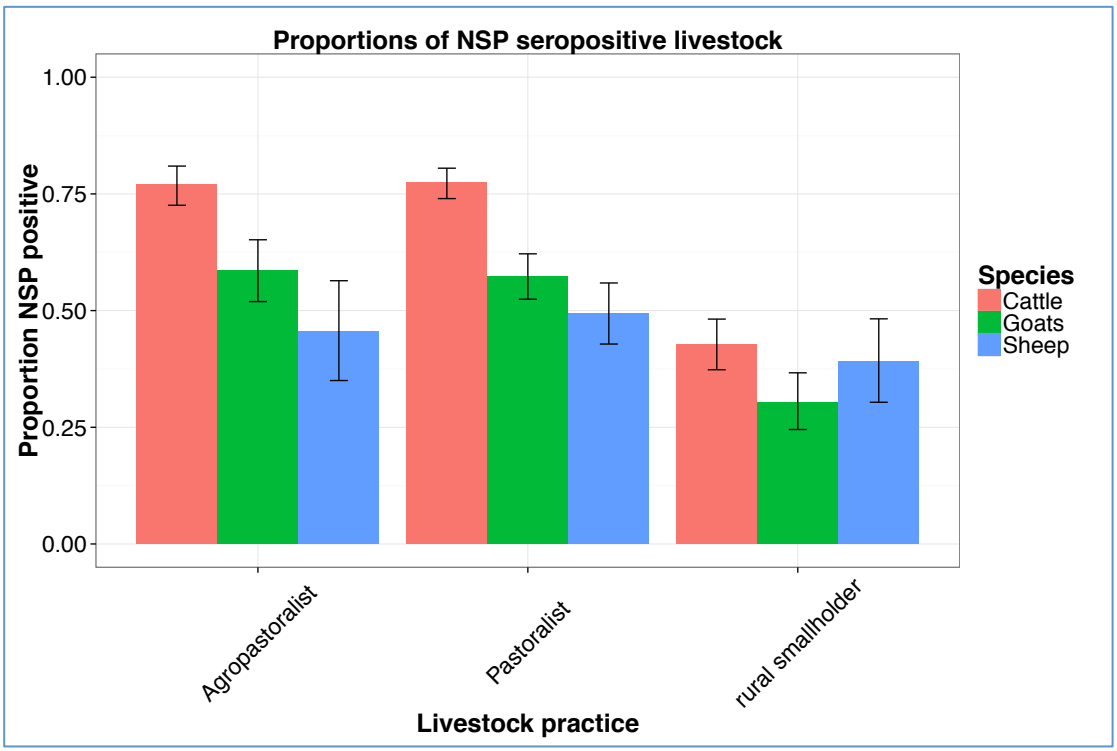


Figure 3.3: Proportions of cattle, goats and sheep belonging to agropastoralists, pastoralists and rural smallholders that tested positive for FMDV non-structural protein (NSP) antibodies.
Bars represent 95% confidence intervals.

Frequency of FMD outbreaks reported by households

The majority of households from the cross-sectional study (80.5% CI: 70.3-88.4%) reported that they had an FMD outbreak in their herd at some point and 67.1% (CI: 55.8-77.1%) reported an outbreak in the previous year. Greater proportions of agropastoralists and pastoralists reported outbreaks compared to rural smallholders (Figure 3.4). In 81.8% [64.5-93.0%] of pastoral and 80.0% [56.3-94.2%] of agro-pastoral households, at least one FMD outbreak was reported in the past year. Of the herd owners reporting FMD outbreaks in the past year, 39.5% [25.0-56.5%] reported two or more outbreaks, and 25.6% [13.5-41.2%] three or more.

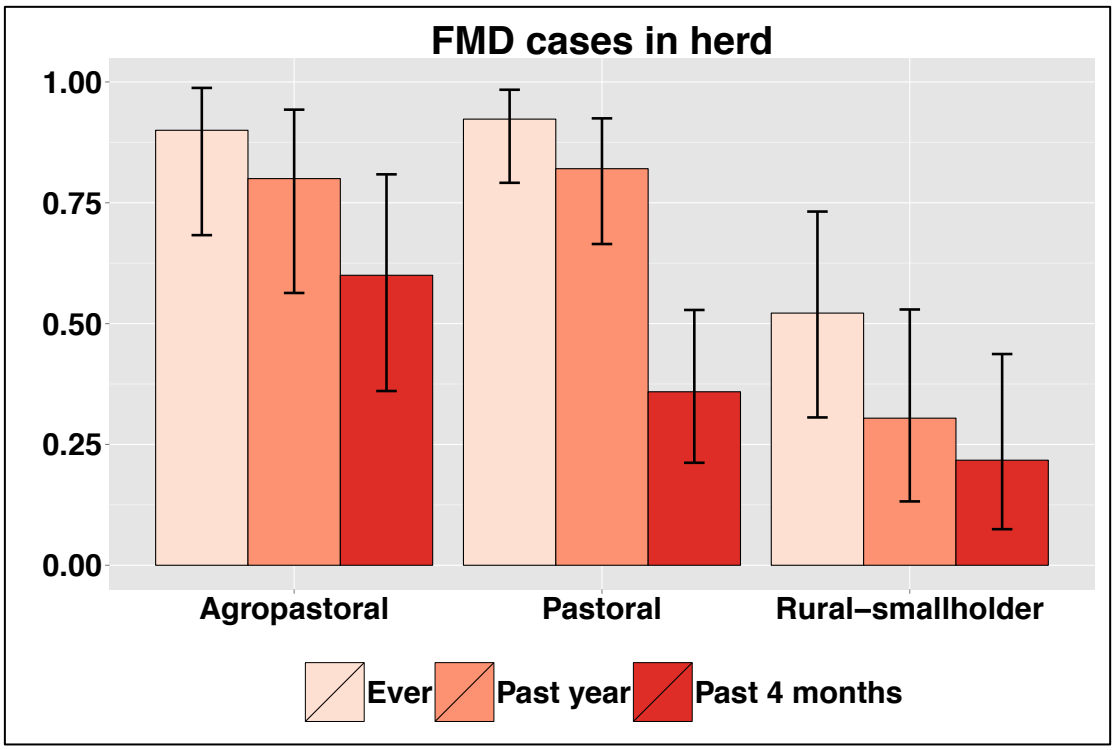


Figure 3.4: FMD outbreaks reported by households in the three management systems investigated.
Bars represent 95% confidence intervals.

Owner reports of FMD outbreaks ever and in the past year helped explain the variation in seroprevalence levels (Table 3.6). When the outcome variable (seropositive or not) was removed, and model inferences based on the coefficients for outbreaks reported were generated, inferences equalled true observations in 65.8% of comparisons (Kappa = 0.28, fair agreement) for outbreaks reported ever. For outbreaks reported in the past year, inferences equalled observations 64.1% of the time (Kappa = 0.25, fair agreement). In contrast, reports of FMD outbreaks in the past four months did not explain FMD seroprevalence (Table 3.6). The effects of animal age and species on the likelihood of livestock seropositivity are described in Chapter 4.

Table 3.6: FMD history as an explanatory variable for FMDV exposure in livestock. Likelihood ratio testing results for a generalised linear mixed model using household reported.

Explanatory variable dropped (whilst maintaining age, species and random effect of herd)	Difference in AIC	Likelihood ratio test X^2	p	Estimate (95% CI)	Odds Ratio (95% CI)
FMD in your herd ever?	-14.7	16.7	<10 ^ -4	1.8 (1-2.7)	6.1 (2.6-14.3)
FMD in your herd in past year?	-9.6	11.7	< 10 ^ -3	1.3 (0.6-2)	3.6 (1.7-7.4)
FMD in your herd in past four months?	0.3	1.7	0.19	0.5 (-0.2-1.2)	1.6 (0.8-3.3)

Active outbreak surveillance further supported the households' reports of frequent FMD outbreaks. For example, a longitudinally tracked herd in the pastoral area was observed to suffer four FMD outbreaks over three years. The minimum interval between the outbreaks in this herd was four months and the maximum was eleven months. In the agropastoral area, fifteen herds were tracked through serial FMD outbreaks (Figure 3.5). Of these, three herds were observed to suffer four outbreaks over the course of two and a half years. A further herd suffered three outbreaks. Eight herds had multiple outbreaks confirmed by virus isolation at the WRL (Figure 3.5).

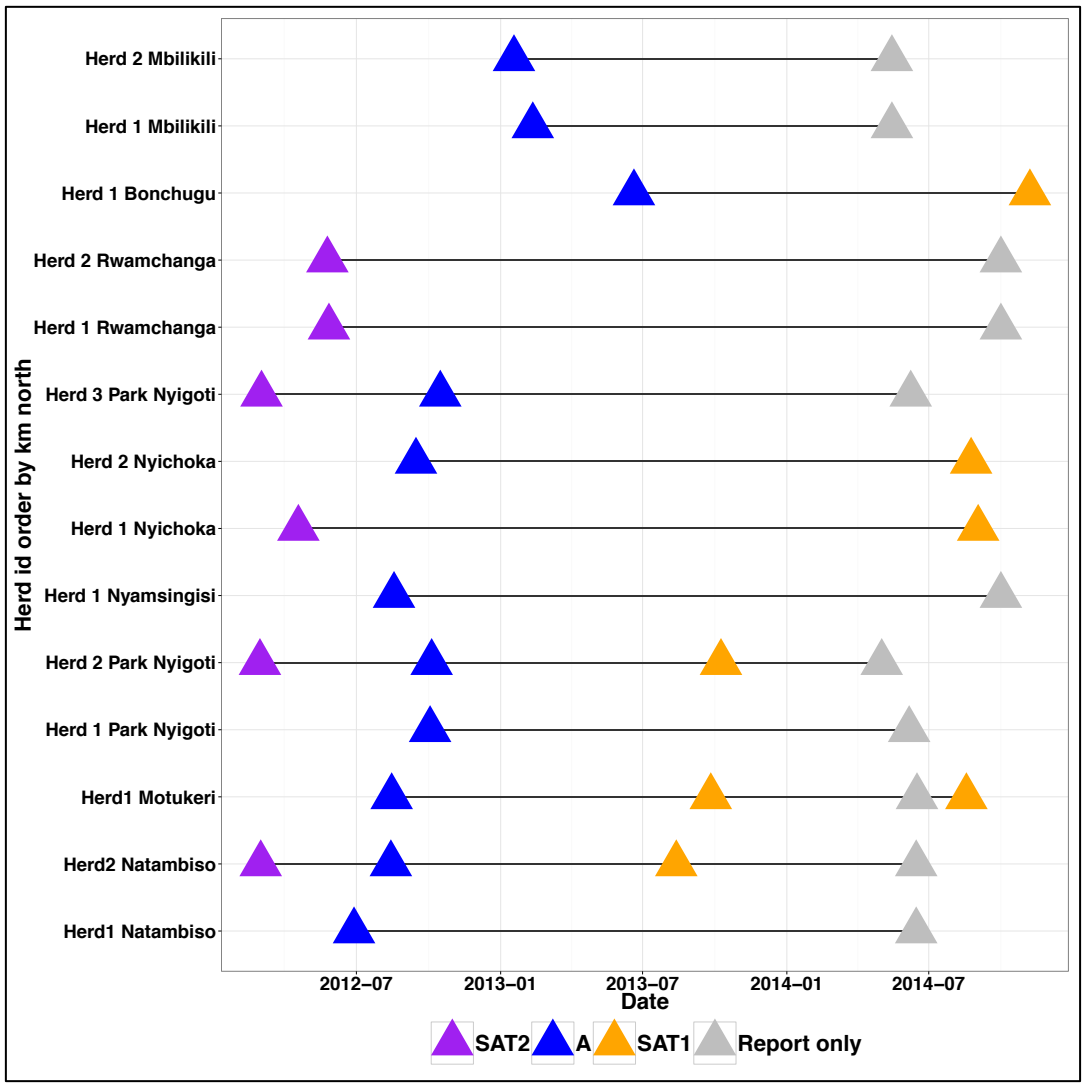


Figure 3.5: Outbreak reports and virus-typing results in fourteen herds followed through serial FMD outbreaks in Serengeti district.
Km = kilometres

Seasonality of reported FMD outbreaks

There were 170 outbreak reports from 90 different households (43 agropastoral, 41 pastoral and 6 smallholder) available for investigation of outbreak seasonality. Due to low numbers, smallholder households were excluded from analyses. The interaction between livestock practice and season explained some of the variation in reported outbreak occurrence, with pastoralists reporting more outbreaks in the rainy season, and agropastoralists reporting more in the dry season (Table 3.7 and Figures 3.6 and 3.7). (Null deviance: 195.2 on 78 degrees of freedom, residual deviance: 167.2 on 75 degrees of freedom)

Table 3.7: the effect of season on the likelihood of an FMD outbreak being reported. *For easier interpretation, coefficients and odds ratios are reported from separate models for pastoralists and agropastoralists rather than from the model with interactions.

Variable	Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds Ratio (95% CI)
Interaction between season and livestock practice	-25.37	27.4	1.7 x 10 ^ -7		
Wet season compared to dry season for agropastoral*				-0.7 (-1.1 - -0.3)	0.5 (0.3-0.8)
Wet season compared to dry season for pastoral*				1.3 (0.6-2)	3.7 (1.8-7.6)

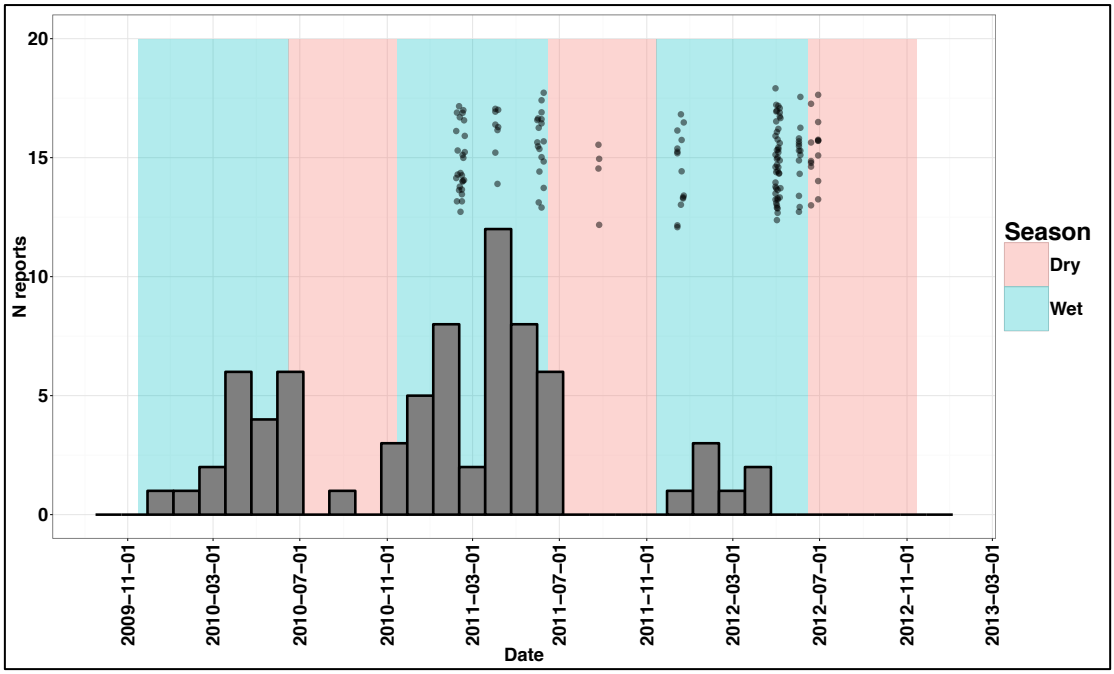


Figure 3.6: A histogram showing when pastoralists reported FMD outbreaks in their area.
The superimposed vertically jittered points represent the questionnaire dates relevant to the pastoral outbreak reports. The background red colouring represents the dry season from 15th of June to 15th of November. The green colouring represents a period with more precipitation from the 15th of November to the 15th of June.

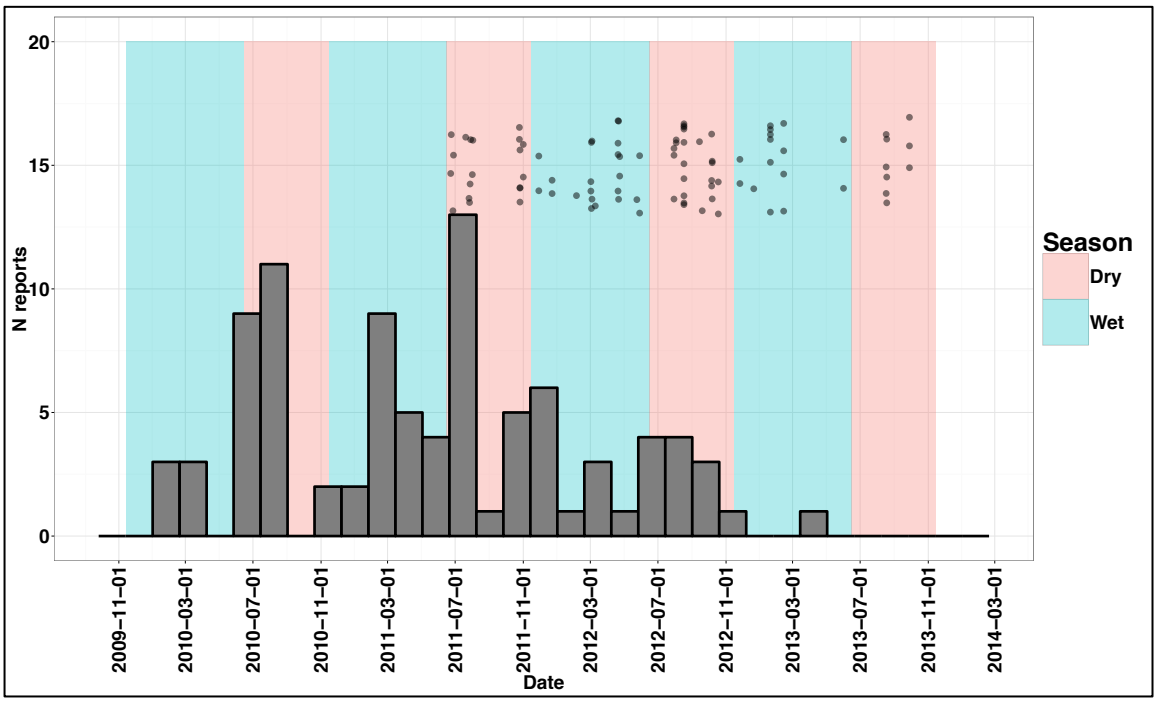


Figure 3.7: A histogram showing when agropastoralists reported FMD outbreaks in their area.
The superimposed vertically jittered points represent the questionnaire dates relevant to the agropastoral outbreak reports. The background red colouring represents the dry season from 15th of June to 15th of November. The green colouring represents a period with more precipitation from the 15th of November to the 15th of June.

In contrast to the model of reported outbreaks, there was no observed outbreak seasonality during active surveillance in Serengeti district (Figure 3.8). The ratio of months classified as dry (mid June to mid November) to wetter months (mid November to mid June) was 1.4, and the ratio of 32 outbreaks in dry months and 22 outbreaks in wetter months was 1.46. There were no active outbreak surveillance data available from pastoral areas. However, of the 14 outbreaks in pastoral areas that were reported and sampled, 11 occurred between March and June (wetter season) and 3 occurred in July (dry season).

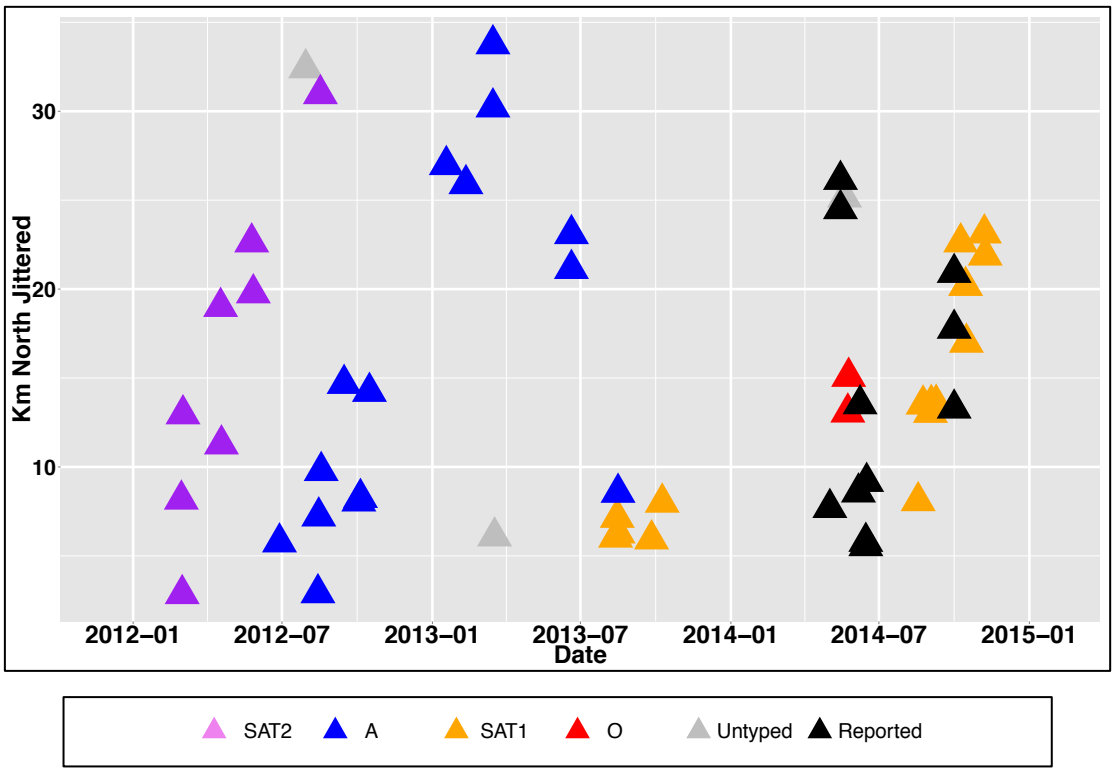


Figure 3.8: Outbreaks detected through active surveillance in Serengeti district. The colours represent virus isolation and typing results and also where outbreaks were visited or reported but no virus typing results were generated.

3.4.7 Morbidity and mortality due to FMD

Drivers of livestock morbidity in FMD outbreaks

The median of the morbidities reported by each individual household surveyed (N = 118) was 42.9% (IQR: 21.9-68.8%) for cattle and 10.2% (IQR: 0 – 56.6%) for small ruminants, highlighting large variation in reported morbidity levels. Reported morbidity in the most recent FMD outbreak did not explain FMDV seroprevalence in the cross-sectional study herds (Table 3.8).

Table 3.8: Morbidity is a poor explanatory variable for FMD seropositivity in livestock.

Explanatory variable dropped (whilst maintaining age and random effect of herd)	Difference in AIC	LRT X^2	p
Reported cattle morbidity	-0.86	1.14	0.286
Reported small ruminant morbidity	-1.35	0.61	0.434

Livestock practice and herd size explained a small amount of the variation in cattle morbidity. Pastoralists reported higher morbidity (Median 67.7% [IQR: 33.5 – 95.4%]) compared to agropastoralists (Median 50.0% [IQR: 13.2 – 100.00%]) and households with larger herds reported lower morbidity (Table 3.9). However, much variation in morbidity remained unexplained (Deviance with random effects and intercept only: 956 on 112 degrees of freedom, residual deviance: 941 on 110 degrees of freedom).

Table 3.9: Variables explaining some of the variation in reported cattle morbidity using all available morbidity data.

Variable	Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds ratio (95% CI)
Livestock practice	-9.08	11.08	0.0009		
Pastoral compared to agropastoral				1.5 (0.6-2.3)	4.3 (1.9-10.0)
Herd size	-3.3	5.3	0.021		
Per extra ten cattle				-0.028 (-0.052--0.005)	0.972 (0.950-0.995)

Livestock practice had a large effect on reported small ruminant morbidity (Table 3.10), with agropastoralists reporting lower morbidity in their small ruminants (Median 0%, IQR: 0.0 -28.7%), compared to pastoralists (Median 50.0, IQR: 13.2 – 100.0%). Similarly to the cattle morbidity model, the small ruminant morbidity model failed to explain much of the variation in the data (Deviance with random effects and intercept only: 466.9 on 88 degrees of freedom, residual deviance: 441.8 on 86 degrees of freedom).

Table 3.10: Variables explaining some of the variation in reported small ruminant morbidity using all available morbidity data.

Variable	Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds ratio (95% CI)
Livestock practice	-23.76	25.08	5.5 x 10 ^-7		
Pastoral compared to agropastoral				8.42 (4.161-12.692)	4565.4 (64.1-325093.6)
Herd size	-5	6.3	0.01		
Per extra ten small ruminants				-0.12 (-0.206--0.034)	0.887 (0.814-0.967)

In order to increase the likelihood of accurate estimates for morbidity, only data from the outbreak visits, and not from the cross-sectional study were used for further analyses. However, even when only the 41 herds that underwent outbreak investigations were considered, there was large variation in reported morbidity (IQR for cattle 21.9-35.8%, for small ruminants 0-50%).

Both cattle morbidity and virus isolation data were available for 31 outbreaks (24 agropastoral and 7 pastoral). Herd size and outbreak serotype explained a small of the variability reported for both cattle (Table 3.11) and small ruminants (Table 3.12), whereas livestock practice and season did not help explain cattle morbidity levels. However, similarly to the morbidity model with the larger dataset, much variation in morbidity remained unexplained (Deviance with random effects and intercept only: 306.5 on 29 degrees of freedom, residual deviance: 290.7 on 26 degrees of freedom).

Table 3.11: Variables explaining variation in cattle morbidity using data from herds with virus isolation and typing.

Variable		Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds ratio (95% CI)
Serotype		-5.96	9.96	0.0068		
	SAT1 relative to A				0.39 (0.14-0.64)	1.48 (1.16-1.90)
	SAT2 relative to A				-0.103 (-0.536-0.329)	0.90 (0.59-1.39)
Herd size		-3.93	5.93	0.0149		
	Per extra ten cattle				-0.046 (-0.083--0.01)	0.955 (0.92-0.99)

Small ruminant morbidity and virus isolation data were available for 16 outbreaks (9 agropastoral and 7 pastoral). For the sample available, livestock practice, herd size, season or outbreak serotype did not help explain the variation in morbidity.

Morbidity levels in different species

Lactating cows had the highest reported levels of morbidity, followed by other types of cattle (Figure 3.9).

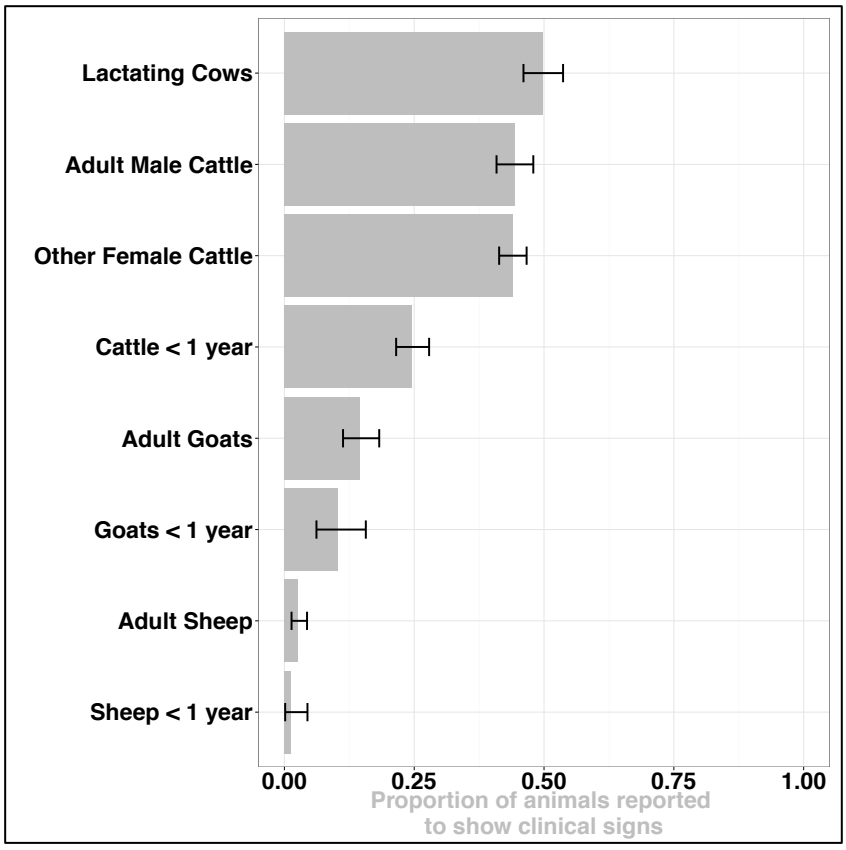


Figure 3.9: Proportion of animals that households reported to show clinical signs of FMD in each species and age group.

The most commonly reported clinical signs of FMD in livestock were foot and mouth lesions, salivation, anorexia and depression. These signs were reported by over 90% of households that had outbreaks. Over 85% reported lameness and weight loss. Milk loss in female cattle, goats and sheep was reported by 90.6%, 90.0% and 69.2% of households, respectively.

Of 36 (15 agropastoral and 21 pastoral) households in the cross-sectional study where information about abortions due to FMD was available, five (13.8%, one agropastoral and

four pastoral) reported animals aborting during FMD outbreaks. Two of these households reported abortions in cattle only and the other three reported abortions in both cattle and small ruminants. The proportion of cows in each herd reported to have aborted ranged from 0.3 to 19.2%. For small ruminants, the proportion ranged from 7.5 to 14.3%.

Of the 89 households in the cross-sectional and longitudinal studies that reported on long-term effects of FMD on their livestock, 22 households (24.7%) reported heat intolerance syndrome in one or more of their livestock.

Clinical signs recorded during veterinary examination

Individual animal data from clinical examinations by veterinarians on the field team were available for 238 animals with FMD lesions from 19 different herds in the outbreak tracking study (11 agropastoral and 8 pastoral). Of the 238 animals, 90.8% had foot lesions and 88.2% had mouth lesions. Of 37 cows over three years that were in their lactation period, four (10.8%) had FMD lesions on their udders. Detailed data on clinical signs were available for 213 of the 238 animals examined for lesions. These are summarised in Table 3.12.

Table 3.12: Clinical signs recorded in animals with FMD lesions that were examined in detail.

Clinical Sign	Number (%) affected
Weight loss	148 (69.5 %)
Lameness	136 (63.8 %)
Anorexia	130 (61 %)
Depression	117 (54.9 %)
Salivation	111 (51.9 %)
Heat intolerance	33 (15.5 %)
Diarrhoea	19 (8.9 %)
Loss of milk (Lactating cows > 3 years old, N=37)	26 (70.3%)
Abortion (Cows in calf > 3 years old, N=36)	1 (2.8%)

Overall, levels of reported livestock mortalities due to FMD were low (median and IQR of 0% for all species). Out of 118 households (84 agropastoral and 34 pastoral) that reported on livestock morbidity and mortality due to FMD, 29 (24.6%) reported that animals in

their herd died due to FMD. There were 23 agropastoral and six pastoral households amongst these.

There were 25 households that reported bovine mortalities due to FMD. Of these, 19 households reported mortalities below 5%. A further three households reported bovine mortalities between 5% and 10%. Two households reported bovine mortalities between 10% and 20%. Only one agropastoral household reported mortality in cattle of 36.5%. Eleven households reported small ruminant mortalities. Of these 3, 6 and 9 had mortalities below 5%, 10% and 20% respectively. Two agropastoral households had small ruminant mortalities above 40% (Figure 3.10). One of these was the same household that reported the high cattle mortalities. The majority of small ruminants that died during outbreaks were juveniles, with 100% mortality in this group in two households. Six of the herds with mortalities had virus isolation data. Three of these had outbreaks caused by SAT2, two by SAT1 and one by serotype A.

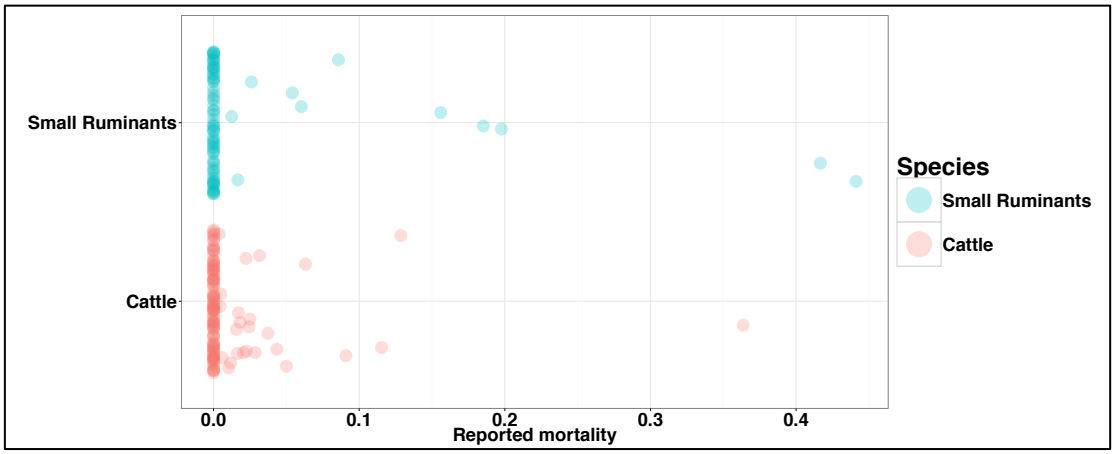


Figure 3.10: Reported livestock mortality due to FMD in 118 households.

Reports of human illness during FMD outbreaks

Of the 88 households that commented on whether or not they perceived people to become ill from FMD during outbreaks in livestock, nine (10.2%) pastoral households reported symptoms in household members that they believed were caused by FMD. Fourteen households (15.9%) reported symptoms in people outside of the household that they attributed to FMD. These households included 11 pastoralist households, two agropastoral and one rural smallholder household. Reported symptoms in people included lesions in the

mouth, on the lips and in the nose, coughing, sneezing, headache, fever and muscle pain. Of 97 respondents commenting on whether they believed people got FMD, 23 (23.7%) believed that people could become infected. When asked how people contracted FMD, ten respondents attributed it to drinking milk and thirteen were unsure. Nineteen respondents of the 23 listed similar clinical signs in people as above and four did not list symptoms.

3.4.8 Impacts of FMD on production

Reduction in milk yield

Agropastoral households quantified milk yield from cattle only (n=47 households), rural smallholders reported milk yield from cattle (n=20) and goats (n=3), and pastoralists reported about cattle (n=47), goats (n=17) and sheep (n=4). Cow milk yield in absence of FMD reported by households in the study is shown in Figure 3.11 and Table 3.13. Agropastoral and pastoral cows yielded significantly less milk compared to rural smallholder cows (Figure 3.11, Tables 3.13 and 3.14).

Decreased cow milk during FMD outbreaks was reported by 90% (CI: 83.5-94.6%) of households. Decreased goat milk was reported by 66% (CI: 51.2-78.8%). Cow milk yield during FMD outbreaks was significantly lower than normal (Paired t-test: $t = 6.8$, $p = 7.3 \times 10^{-9}$, degrees of freedom =54). Goat milk yield data with and without FMD were only available for five pastoral herds, but a decrease in goat milk during outbreaks was suggested (Table 3.13).

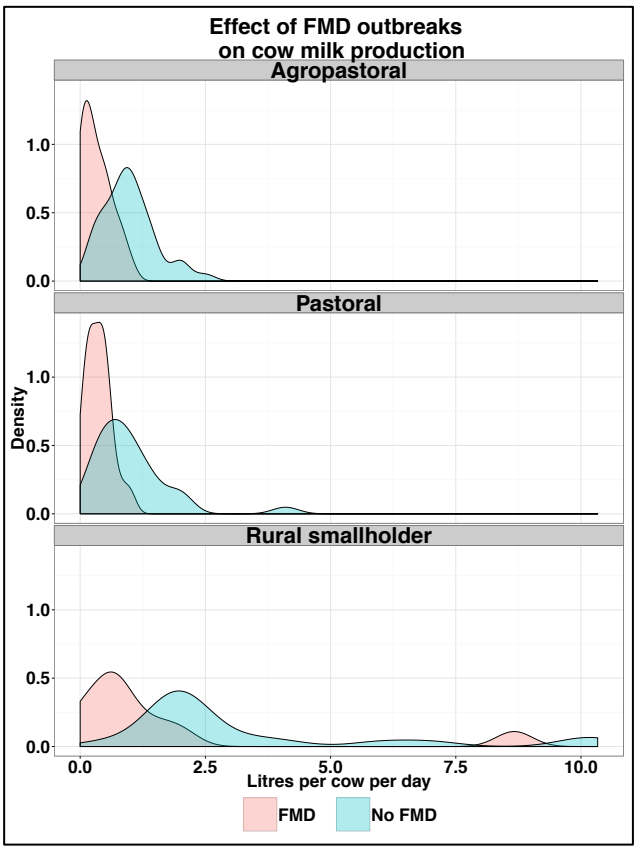


Figure 3.11: Density plots showing reported cow milk production in the three different management systems with (red) and without (green) FMD.

Table 3.13: A summary of cattle, goat and sheep milk yields with and without foot-and-mouth disease.
Where the same herd reported milk yields with and without FMD, FMD milk yield was expressed as a proportion of normal milk yield at herd level and then summarised for all herds with these data available. No data were available for sheep milk yield with FMD.

Summary of milk production (Median and interquartile ranges of litres per animal per day)							
	Cattle			Goats			Sheep
	Normal	During FMD	Herd level FMD/Normal	Normal	During FMD	Matched herd level FMD/Normal	Normal
Agropastoral	1, 0.65 - 1.25	0.25, 0.08 - 0.5	0.30, 0.08 - 0.52	NA	NA	NA	NA
Pastoral	0.83, 0.50 - 1.27	0.37, 0.16 - 0.50	0.35, 0.33 - 0.50	0.14, 0.12 - 0.20	0.07, 0.04 - 0.11	0.40, 0.29 - 0.54	0.13, 0.08 - 0.20
Rural smallholder	2.00, 1.94 - 3.5	0.67, 0.50 – 1.00	0.21, 0.17 - 0.27	0.00, 0.00 - 0.50	NA	NA	NA

Table 3.14: The results from a general linear model explaining milk per cow with livestock practice.
(Null deviance: 157.9 on 54 degrees of freedom,
Residual deviance: 93.3 on 52 degrees of freedom)

Variable	Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds Ratio (95% CI)
Livestock practice	-24.93	28.9	5.2 x 10 ^-7		
Agropastoral compared to rural smallholder				-2.9 (-3.9--1.9)	0.1 (0-0.2)
Pastoral compared to rural smallholder				-3 (-4--1.9)	0.1 (0-0.1)

Drivers of milk loss

For the 55 herds with data available about milk per cow (at herd level) with and without FMD (Table 3.13), the following explanatory variables were examined: livestock practice, estimated milk yield and herd size. In addition to these variables, data about outbreak season and morbidity were available for 35 and 14 data points respectively.

Herd size, livestock practice, estimated litres of milk produced per animal and season of the outbreak did not help explain the variation in milk loss due to FMD. Reported

morbidity during the FMD outbreak helped explain a small amount of the variation in milk losses (Pseudo R-squared: 0.11) (Table 3.15).

Table 3.15: Results of GLMs explaining variation in milk loss due to FMD. N = number of, AIC = Akaike information criterion, LRT = likelihood ratio test, CI = confidence interval.

	Difference in AIC	LRT X^2	p	Estimate (95% CI)	Odds Ratio (95% CI)
				Negative = more milk loss	
Morbidity	-2.05	4.0457	0.04428		
Per extra unit (1/100) morbidity				-1.397 (-2.679--0.114)	0.247 (0.069-0.892)

Impact on milk sales and consumption

The majority (63.9%, CI: 53.5-73.5%) of households stopped selling milk during FMD outbreaks. Fewer households (24.7%, CI: 16.5 -34.5%) reported that they stopped consuming milk (Figure 3.12). There were similar patterns of cessation of milk sales and consumption during FMD outbreaks in all three management systems (Figure 3.12).

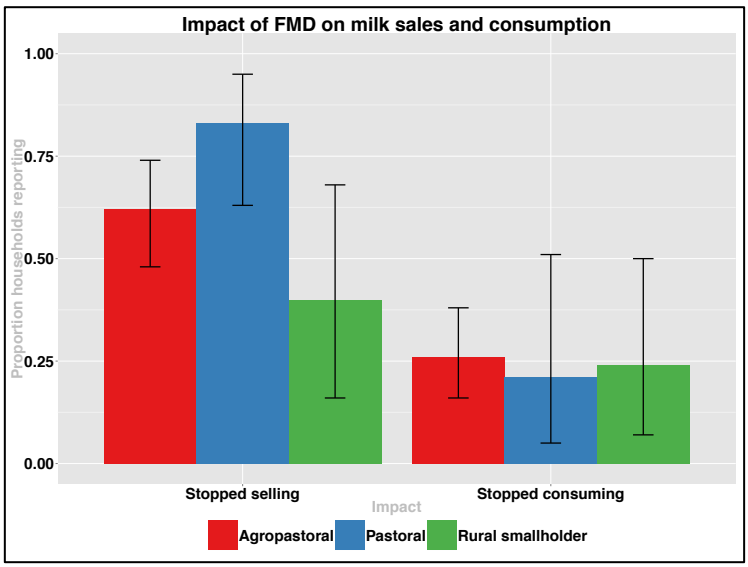


Figure 3.12: Proportions of households in the three management systems that stopped selling and consuming milk during FMD outbreaks.
Bars represent 95% confidence intervals.

Oxen's ability to pull carts and ploughs

Of the households that used cattle for draught purposes, 70.5% (CI: 61.2-78.8%) reported that their animals' ability to pull carts and ploughs was affected by FMD, whereas 65.7% reported that their crop production was affected (Figure 3.13). These trends were similar across the production systems (Figure 3.13).

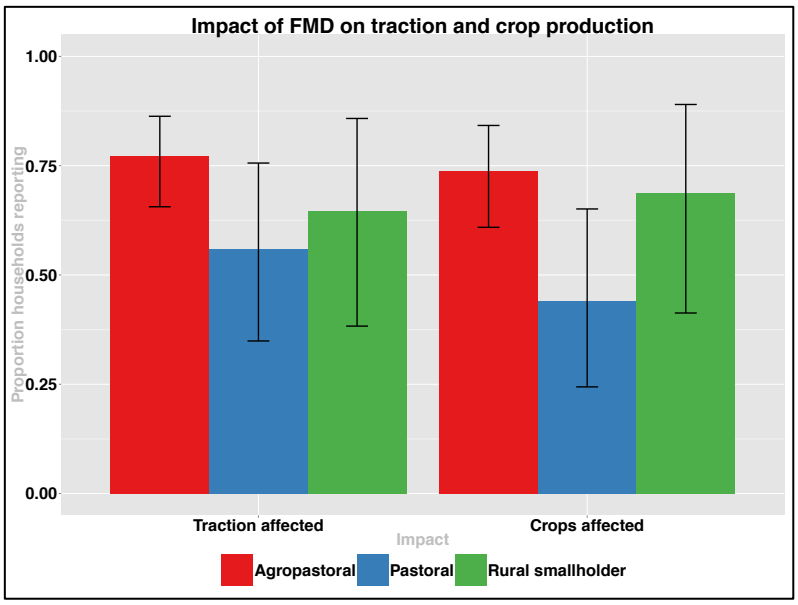


Figure 3.13: Proportions of households in the three management systems reporting impacts on the draught ability of their animals and on crop production due to FMD. Bars represent 95% confidence intervals.

Impact on livestock management and sales

Time spent tending to livestock was altered due to FMD in 83.8% (CI: 75.6-90.1%) of households, and the main reason for this was increased time spent looking after and treating sick animals (Figure 3.14).

Only 17.1% (CI: 10.8-25.2%) of households reported that they changed grazing and watering areas due to an FMD outbreak. Households changed their grazing and watering practices to avoid perceived disease risk and due to affected livestock's inability to walk longer distances. Households that did not change their practices indicated that this was due to a lack of access to alternative grazing or watering points.

In the case of alterations in livestock sales, 12% (CI: 6.9-19%) of households reported FMD to have an impact. The most frequently described reason for changing plans for selling livestock was because animals that were thin or sick due to FMD would be more difficult to sell and would fetch a lower price.

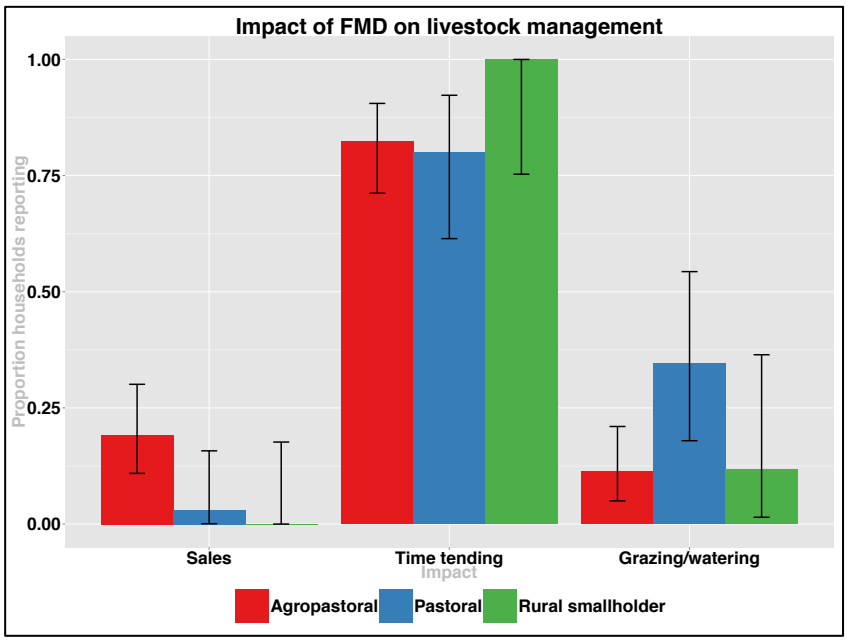


Figure 3.14: The proportion of households in the three management systems reporting impacts on animal sales, time tending to livestock and grazing and watering practices due to FMD.
Bars represent 95% confidence intervals.

Duration of FMD impacts

Information about the duration of FMD impacts was available from four agropastoral households in the outbreak follow-up study that had suffered serial FMD outbreaks. Duration of lameness due to FMD lesions was reported to be 1-2 weeks. However, draught animals were unable to pull carts or ploughs for 1-2 months after each FMD outbreak. Milk yield remained lower for 1-2 months after the outbreaks and it took the livestock 3 -4 months to regain weight lost during an FMD outbreak.

3.4.9 Perceived impact of FMD compared to other livestock diseases

Out of seven common livestock diseases investigated, agropastoralists ranked FMD as the most important disease (Figure 3.15). Pastoralists ranked it second after East Coast Fever (Figure 3.16) and rural smallholders ranked it third after anthrax/blackleg and East Coast Fever (Figure 3.17).

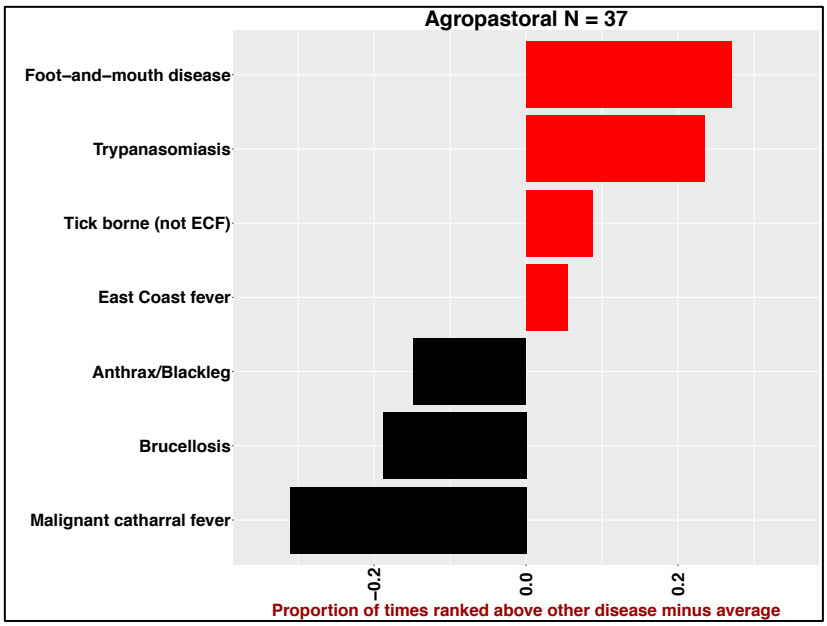


Figure 3.15: Impact of seven common livestock diseases as perceived by agropastoralists (n= 37) in the study area, measured by pairwise ranking.

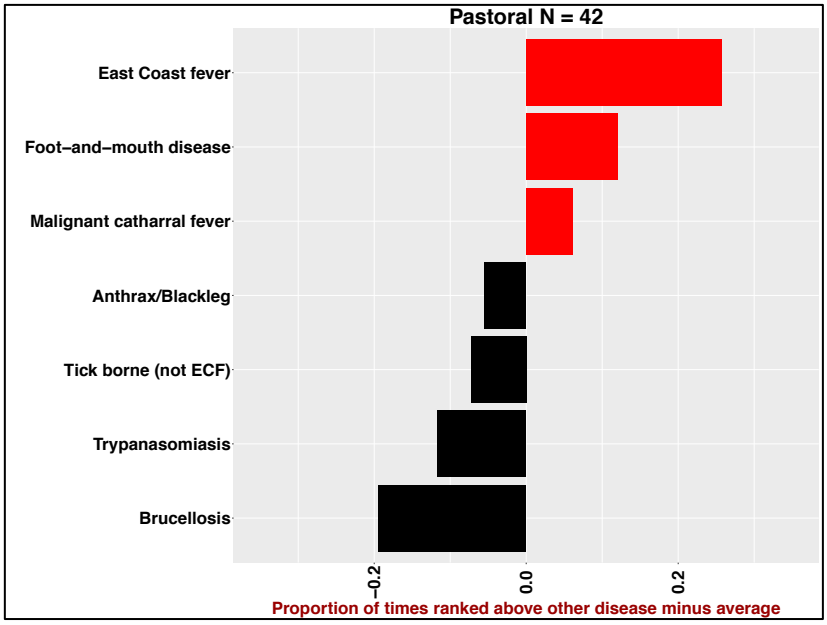


Figure 3.16: Impact of seven common livestock diseases as perceived by pastoralists (n= 42) in the study area, measured by pairwise ranking.

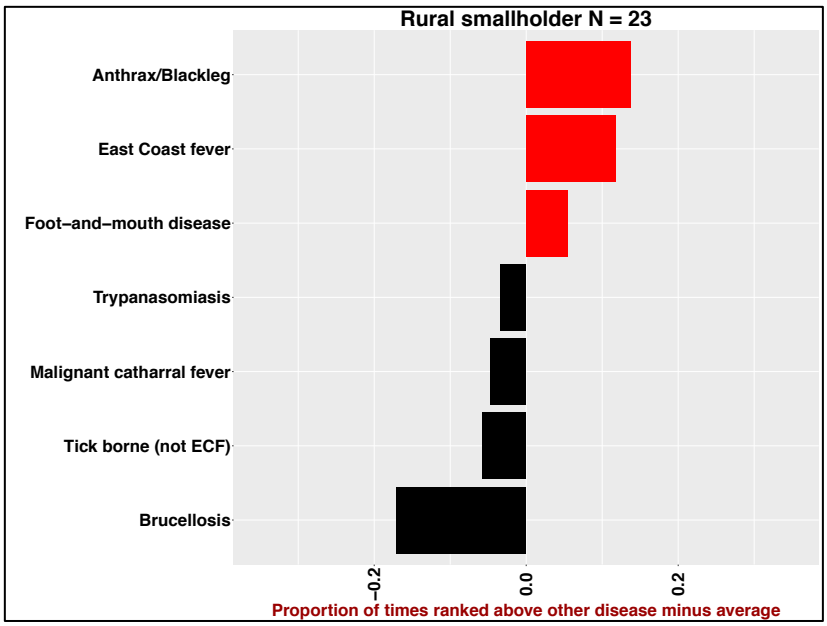


Figure 3.17: Impact of seven common livestock diseases as perceived by rural smallholders (n =23) in the study area, measured by pairwise ranking.

3.5 Discussion

This work addresses the impacts of endemic FMD on some of the poorest sections of society in a developing country. Until recently, this area has received scant coverage in the literature despite FMD affecting a large number of animals and the importance of livestock to rural livelihoods and food security in these countries (Knight-Jones & Rushton, 2013).

Key conclusions from this chapter are

a) Livestock play a vital role in the livelihoods of rural communities in northern Tanzania. In this study, livestock sales were the most important mechanism of cash release. The majority of households relied on milk from their livestock for daily consumption and as source of income. In addition, oxen were depended upon to pull ploughs and carts for crop production.
b) People in the study area were familiar with FMD in their livestock and their reports of frequent outbreaks were supported by findings from active surveillance and by FMD seroprevalence patterns.
c) Households in all three livestock practices in northern Tanzania reported a wide range of impacts on the productivity of their livestock due to FMD.
d) Consistently with the impacts that were quantified, FMD was perceived as one of

the most important livestock diseases in the region.

The serious impacts due to FMD on milk and crop production reported by the households in this study resonate with those of households in Ethiopia and South Sudan (Barasa *et al.*, 2008; Bayissa *et al.*, 2011; Jemberu *et al.*, 2014). The significant effect of FMD outbreaks on milk production and drought capacity are consistently reported in these studies. However, the seroprevalence of FMDV in cattle in the present study (69%) was three times higher than reported in an Ethiopian study (23%) (Bayissa *et al.*, 2011).

FMD has impacts on the emerging economy of intra-regional livestock trade that has the potential to empower the rural poor (Little, 2009). For example, weight loss in livestock due to FMD influences decisions about when to sell. Pastoral and agropastoral households reported durable (1 -2 month) impacts on crops, milk, livestock weight and draught capacity and multiple FMD outbreaks per year. This would explain why FMD is ranked as the most important livestock disease by agropastoralists and second only to East Coast Fever by pastoralists. Several other studies have similarly reported high ranking of FMD amongst livestock diseases of importance in East Africa (Bedelian *et al.*, 2007; Cleaveland *et al.*, 2001; Jost *et al.*, 2010; Ohaga *et al.*, 2007). As well as impacts due to acute FMD, almost a quarter of households reported one or more animals with chronic heat intolerance syndrome. This chronic condition has been previously documented in Tanzania and elsewhere in East Africa (Barasa *et al.*, 2008; Bayissa *et al.*, 2011; Catley *et al.*, 2004; Rufael *et al.*, 2008), and is reported to cause reduced milk yield and draught capacity and increased calving interval for the lifetime of affected livestock (Bayissa *et al.*, 2011). On top of production lossess, and consistently with other studies (Subramaniam *et al.*, 2013), the majority of livestock owners reported altered work patterns due to the care requirements of FMD infected livestock, contributing to FMD induced attrition of resources.

A strong similarity was evident between patterns suggested by household reports about FMD, and those detected by laboratory analyses and longitudinal studies. Seroprevalence patterns, observations of serial outbreaks in the same herds and clinical examinations by veterinarians were consistent with the households' reports of frequent outbreaks with high morbidity. The clinical signs reported by respondents were consistent with findings from veterinary examinations by the field team and correspond to other reports (Kitching & Hughes, 2002; Kitching, 2002). Species and district level reported morbidity, as well as

frequency of outbreaks were supported by laboratory data. Given that livestock may remain NSP seropositive for three years or more after an FMD infection (Elnekave *et al.*, 2015), it makes sense that FMD outbreaks ever or in the past year were better explanatory variables for seropositivity than FMD outbreaks in the past four months.

These consistencies between household reports and laboratory analyses increase confidence in conclusions based on household reports of FMD, including the very high variation in reported morbidity in this study. Furthermore, even when including only the herds that were clinically examined, the high variation in morbidity remained, and other studies in endemic countries have reported similarly high variability in morbidity (Gonzales *et al.*, 2014)(Klein *et al.*, 2008). Median herd level reported morbidity for cattle (42.9%) was lower than that reported for a European breed dairy herd in Kenya (62.1%) (Lyons et al., 2015) and for Ethiopian cattle (60.8-74.3%) (Jemberu *et al.*, 2014).

The variation in reported FMD morbidity within and between studies may be partially explained by subclinical infections. Early experimental studies recognised that when cattle were serially infected with different serotypes of FMD (as is likely to happen to northern Tanzanian cattle), clinical signs were milder in latter infections (Cottral & Gailunas, 1971). This may explain the lack of association between seropositivity and reported morbidity in this study. Subclinical FMD infections were also suggested in a study of FMD outbreaks in a partially immune population of Bolivian cattle where there were FMDV NSP positive results in cattle with no recorded clinical signs (Gonzales *et al.*, 2014). A recent study in Uganda also reported SAT1 infection in cattle in absence of any observed clinical signs (Dhikusooka *et al.*, 2016). Further studies are needed to investigate post-infection immunity to FMD and the proportion of infected animals that show clinical signs of FMD through serial outbreaks.

Similarly to this study's finding of higher reported morbidity in pastoral settings, an Ethiopian study also demonstrated that pastoralists reported higher morbidity in their livestock compared to crop-livestock mixed systems (Jemberu *et al.*, 2014). Pastoralists may have reported higher morbidity in their small ruminants because of their greater dependence on these species for milk, as evidenced by the livestock usage described in this study. Subsequently, they may have monitored the health of their sheep and goats more closely. The higher reported morbidity in both cattle and small ruminants could also be due

to pastoral livestock being more vulnerable to FMD due to the more challenging conditions which they live in, which could influence susceptibility to disease.

The finding that the SAT1 serotype caused outbreaks with higher morbidity levels than serotype A warrants further investigation over a longer period of time. More serotype A outbreaks occurred in the dry season, but even when season was investigated with a univariable model, it did not explain morbidity. Further, SAT1 outbreaks occurred in both wet and dry seasons, but still had the greatest positive effect on reported morbidity. Given the frequency of FMD outbreaks in the study area, herd immunity to the different serotypes may also have played a role in the relative morbidity. It is also possible that the serotype A virus was a less virulent virus than the SAT viruses that caused the outbreaks during this study.

The negative effect of herd size on reported morbidity could be due to the increased difficulty for the owners of large herds to examine every animal in detail. This could potentially result in under-reporting of animals with clinical signs in large herds. Further, in low morbidity outbreaks, a single animal observed with clinical signs would make up a larger proportion of a small herd compared to a large herd.

As well as differences in morbidity, this study also suggested a difference in the seasonality of outbreaks reported by pastoral and agropastoral respondents. Pastoral households reported more FMD outbreaks in the wetter months, and agropastoralists reported more in the dry months. However, as inferences were dependent on recollected outbreak dates, further active surveillance is necessary to endorse this idea. Gaining insight into the timing of FMD outbreaks is important to better understand their impacts as well as their epidemiology. For example, outbreaks during the “hunger gap” at the end of the dry season in South Sudan have maximal negative impact on human nutrition (Barasa *et al.*, 2008).

Like the agropastoral area in this study, a study in Ethiopia reported more outbreaks in the dry season and suggested this to be due to increased cattle movements in search of grazing and pasture (Rufael *et al.*, 2008). Both pastoralists and agropastoralists move their livestock in search of grazing and water in the dry season, increasing potential FMDV transmission opportunities. An earlier Tanzanian study in the areas surrounding Lake

Victoria indicated that more FMD outbreaks were reported between May and July (after the long rains) and in January and February (after the short rains) and attributed this to increased livestock movements (Genchwere *et al.*, 2014). In the relatively more arid pastoral areas, FMDV may be more vulnerable to desiccation during the dry season, and subsequently reduced transmission. FMDV is known to be more stable and to retain its infectivity for longer at higher humidity (Donaldson, 1973). This has been suggested as an explanation for increased wet season FMD outbreaks in India and Pakistan (Subramaniam *et al.*, 2013; Klein *et al.*, 2008).

Differences in pastoral livestock management practices throughout the year could also explain why they may have more FMD outbreaks during the wetter months. Wildebeest calving occurs in the pastoral areas of the study area between February and June. To avoid MCF associated with calving wildebeest, pastoralists move their cattle (Bedelian *et al.*, 2007; Cleaveland *et al.*, 2001; Lankester *et al.*, 2015b), and herds from multiple different areas congregate elsewhere. This movement and mixing of cattle during the wetter season could explain why pastoralists reported more FMD in the wetter season. In contrast, wildebeest do not go to the agropastoral area for calving, meaning that agropastoralists do not need to move their cattle during these months. Anecdotally, Simanjiro pastoralists report increased livestock diseases, including FMD, when cattle from different areas congregate on the hilly areas to avoid the wildebeest calving (Dr Ahmed Lugelo, personal communication). This raises the potential for an interplay between the impacts of FMD and MCF.

Unlike morbidity and seasonality, milk losses were similarly high in all three management systems in our study. Even in absence of FMD, the milk production reported compares poorly to what would be expected from native breed cattle (Kurwijila, 2001), especially in pastoral and agropastoral systems, and is on a different scale to what more intensively managed European breed cattle can produce in East Africa (Lyons *et al.*, 2015b). Rural smallholder livestock produced more milk than those in the pastoral or agropastoral areas in this study and this resonated with better child health status and food security reported for smallholders versus pastoralists in another study in the same area (Lawson *et al.*, 2014). Whilst smallholders owned fewer animals, they had a greater proportion of exotic breeds, higher milk yield per animal and were likely to benefit from milk sales in the adjacent Arusha urban area. This management group were also less affected by FMD. The lower

seroprevalence (37.1%) of FMD in smallholder livestock was consistent with the lower rank (third in importance) that smallholders attributed to this disease. Never the less, smallholders reported similar milk losses to the other systems when outbreaks occurred. Similarly to this study, a study in Ethiopia also reported higher FMD related impacts on pastoralists compared to smallholders (Jemberu *et al.*, 2014).

Pastoral livestock have low milk outputs but this is the management system that can least afford reduced milk yield due to FMD. Whilst many pastoral households reported some degree of crop production, this is on a small scale compared to smallholder or agropastoral systems (Tanzania Natural Resource Forum, 2011). Pastoralists were the only management system where some households milked their sheep as well as goats and cattle, adding evidence to the degree to which they rely on milk as a food source. None of the pastoralists in the Loliondo area reported eating meat at regular intervals, highlighting the role of milk as a vital source of protein in their diet. A study of child health in northern Tanzania similarly highlighted that pastoralists were most dependent on milk for protein and most vulnerable to food insecurity (Lawson *et al.*, 2014). This emphasises the severity of FMD's impact on this management system, as has been reported in other parts of East Africa (Barasa *et al.*, 2008; Bayissa *et al.*, 2011; Jemberu *et al.*, 2014; Rufael *et al.*, 2008), with potential repercussions for human nutrition. For example, milk reductions due to severe drought on child mortality have been clearly documented (Seaman *et al.*, 1978) and further studies are needed to investigate the association between FMD related milk decreases and human health. As well impacts on human nutrition, it is also likely that milk reduction may cause mortality in young animals during outbreaks, as has been reported in this study.

In addition to implications of FMD for human nutrition, pastoral households reported potential human infections due to FMD, and households in all three management systems were aware of this potential. It is known that humans can contract FMD from drinking the milk of infected animals (Bauer, 1997) and this is how the majority of respondents in this study believed that people contracted FMD. It is also possible that people succumb to respiratory infections secondary to nutritional stress during outbreaks or that they better recollect their own illnesses when their livestock are ill. Further studies are required to investigate the potential for FMD to cause disease in people in these study systems.

In conclusion, this study demonstrates significant impacts of FMD on traditional livestock keeping systems in northern Tanzania. People living in this region are already faced with high levels of poverty in a challenging environment of increasing human populations, decreased land availability and climate change (Upton, 2004). Their livelihood strategies show resilience, and optimal use of their livestock may represent a pathway out of poverty. Control of FMD would allow them to invest extra resources on pursuing this path. Livestock movement, whilst a risk factor for FMD, is integral to the pastoral way of life and allows maximum benefit to be derived from land as well as being the least harmful system to wildlife conservation (Castel, 2006; Nelson, 2012). Supporting FMD control in a way that supports traditional livestock keeping systems is therefore justified upon the grounds of conservation and sustainable land use as well as on a humanitarian basis.

Chapter 4: Risk factors for foot-and-mouth disease at the wildlife-livestock interface in northern Tanzania

4.1 Summary

Despite significant impacts of FMD on rural livelihoods, its epidemiology in endemic countries is poorly understood. In East Africa, elucidating the drivers of FMD transmission is complicated by the presence of large numbers of susceptible wildlife. Southern African studies have implicated buffalo (*Syncerus caffer*) as a reservoir of FMDV for livestock. However, in these settings FMD is tightly controlled in livestock, contrasting with endemic circulation in East African livestock. The veterinary fencing currently utilised in southern Africa to reduce potential disease transmission between wildlife and livestock could potentially be damaging to conservation and optimal land use in East Africa, where more environmentally sensitive management policies are used. In order to devise disease control strategies appropriate for the eastern African context, it is therefore essential to understand how important wildlife contact related risk factors are and whether wildlife play a role as a source of FMDV for livestock.

Cross-sectional (n=84 households) and case-control (n = 70 households) studies were used to collect data from livestock-keeping households in northern Tanzania about potential risk factors for FMD infection and outbreaks, respectively, including factors related to livestock management and wildlife contact. Serological evidence of FMDV infection in cross-sectional study livestock (n=2738) was measured using a commercial FMDV non-structural protein ELISA. For the case-control study, FMD outbreaks were confirmed at village level by virus isolation from FMDV lesion material at the WRL-FMD.

Older livestock were more likely to be seropositive for FMD (Odds Ratio [OR] 1.4 [95% CI: 1.4-1.5] per extra year) and cattle (OR 3.3 [95% CI: 2.7-4.0]) more than sheep and goats. In addition, livestock managed by agro-pastoralists (OR 8.1 [95% CI: 2.8-23.6]) or pastoralists (OR 7.1 [95% CI: 2.9-17.6]) were more likely to be seropositive compared to smallholders' livestock. Larger herds (OR: 1.02 [95% CI: 1.01-1.03] per extra bovine), and

those that recently acquired new livestock (OR: 5.57 [95% CI: 1.0 – 30.9]) had increased odds of suffering an FMD outbreak. Measures of potential contact with buffalo or with other FMD susceptible wildlife did not increase the likelihood of FMD in livestock.

Both approaches used in this study pointed towards livestock management rather than wildlife contact-related risk factors as being the dominant drivers of FMD epidemiology in northern Tanzania. Buffalo-to-livestock transmission is likely to be negligible compared to the manifold opportunities for livestock to act as sources of infection for other livestock.

4.2 Introduction

FMDV is amongst the top ten diseases constraining pro-poor growth (Perry & Rich, 2007). Household and country level impacts due to endemic FMD circulation are likely to be considerable (Knight-Jones & Rushton, 2013, Chapter 3). A better understanding and control of FMD in these settings would result in reductions in disease burden and therefore improve rural livelihoods and animal welfare. Yet, although FMDV was the first virus of animals to be discovered (Loeffler & Frosch, 1898), and epidemiology of FMD outbreaks in developed countries has been extensively studied (Boender *et al.*, 2010; Bouma *et al.*, 2003; Cottam *et al.*, 2008a, b; Gibbens & Wilesmith, 2002; Gibbens *et al.*, 2001; Haydon *et al.*, 2004), surprisingly little is known about the drivers of disease in endemic settings (Vosloo *et al.*, 2002b).

High prevalence of FMD has been reported in East (Ayebazibwe *et al.*, 2012; Genchwere *et al.*, 2014; Kasanga *et al.*, 2012; Kivaria, 2003; Namatovu *et al.*, 2013a; Picado *et al.*, 2011) and West (Bronsvoort *et al.*, 2006a) Africa. Both livestock management practices and wildlife contact have been suggested as risk factors for FMD in east Africa (Kivaria, 2003). A recent study based on passive surveillance highlighted a greater intensity of FMDV reports near borders, roads and railways, suggesting the importance of transport networks and movements (Picado *et al.*, 2011). However, as this study analysed passively reported FMD outbreaks only, as recorded by the central veterinary authority in Tanzania, there was a potential for reporting bias. For example, somebody living near a main road may have more opportunity to report an FMD case and be visited by a veterinary team compared to somebody in a remote area. Later studies reported high FMD seroprevalence and hypothesized about both wildlife and livestock related drivers of infection (Genchwere

et al., 2014; Mkama *et al.*, 2014). However, comprehensive studies on risk factors for FMD based on active surveillance are scant in eastern African literature.

Tanzania, the country where the study described in this chapter is based, has many characteristics that could facilitate FMDV circulation. It has large, mobile populations of susceptible hosts for FMDV including the third largest cattle population in Africa (estimated to be 21,280,875 head in 2008) (Chapter 2, FAO, 2013b; Tanzanian Misistry of Agriculture, 2012; Robinson *et al.*, 2007) and the highest African buffalo (*Syncerus caffer*) population (estimated to be >342,450 head in 1999 and reported to be increasing in the most recent Tanzanian wildlife census) (Chapter 2, East, 1999; TAWIRI, 2014). Our study is focused in northern Tanzania, a region with vast livestock and wildlife movements that are integral to wildlife conservation and rural livelihoods. There is no physical separation between livestock and wildlife. Livestock management practices in interface areas entail frequent animal movements, shared resources and a vibrant rural livestock trade, presenting a web of potential risk factors for FMD (Figure 4.1).

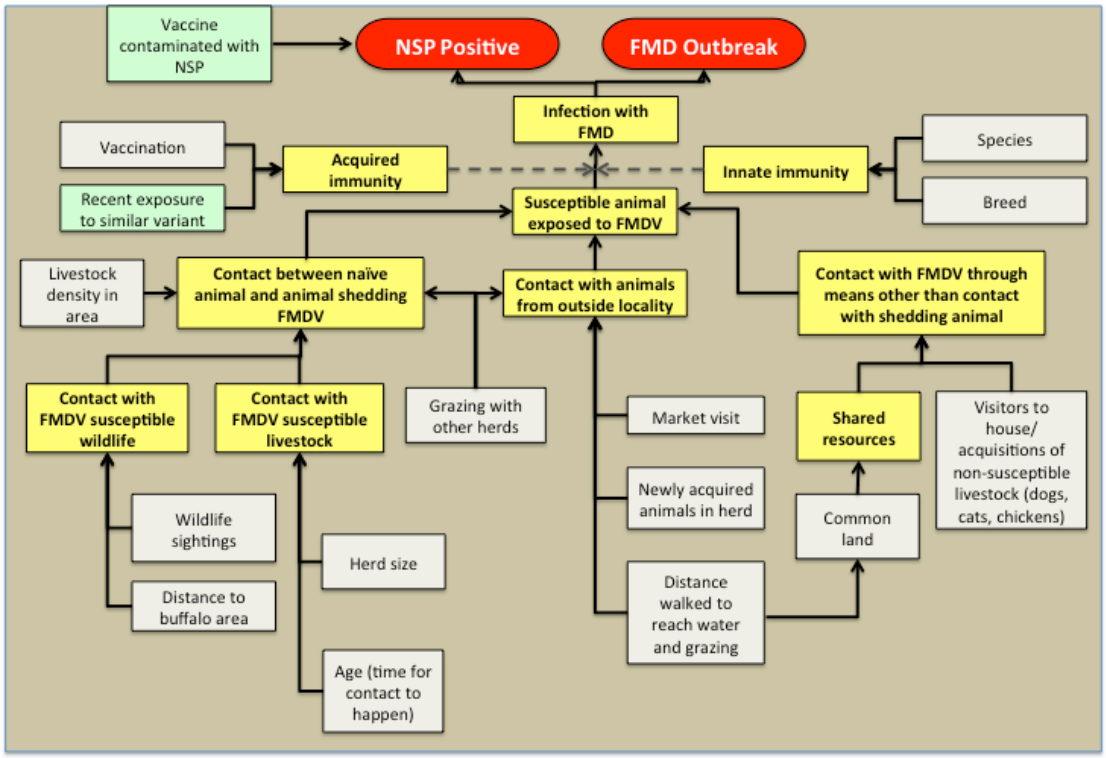


Figure 4.1: A summary of possible risk factors for FMD infection with or an outbreaks.
The red colour means a measurable outcome. The yellow means possible reasons for the outcome and the grey means measurable drivers for these reasons. The green colour shows drivers that are not measurable in the current study. NSP = Foot-and-mouth disease non-structural protein ELISA.

The epidemiology of FMD in African livestock-wildlife interface areas may be complicated by the presence of FMD susceptible buffalo (*Syncerus caffer*) populations. In contrast to other wildlife, African buffalo populations have been consistently reported to have high prevalence of FMD infection in both Southern (Jori et al., 2016; Miguel *et al.*, 2013; Thomson, 1995; Thomson *et al.*, 1992) and East Africa (Anderson *et al.*, 1979; Ayebazibwe *et al.*, 2010b; Bronsvoort *et al.*, 2008; Hamblin *et al.*, 1990; Mkama *et al.*, 2014). In Southern Africa, where FMD is tightly controlled in livestock, infected buffalo are considered to be the main source of infection for livestock (Vosloo *et al.* ,2002a, Vosloo *et al*., 2002b, 2010; Thomson *et al*., 2003; Hargreaves *et al*., 2004; Jori *et al*., 2009; Miguel *et al*., 2013; Caron *et al*., 2013). However, different patterns might apply in east Africa where FMD is endemic in livestock. A study in Kenya reported unrelated lineages of FMDV being isolated from cattle and buffalo, albeit with limited sample numbers (Wekesa *et al.*, 2015). Understanding the relative role of livestock versus wildlife related risk factors is important for devising FMD control strategies. Measures to separate wildlife and livestock, such as wildlife fencing traditionally used in southern Africa, could have profound impacts on wildlife and livestock mobility in East Africa (Durant *et al.*, 2015; Ferguson & Hanks, 2010; Ferguson *et al.*, 2013).

The key aim of this study was to understand risk factors for FMD infection and outbreaks in northern Tanzanian livestock.

4.3 Materials and methods

4.3.1 Data for analysis of risk factors for FMD infection

Cross-sectional study

A cross-sectional survey of 85 households in 40 villages in northern Tanzania (Chapter 2, Page 53, Figure 2.16) was conducted using a stratified random sampling design as described in Chapter 2. Agro-pastoralists in Serengeti and Bunda districts (n=20 herds), pastoralists in Monduli, Ngorongoro and Simanjiro districts (n=42 herds), and rural smallholders near Arusha urban area (n=23 herds) were surveyed. The questionnaire (Appendix 2) was designed to collect information relevant to potential livestock exposure

to FMDV as shown in Figure 4.1, including location, tribe, management practice, livestock owned, livestock movements, births, deaths, purchases, sales, disease, shared resources (i.e. grazing, watering, dipping, herd owners looking after each other's animals), distance travelled to reach grazing and water and contact with wildlife.

Serum samples were available from 1410 cattle, 877 goats and 451 sheep. The median proportion of livestock sampled per herd was 31.9% (IQR: 17.0-58.2%). Each sampled animal was given an ear-tag with a unique identifier and its age, species, breed, origin, movement, vaccination and disease history were recorded. One of the 85 households had livestock sampled but a questionnaire was not conducted, leaving 84 households from 40 different villages contributing data to the risk-factor study.

Data relating to buffalo and livestock density

Cattle, sheep and goat densities in the study area were approximated using data from the Food and Agriculture Organisation's (FAO) "Gridded Livestock of the World," resource (Robinson *et al.*, 2007). The cattle, goat and sheep data for the study area were mapped (chapter 2) and the cattle density at each study household location was recorded.

Areas estimated to have buffalo present were mapped in the R statistical environment (R development core team, 2008) as described in Chapter 2. Proximity to buffalo was measured by calculating the minimum geographic distance of each household to the centre of the closest 1km squared raster cell classified as a buffalo area.

4.3.2 Laboratory methods

Sera from the cross-sectional study were tested at the Pirbright Institute with a commercial blocking enzyme linked immunosorbent assay (PrioCHECK FMDV NS[10]) to detect antibodies against FMDV non-structural protein (NSP ELISA) (Chapter 2, Chung *et al.*, 2002; Sorensen *et al.*, 2005). A positive serological result was defined as one with a percentage inhibition of 50% or greater, as per the manufacturers' recommendations.

[10] PrioCHECK®, Life Technologies™, Thermo Fisher Scientific Inc, Platinastraat 33 Lelystad, Netherlands

4.3.3 Analysis of risk factors for FMD seropositivity

Data management

The questionnaire and spatial data were collated in an SQL database specifically constructed for the study and summarised in the R statistical environment. Data were summarised and associations between potential explanatory risk factors were identified. These data were than merged with the NSP ELISA results for each animal using unique identifier ear-tag numbers.

Questionnaire data about potential risk factors for exposure to FMDV and serological results were summarised and compared for the three different livestock practices (agropastoral, pastoral and rural-smallholder).

Generalised linear mixed model to explain patterns of FMDV sero-prevalence

A generalised linear mixed effects model (GLMM) with a logit link function was used to investigate the effects of explanatory variables on the likelihood of a positive NSP ELISA result. After initial descriptive analyses of all questionnaire variables shown in Figure 4.1, seven potential explanatory variables were selected for the initial trial model based on the strongest biological rationale and avoiding extreme colinearity between variables. The variables were 1) animal age, 2) species, 3) livestock practice, 4) maximum time walked to reach grazing and water, 5) herd size, 6) proximity to wildlife area and 7) wildlife sightings.

Positive or negative serological results ($y_{a,j,v}$) from animal a in herd j and village v were assumed to follow a Bernoulli distribution based on a probability of $p_{a,j,v}$ of being seropositive.

$$\boldsymbol{y_{a,j,v} \sim Bernoulli(p_{a,j,v})}$$

A logit function was used to link $p_{a,j}$ to the GLMM as $\eta_{a,j}$.

$$\boldsymbol{\eta_{a,j,v} = \log\left(\frac{p_{a,j,v}}{1 - p_{a,j,v}}\right)}$$

The initial GLMM is shown as Model 4.1 below.

$$\eta_{a,j,v} = \beta_0 + \beta_1 x_{a,1} + \beta_2 x_{j,1} + \beta_3 x_{j,2} + \beta_4 x_{j,3} + \alpha_s + \omega_h + \lambda_l + \gamma_j + \nu_v$$

Model 4.1

a = animal
j = herd
v = village
$x_{a,1}$ = Age of animal
$x_{j,1}$ = Maximum minutes walked to reach grazing and water by herd
$x_{j,2}$ = Distance to buffalo area of herd
$x_{j,3}$ = Number of cattle in herd
s = Bovine or small ruminant
h = Buffalo sightings weekly or not
l = Agropastoral, pastoral or rural smallholder livestock practice

The herd and village level random effects in Model 4.1 were assumed to follow a normal distribution with a mean of 0.

$$\gamma_j \sim Normal(0, \sigma_\gamma^2)$$

$$\nu_j \sim Normal(0, \sigma_\nu^2)$$

Number of cattle, minutes walked and distance to buffalo area had large ranges and caused convergence problems for the model. These variables were logged in order to rescale them and ease convergence.

Model selection

For model selection, variables were dropped in a stepwise fashion with the least significant variable upon likelihood ratio testing (LRT) being dropped first. For each step, the LRT was repeated for the remaining variables.

Model validation and power analysis

The model predictions (with and without random effects) for each of the 2694 animals' NSP ELISA results were compared to the true results from the data to assess the explanatory ability of the model for the data. The distribution of random effects on the

intercept was described by plotting. The statistical power of the model was assessed retrospectively as described by (Johnson *et al.*, 2015).

Power analysis for the cross-sectional study was performed retrospectively by simulation as described by (Johnson *et al.*, 2015). Simulations of between 1000 and 5120 livestock sampled from between 40 and 160 herds was made and buffalo sighting data were randomly generated based on a Bernoulli distribution and with a probability of 0.5 of a buffalo sighting weekly or more often. Simulated village levels were generated based on two herds per village. A scenario was investigated where the baseline probability of livestock being positive for NSP antibodies was 0.5 based on FMDV sero-prevalence estimates from Tanzania, Uganda and Kenya (Ayebazibwe *et al.*, 2012; Kibore *et al.*, 2013; Mkama *et al.*, 2014; Namatovu *et al.*, 2013a). Simulated effects of buffalo sightings were created where weekly buffalo sightings by the household increased the probability their livestock being seropositive by between 0 and 0.45 (or buffalo sightings increased the odds of being seropositive by a ratio between 1 and 19). A variance of 1 was assumed for the herd and village level random effects. A GLMM was run with the simulated data:

Positive or negative serological results ($y_{a,j,v}$) from animal *a* in herd *j* and village v were assumed to follow a Bernoulli distribution based on a probability of $p_{a,j}$ of being seropositive. In the simulation, a logit function was used to link the probability of an animal being seropositive, $p_{a,j,v}$ to the GLMM as $\eta_{a,j,v}$.

The simulated GLMM for power analysis is shown below.

$$\eta_{a,j,v} = \beta_0 + \omega_h + \gamma_j + \nu_v$$

a = animal
j = herd
v = village
h = buffalo sightings weekly or not
v = village

The p value from a Wald test was recorded. This procedure was repeated with 1000 simulated responses for each size of buffalo sighting effect and for sample sizes of 40, 84 and 160 herds with 25 livestock per herd. The proportion of times that the p value was less than 0.05 was calculated.

4.3.4 Data for analysis of risk factors for FMD outbreaks

Case-control study

As described in Chapter 2, case-control questionnaires were conducted involving 70 households in Serengeti district. These households were sampled from 7 villages suffering FMD outbreaks. In each village, five herds with outbreaks were randomly selected (two of which had samples taken to confirm FMD in the WRL), and five herds with no reported evidence of FMD during the outbreak in the village were selected. Case herds and control herds were matched for FMD risk period. Questionnaires (Appendix 3) were conducted to obtain information about livestock management and wildlife contact risk factors during the month prior to the first observed FMD case in the village FMD outbreak, and therefore matched for the risk period. Similarly, cases and controls were matched in location (the same village). Control herds were revisited after six weeks to check that the animals had not shown clinical signs of FMD since the initial visit. If a control herd had an FMD outbreak within six weeks from the initial visit, it was excluded from the study. The reminder of the case-control study design from Chapter 2 is presented on Table 4.1. One household was excluded from analysis as the timing of its FMD outbreak fell outside the risk period for the village. Two villages had six cases and four controls due to a shortage of herds that were unaffected by the FMD outbreak. This left 36 cases and 33 controls for risk-factor analysis.

Table 4.1: Summary of the case-control study design.

Source population	Livestock owning households in Serengeti district living in villages where the village leader reported an FMD outbreak during active surveillance by the project FMD field team
Risk period	One month prior to the first case observed in the village associated with the reported outbreak
Matching Criteria	Matching was done at village level – five cases and five controls per village. Questionnaires of cases and controls at village level were conducted within a short time-span (one week maximum) and covered the same risk period.
Case definition	A household that reported livestock in their herd with FMD lesions during a laboratory confirmed village outbreak
Control definition	A household in the same village as a case that reported that no livestock in their herd had clinical signs of FMD in the village outbreak and reported that no clinical signs of FMD were observed in their livestock in the six weeks after the initial questionnaire visit.
Case validation	Two of the five cases per village had their livestock clinically examined and FMD lesion material sampled and sent for virus isolation and typing at the WRL

Herd size and composition were recorded in case and control herds. For the risk period, variables investigated included 1) newly acquired animals, 2) distances travelled for grazing and water, 3) visits to the dip tank, 4) number of different herds contacted and number of different villages that these herds came from, 5) sightings of buffalo and other wildlife near the livestock, 6) visits from livestock trucks, milk collectors, vets, agricultural officers and animal carers. Herd-owners were also asked about previous FMD outbreaks in their herd, their village and in other villages.

4.3.5 Analysis of risk factors for FMD outbreaks

Case control data were collated in an SQL database and analysed in R. Due to relatively few data-points (69) compared to the multiple possible explanatory variables and due to colinearity between variables, combination measures of livestock contacts were created for grazing, watering and dipping.

Combination measure for livestock contacts during grazing and watering

Levels of 1 and 2 were assigned to herds that walked for less than and hour and more than an hour (respectively) to reach grazing and water. Levels of 1, 2 and 3 were assigned for contacting zero, 1-5 herds and greater than 5 herds during grazing or watering. Village contact levels were assigned according to the number different villages that herds contacted during grazing or watering came from. The combination measure for livestock contacts during grazing or watering was then calculated as:

Distance walked level * Herds contacted level * Villages contacted level

Combination measure for livestock contacts during dipping

Levels were assigned to whether or not livestock were taken to the dip tank in the past month and also to the number of villages that herds encountered at the dip tank originated from. Numbers of herds encountered at the dip tank was not incorporated as all but one of the households encountered more than five herds. The combination measure for livestock contacts at the dip tank was calculated as:

Level for dipping or not * Level for villages contacted at the dip tank

Conditional logistic regression model for analysis of case-control data

The case-control data were analyzed using a conditional logistic regression model (Model 4.2) with village level strata (Gail *et al.*, 1981; Therneau & Lumley, 2015).

The probability of herd j being a case in village v was linked to the conditional likelihood model with a logit function as $\eta_{j,v}$.

$$\boldsymbol{\eta_{j,v}} = \boldsymbol{\beta_0} + \boldsymbol{\beta_1 x_{j,1}} + \boldsymbol{\beta_2 x_{j,1}} + \boldsymbol{\beta_3 x_{j,2}} + \boldsymbol{\beta_4 x_{j,3}} + \boldsymbol{\alpha_n} + \boldsymbol{\omega_h} + \boldsymbol{\lambda_g} + \boldsymbol{\mu_f} + \boldsymbol{\nu_v} \quad \text{Model 4.2}$$

j = herd

v = village

$\boldsymbol{x_{j,1}}$ = Number of cattle in the herd

$\boldsymbol{x_{j,1}}$ = Maximum minutes walked to reach grazing and water by herd

$\boldsymbol{x_{j,2}}$ = Measure of contacts during grazing/watering

$\boldsymbol{x_{j,3}}$ = Measure of contacts during dipping

n= New animals acquired during risk period (yes or no)

h = Buffalo sightings weekly or not

g = Grazing/watering location different from usual (yes or no)

f = visitors to livestock during risk period (yes or no)

$\boldsymbol{\nu_v}$ = stratification at village level for conditional liklihood

Model selection was based on likelihood ratio testing, with the variables adding least to the explanatory ability of the model being dropped first. Analysis of the statistical power of the model was performed retrospectively using Monte Carlo simulation as described by (Haneuse *et al.*, 2011).

4.4 Results

4.4.1 Patterns of FMDV seroprevalence in the cross-sectional study

Of the livestock sampled, 59.0% (95% CI: 57.1-66.1%) were seropositive for antibodies against NSP. Figure 4.2 shows the distribution of NSP ELISA percentage inhibition (PI) results for the 2694 sera that were tested. A higher proportion of cattle (69.0%, CI: 66.5 – 71.4%) were seropositive compared to small ruminants (48.5%, CI: 45.7-51.3%). Higher proportions of livestock belonging to agro-pastoralists (67.2%, CI: 63.6-70.7%) and pastoralists (65.5%, CI: 63.2-68.4%) were seropositive compared to livestock belonging to smallholders (37.1%, CI: 33.5-40.9%). Seroprevalences of FMDV by species in the three production systems are shown in Figure 4.3.

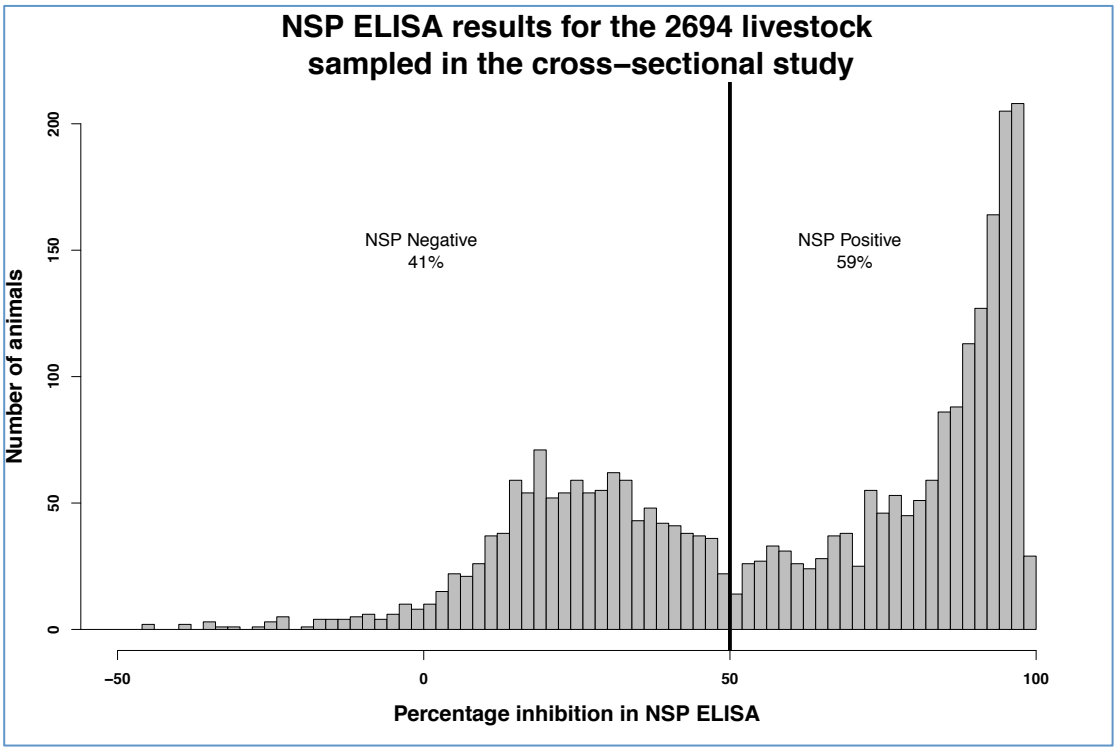


Figure 4.2: A histogram summarising the results of non-structural protein (NSP) ELISA testing of sera from the cross-sectional study livestock. The vertical line represents the manufacturer recommended cut-off between results considered positive and negative.

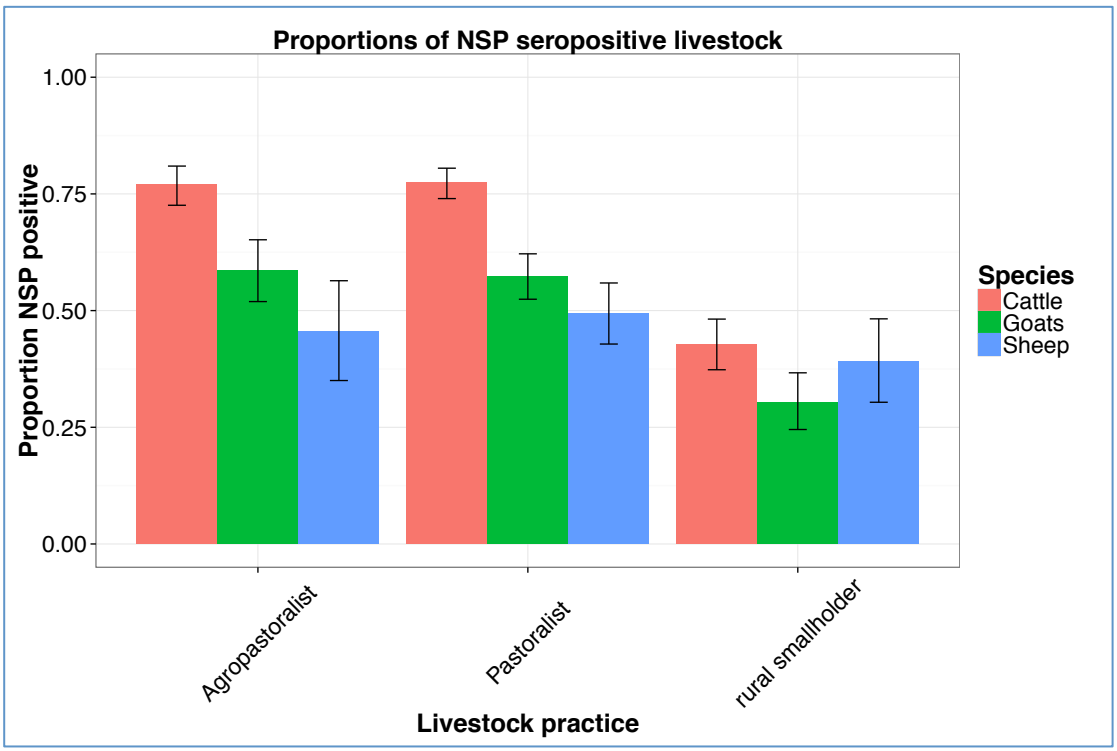


Figure 4.3: Proportions of cattle, goats and sheep belonging to agropastoralists, pastoralists and rural smallholders that tested positive for FMDV non-structural protein (NSP) antibodies.
Bars represent 95% confidence intervals.

Figure 4.4 illustrates that the majority of herds had a high proportion of seropositive livestock. Only six out of 84 herds (7.1%) had no seropositive livestock and 15 (18.1%) of the 83 herds with cattle aged two-and-a-half years old or less ("young cattle") had no seropositive animals in this age-group (Figure 4.4).

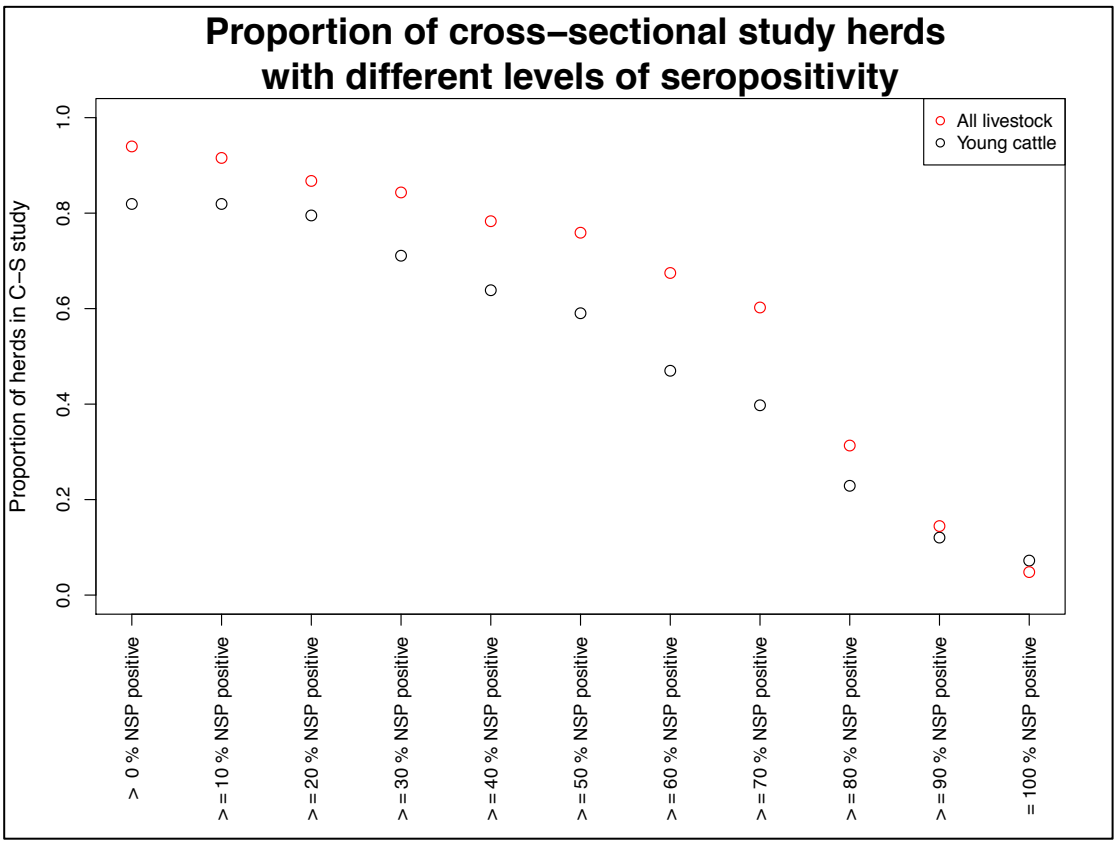


Figure 4.4: Levels of sero-positivity in the 83 study herds that owned young cattle (cattle aged 2.5 years old or less).
The x-axis shows 11 different levels of sero-positivity for the herds ranging from any animal tested from the herd being seropositive to 100% of animals tested being seropositive. The y-axis shows the proportion of the total herds in the study that fitted this category. The black points relate to the proportions of herds with different levels of sero-positivity in young cattle. The red points relate to different levels of sero-positivity in all livestock.

4.4.2 Potential risk factors for FMD infection in agropastoral, pastoral and rural smallholder livestock

Table 4.2 shows that agropastoralists and pastoralists had larger herds, acquired more livestock and walked farther to reach grazing and water compared to rural smallholders. Pastoralists reported more non-buffalo FMD susceptible wildlife sightings compared to agropastoralists and smallholders. The frequency of buffalo sightings reported by pastoralists and rural smallholders was comparable. All three management practices reported buffalo sightings more rarely than sightings of other wildlife. Rural smallholders were located closer to the Arusha NP buffalo area than the pastoralists or agropastoralists were to the buffalo areas in their districts.

Table 4.2: A comparison of potential FMD risk factors in agropastoral, pastoral and rural smallholder management systems.
Young cattle = cattle aged two-and-a-half years old or less

Observation		Livestock Practice			Comment
		Agro-pastoralist	**Pastoralist**	**Rural smallholder**	
Number of households		20	41	23	
Proportion of households that had > = 50% of their young cattle seropositive	Comparison	HIGHER		LOWER	
	Proportion	0.68	0.66	0.26	
	95% CI	0.61 - 0.74	0.63 - 0.68	0.18 - 0.35	
Proportion of households that had > = 50% of livestock seropositive	Comparison	HIGHER		LOWER	
	Proportion	0.67	0.66	0.37	
	95% CI	0.64 - 0.71	0.63 - 0.68	0.33 - 0.41	
Number of cattle per herd	Comparison	HIGHER		LOWER	
	Mean	86.38	122.98	16.74	
	Median	58	75	17	
	IQR	41-88.5	27-150	12 - 20	
Number of small ruminants per herd	Comparison	LOWER	HIGHER	LOWER	
	Mean	57.21	264.51	35.55	
	Median	45	109	27	
	IQR	22-78	60-250	22-42	
Proportion of households that saw buffalo weekly or more frequently	Comparison	LOWER	HIGHER		Households that walked further for grazing and water reported more frequent buffalo sightings
	Proportion	0.05	0.39	0.3	
	95% CI	0-0.25	0.24 - 0.55	0.13 - 0.53	
Proportion of households that saw non-buffalo FMD susceptible wildlife weekly or more frequently	Comparison	LOWER	HIGHER	LOWER	
	Proportion	0.25	0.83	0.39	
	95% CI	0.09-0.49	0.68-0.93	0.2-0.61	
Hours walked to reach grazing and water	Comparison	HIGHER		LOWER	Answers were in both distance and time, with the majority in time - 1 hour and 3 Km were considered equivalent
	Mean	2.01	3.78	0.8	
	Median	2	2.33	0.5	
	IQR	1.5-2	1.33-3.5	0.17-1	
Number of acquired animals	Comparison	HIGHER		LOWER	Moderately collinear with hours walked for grazing and water (r = 0.47)
	Mean	23.95	18.73	2.53	
	Median	19	10	0	
	IQR	7- 48	1 - 24	0 - 4	
FAO predicted cattle density at households location (proportions of household at each density)	Cattle per km^2				
	<20	0.1	0.41	0.09	
	20-50	0	0.59	0.7	
	>50	0.9	0	0.22	
Distance to buffalo area (Km)	Comparison	HIGHER		LOWER	
	Mean	18.4	19.04	6.82	
	Median	16.58	17.14	4.74	
	IQR	10.79 - 19.29	6.96 - 26.43	1.12 - 9.98	

Observation		Livestock Practice			Comment
		Agro-pastoralist	Pastoralist	Rural smallholder	
Proportion of livestock that are foreign breeds	Comparison	LOWER		HIGHER	
	Proportion	0.001	0.004	0.324	
	95% CI	0-0.008	0.001-0.009	0.289 – 0.360	

Livestock moved in and out of households

A count of the livestock species involved in animal movements is summarised on Table 4.3. Based on these counts, cattle made up the largest proportion of livestock being sent to other households for care and are also the species most commonly purchased. Goats made up the largest proportion of animals sold and given as gifts.

Table 4.3: A count of each species reported to be moved in and out of households.

Livestock movement type		Species count from 84 households		
		Cattle	Goats	Sheep
Livestock owned but being cared for elsewhere		841	1	6
Livestock belonging to somebody else being cared for with herd		254	114	22
Livestock movements in the past 4 months	Purchased	192	89	87
	Given as gifts	17	33	15
	Sold	222	393	160
	Gone to market and back as not sold	25	8	7
	Livestock moved into herd	105	23	18

Measures of wildlife contact

Number of acquired livestock was moderately correlated with hours spent walking for grazing and water (r = 0.47). Households that walked farther for grazing and water also tended to report more frequent sightings of buffalo (Figure 4.5). This trend was not so pronounced in the case of other wildlife species (Figure 4.5). Households in all three management practices reported wildlife and buffalo sightings. However other FMD susceptible wildlife were seen more frequently than buffalo (Figure 4.6). The distribution of cross-sectional study household distances from buffalo areas is summarised on Figure 4.7.

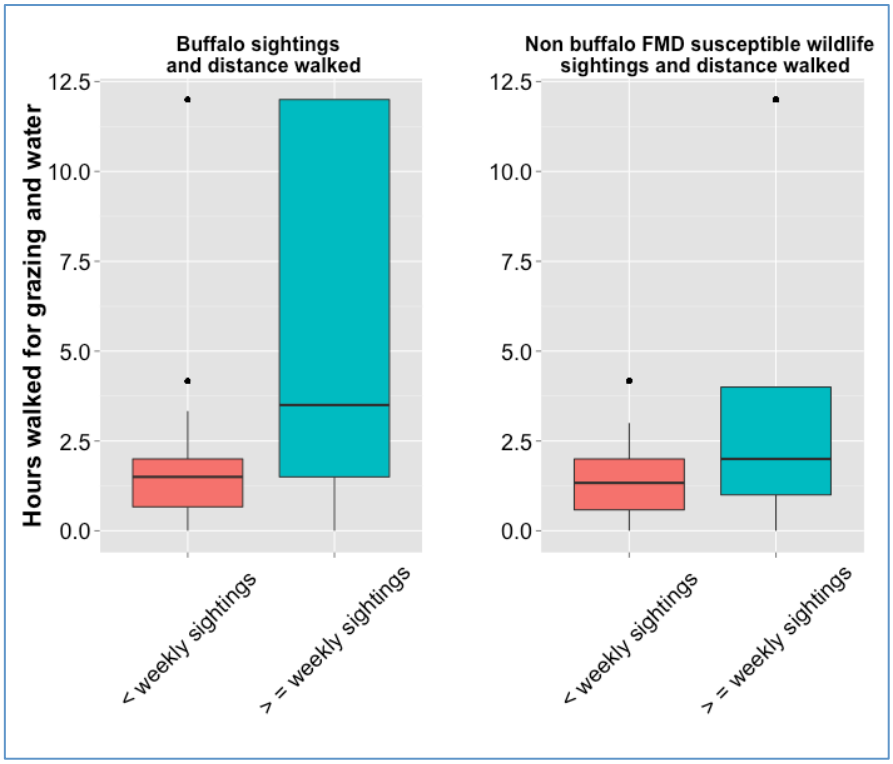


Figure 4.5: Distance walked by livestock to reach grazing and water compared to frequency of wildlife sightings.

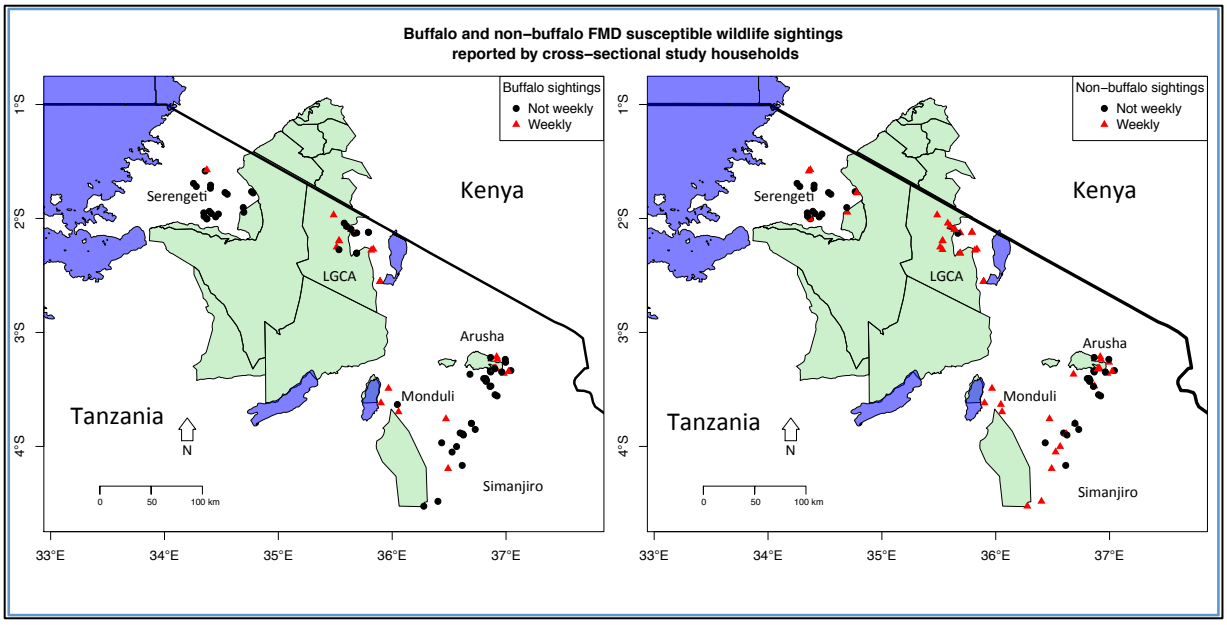


Figure 4.6: Locations of cross-sectional study households highlighting those that reported frequent wildlife sightings.
The map on the left relates to buffalo sightings. The map on the right relates to non-buffalo FMD susceptible wildlife sightings. LGCA = Loliondo game controlled area. Arusha is the rural smallholder area. LGCA, Monduli and Simanjiro are pastoral areas. Serengeti district is an agropastoral area.

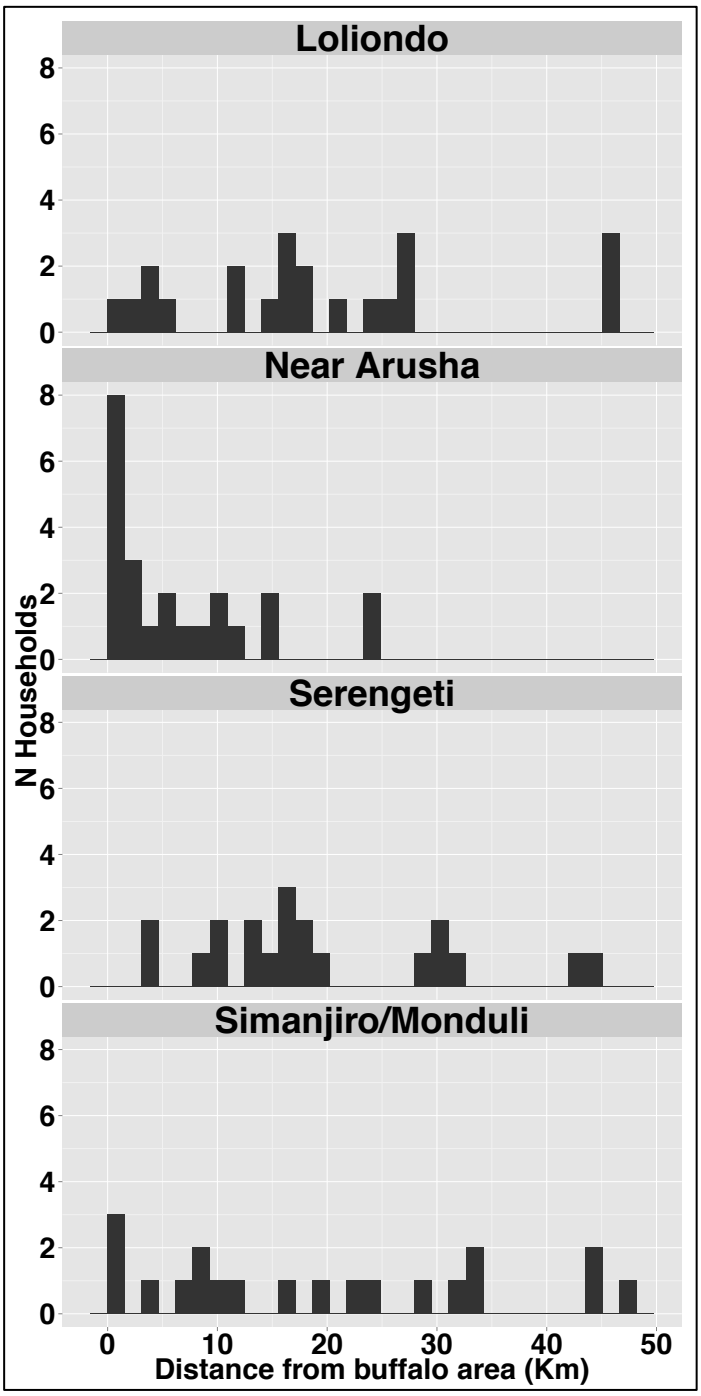


Figure 4.7: The distribution of cross-sectional study household distances from buffalo areas.

4.4.3 Final model to explain seropositivity to FMDV

After the LRT guided stepwise process to identify important explanatory variables for an animal being NSP seropositive, the final model included animal age, species, livestock practice and random effects of household and village.

Variables that explained sero-prevalence patterns

Older animals were more likely to be seropositive, as well as cattle compared to small ruminants to be seropositive. Livestock managed by agropastoralists and pastoralists were more likely to be seropositive than those managed by rural smallholders (Table 4.4, Figure 4.8).

Table 4.4: Significant explanatory variables from the final generalized linear mixed model inferring FMDV seropositivity.
LRT = Likelihood ratio test, CI = Confidence interval

	LRT χ^2	p	Coefficient (95% CI)	Odds Ratio (95% CI)
Age (per extra year)	219.6	<10^-6	0.4 (0.3-0.4)	1.4 (1.4-1.5)
Species	144.9	<10^-16		
Cattle compared to small ruminants			1.2 (1-1.4)	3.3 (2.7-4)
Livestock practice	17.1	0.0002		
Agropastoral compared to smallholder			2.1 (1-3.2)	8.1 (2.8-23.6)
Pastoral compared to smallholder			2 (1.1-2.9)	7.1 (2.9-17.6)

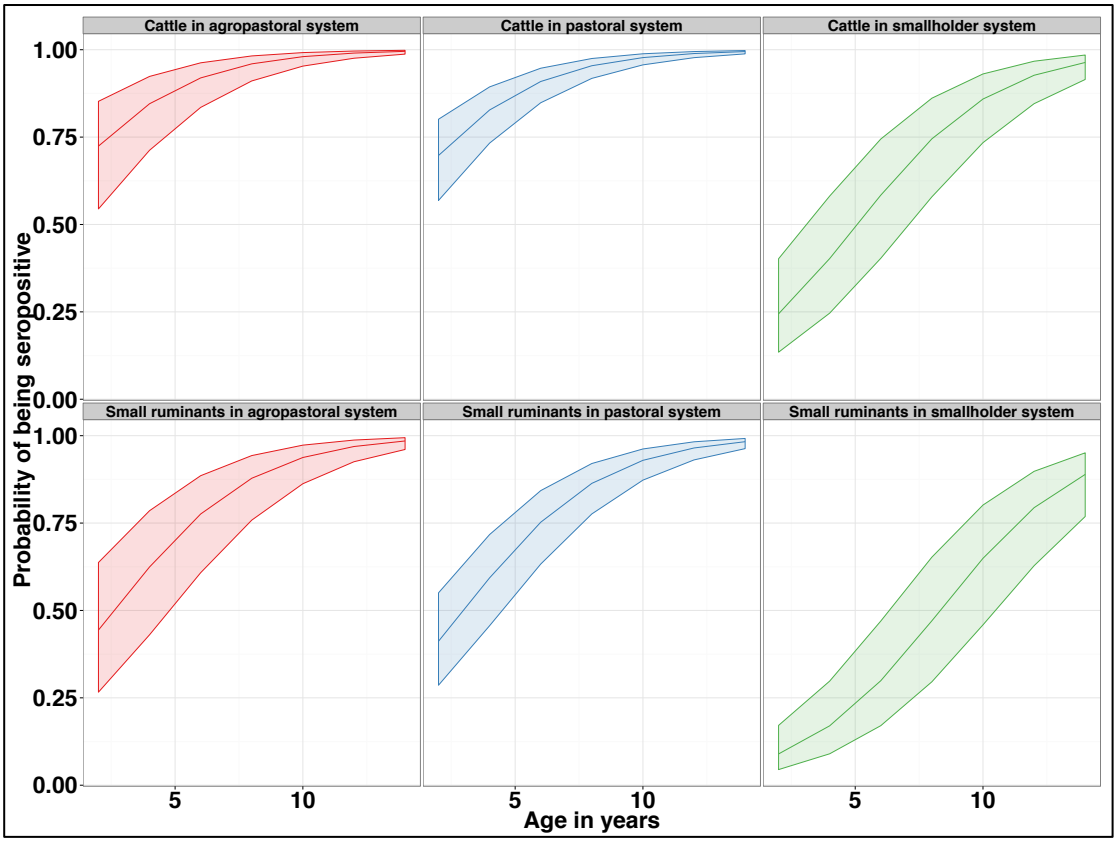


Figure 4.8: The age-related increase in probability of cattle and small ruminants being NSP seropositive in the three management systems as inferred by the final model.
The darker lines indicate the mean probability and the lighter shading indicates 25% and 75% quartiles.

Variables that did not explain sero-prevalence patterns

The results for variables with no observed effects on the likelihood of livestock being seropositive for FMDV are shown on Table 4.5.

Table 4.5: Non-significant explanatory variables from the generalised mixed linear model trials inferring FMDV seropositivity.
Likelihood ratio testing was performed and coefficients were estimated by adding each listed variable to the model with the significant variables present.
LRT = Likelihood ratio test, CI = Confidence interval

	LRT χ^2	p	Coefficient (95% CI)	Odds Ratio (95% CI)
Log (Total cattle)	2.76	0.1	0.3 (0 - 0.6)	1.3 (1-1.8)
Log (Maximum minutes walked to reach grazing and water)	2.37	0.12	0.1 (0 -0.3)	1.1 (1-1.3)
Buffalo sighting weekly or more often	1.32	0.3	-0.4 (-1 - 0.3)	0.7 (0.4-1.4)
Log (Distance to buffalo area)	0.09	0.75	0 (-0.3 - 0.2)	1 (0.7-1.3)
Acquired livestock in the past four months (Y or N)	0.6	0.44	0.2 (-0.3 - 0.8)	1.2 (0.7-2.1)

4.4.8 FMD seropositivity model validation results

Comparison between model predictions and data

There were 58.9% seropositive results amongst the 2694 livestock. When the significant fixed effects (age, species and livestock practice) were used to predict seropositive results, 65.5% were predicted positive (Figure 4.9). The fixed effects prediction equalled the true result 68.2% of the time and the Kappa statistic for agreement between the prediction and reality was 0.326 (fair agreement). When random effects (village and herd) were added to the significant fixed effects, 66.9% of the livestock were predicted to be seropositive. The model prediction equalled the true result 77.6% of the time and the Kappa statistic was 0.52 (moderate agreement).

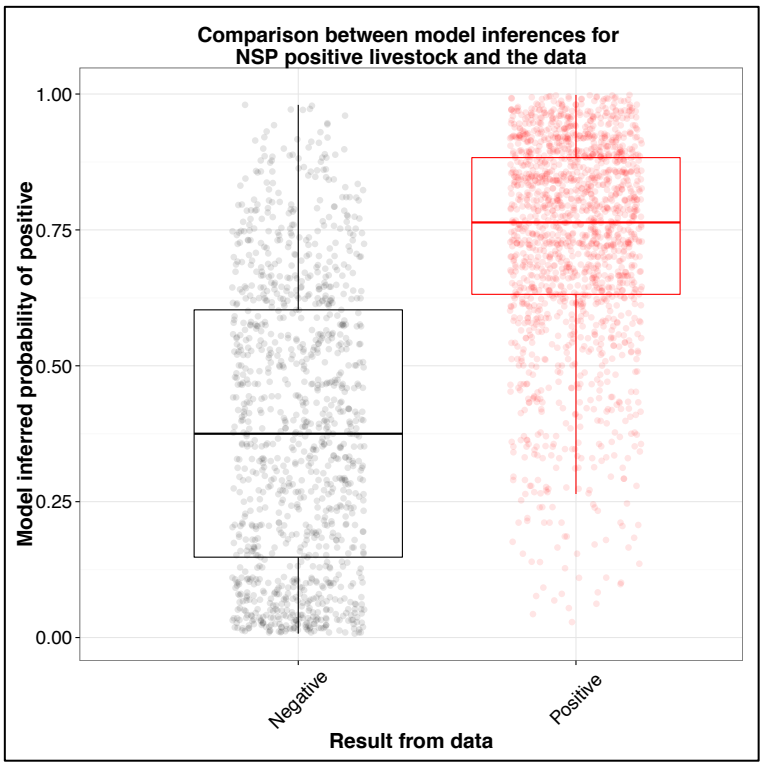


Figure 4.9: A comparison between the laboratory data and the model inference for each animal.
NSP positive or negative (x-axis) and probability of being NSP positive (y axis).

When 2694 data-points were compared to model predictions, 602 (22.3%) of the model predictions did not agree with the data (Figure 4.9). There was a greater proportion of small ruminants compared to cattle amongst the 197 positive data points that were predicted to be negative. Pastoralist livestock made up the greatest proportion of the 403 negative data points predicted to be positive followed by agropastoral livestock, with smallholder livestock making up the smallest proportion (Table 4.6). The kappa statistic for agreement between the model prediction and the cross-sectional serology result was 0.53 (moderate agreement). When the model's predictions were based only on fixed effects (i.e. without herd and tribe level random effects), the prediction was the same as the data 68.6% of the time and the kappa statistic (0.34) reflected fair agreement.

Table 4.6: Data-points where model inference did not match the NSP result from the data.
NSP = Foot-and-mouth disease non-structural protein antibody ELISA.

Number (Percentage of total wrong positive or negative predictions)	Predicted negative but really positive	Predicted positive but really negative
Total wrong predictions out of 2694	198	404
Herds with one or more livestock with wrong predictions	51	73
Cattle	50 (25.3%)	225 (55.56%)
Small ruminants	148 (74.7%)	179 (44.2%)
Agropastoral livestock	49 (24.7%)	132 (32.59%)
Pastoral livestock	78 (39.4%)	222 (54.81%)
Rural smallholder livestock	71 (35.9%)	50 (12.35%)

Outliers amongst the random effects

Three villages in the rural smallholder area were identified as having more seropositive animals than would be expected given the fixed effects in the model (Figures 4.10 and 4.11). The two herds with the highest positive effect on the intercept were from two of the outlier villages (Figure 4.10).

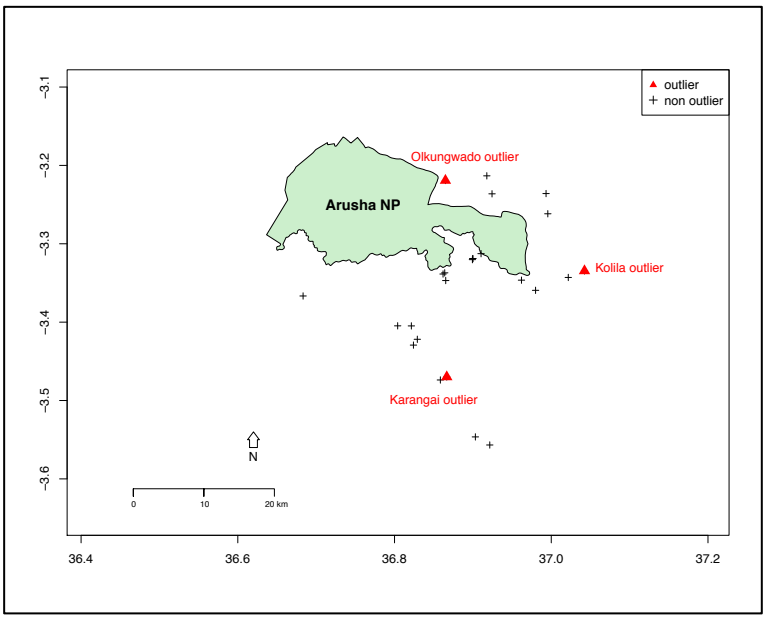


Figure 4.10: A map of the smallholder herds around Arusha National Park highlighting the three outliers amongst the household level random effects. These two herds had more NSP positive animals than was expected given their explanatory variables in the final model.
NSP = Foot-and-mouth disease non-structural protein antibody ELISA.

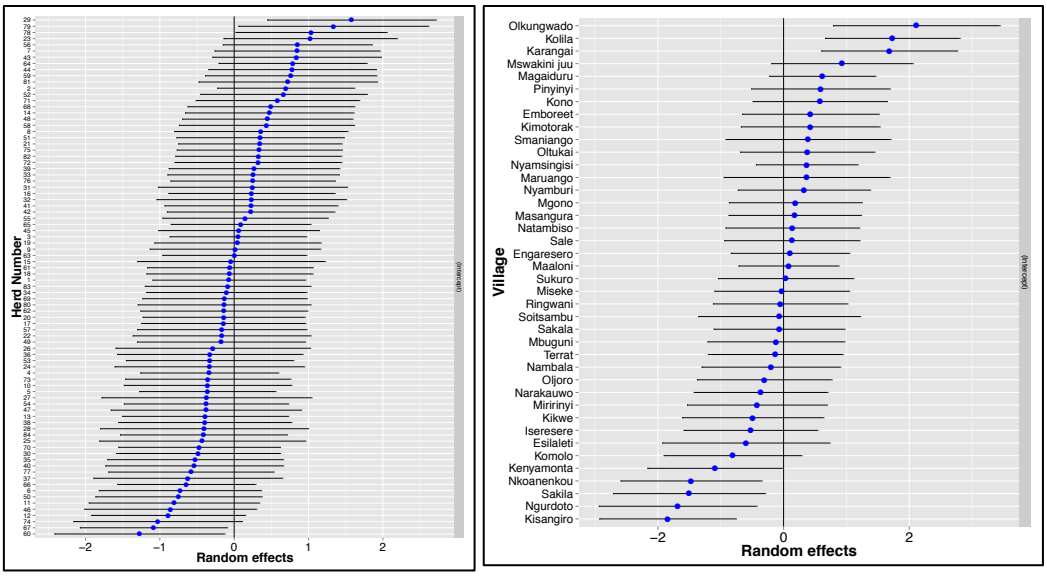


Figure 4.11: A plot of the distribution of household related random effects. Household level random effects from the final model are on the y-axis with bars indicating their 95% confidence intervals.

The villages defined as outliers amongst the random effects were Kolila, Olkungwado and Karangai in the Arusha smallholder area (Figure 4.10).

In Kolila village, the one household had a high proportion of seropositive animals, which would not be expected based on the final model, but the other household had lower levels of seropositivity. Possible reasons for the difference between the two households were investigated (Table 4.7). The only difference detected was that the outlier household was the only Maasai household amongst the rural smallholders. As Maasai people are conventionally pastoralists, it is possible that this outlier household had some different management practices that were not picked up upon through the questionnaire.

Table 4.7: A comparison between management factors and seropositivity in the Kolila rural-smallholder outlier household compared to the other household in Kolila.

	Outlier	Non-outlier
Tribe	Maasai	Mmeru
Number of cattle	21	20
Number of young cattle (<=2.5 years)	9	6
Number of small ruminants	27	22
Time walked to reach grazing and water	10 minutes	3 hours
Number of acquired animals in past 4 months	0	14
Buffalo sighted weekly or more frequently	No	Yes
Non buffalo FMD susceptible wildlife sighted weekly or more frequently	No	Yes
Proportion of young cattle positive	77.7%	0%
Proportion of total cattle positive	92.3%	47.2%
Distance to buffalo area (Km)	7.16	5.05
Proportion of total livestock positive	92%	47%
Distance to buffalo area (Km)	7.16	5.05

In Olkungwado village, only one herd, the outlier that had unexpectedly high levels of seropositivity, was sampled. This household was based less than 500 m away from the boundary of Arusha NP. In contrast to the other households in Arusha, it was sampled in May 2012 rather than in late 2011.

In Karangai, the third outlier village, the two herds sampled had seroprevalences of 70% and 83% respectively, which is higher than expected for the rural smallholder area.

Power analysis for the cross-sectional survey

The results of the retrospective power calculation for the cross-sectional study are summarised on Figure 4.12. For 2688 livestock from 84 herds and 42 villages, when buffalo sightings had no effect, Wald p values were less than 0.05 for 6.1% of simulations. When buffalo sightings increased the probability of livestock in the herd being seropositive by 0.2, Wald p values were less than 0.05 for 85.8% of simulations. When the probability increased by 0.25, p values were less than 0.05 for 96.1% of simulations. This power analysis suggests that the sample size of the cross-sectional study was acceptable.

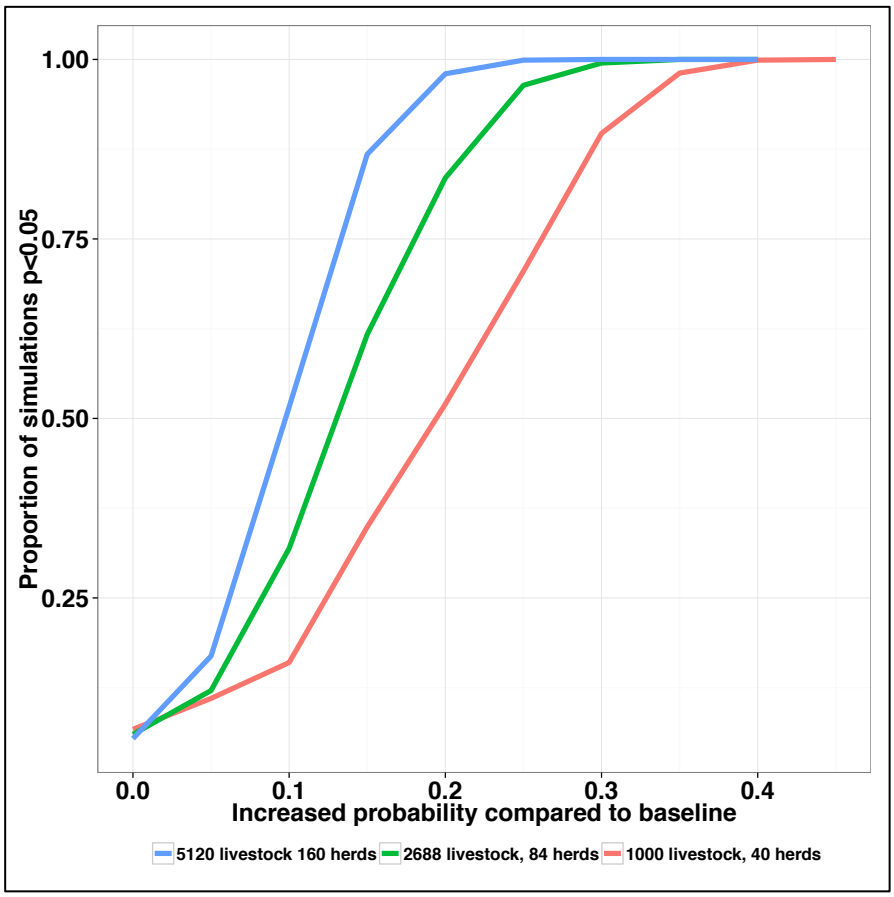


Figure 4.12: Results of power analysis by simulation for detecting an effect of buffalo sightings on the probability of being seropositive (with baseline probability seropositive = 0.5).

4.4.9 Validation of outbreak reports in the case-control study

All of the villages that reported FMD outbreaks in the case-control study had animals with FMD pathognomonic signs present and had confirmation of FMD by virus isolation in the WRL. Virus typing results from the WRL from the seven case-control study villages are summarised in Figure 4.13.

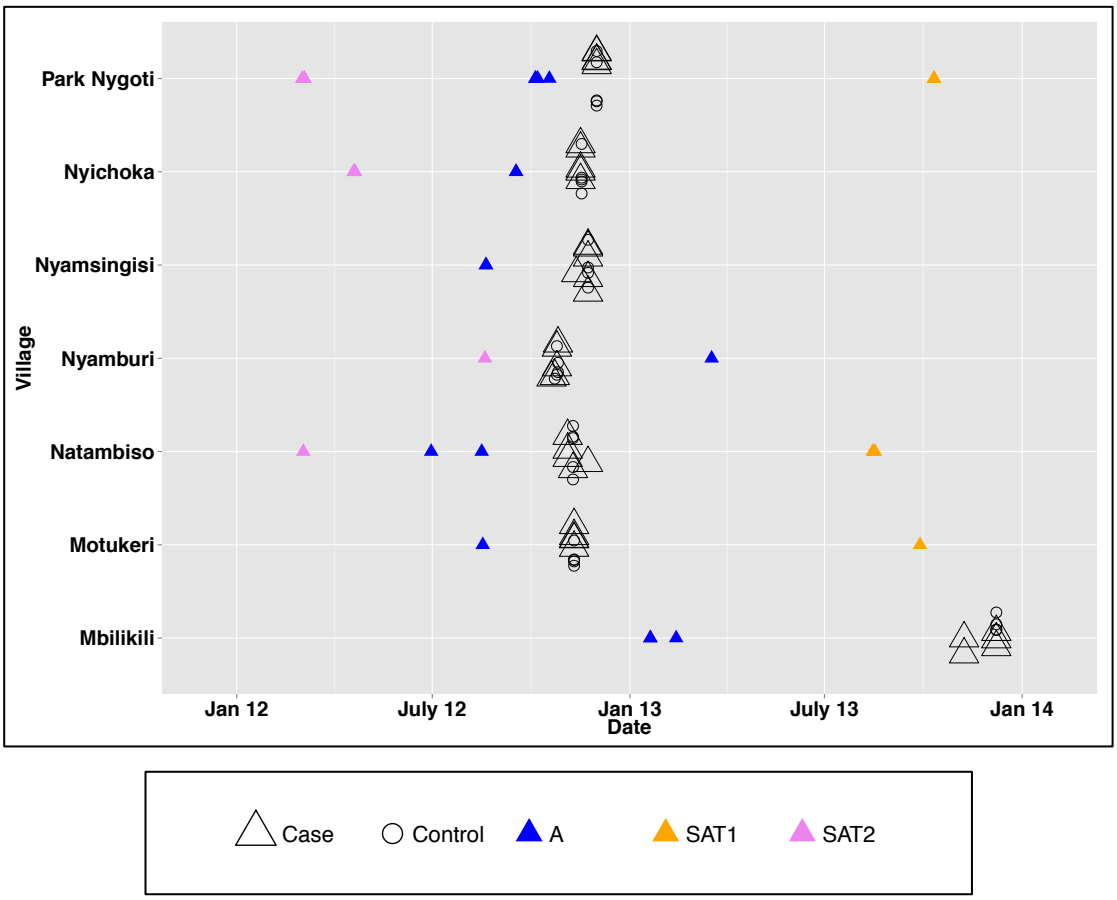


Figure 4.13: World reference laboratory virus typing results from the seven villages in the case control study.

4.4.10 Comparison between potential risk factors for FMD in cases and controls

Case herds were larger than control herds, containing larger numbers of both cattle and small ruminants (Table 4.8). A greater proportion of case households also reported acquisition of new animals compared to controls. Eight of the eleven herds reporting animal acquisitions gave details of this. Sixteen out of the 31 acquired animals were cattle, eleven were sheep, two were goats and two were pigs. There were twenty-two purchases, three gifts and two loans reported and all animals were transported on foot. Descriptive statistics also showed that a greater proportion of case herd owners reported sightings of buffalo with their livestock compared to controls, but this was not justified as a significant explanatory variable in the conditional regression model (see below).

Table 4.8: A comparison between case and control herds.

		Case	Control
Number of households		36	33
Number of cattle	Comparison	HIGHER	LOWER
	Mean	91.3	27.4
	Median	45	16
	Interquartile range	26-83	12 -22
Number of small ruminants	Comparison	HIGHER	LOWER
	Mean	34.2	24.4
	Median	19	13
	Interquartile range	0-45	7-30
Were new animals acquired in past month?	Comparison	HIGHER	LOWER
	Proportion	0.22	0.06
	95% CI	0.12-0.41	0.01-0.2
Livestock near buffalo in past month	Comparison	HIGHER	LOWER
	Proportion	0.32	0.18
	95% CI	0.18-0.5	0.07-0.35
Livestock near other susceptible wildlife in past month	Comparison		
	Proportion	0.59	0.39
	95% CI	0.42-0.75	0.23-0.58
Grazing or watering different from usual in paste month	Comparison	SIMILAR	
	Proportion	0.51	0.67
	95% CI	0.34-0.68	0.48-0.82
Walk for greater than one hour to reach grazing and water	Comparison	SIMILAR	
	Proportion	0.7	0.58
	95% CI	0.53-0.84	0.39-0.75
Meet more than five other herds during grazing and watering	Comparison	SIMILAR	
	Proportion	0.92	0.88
	95% CI	0.78-0.98	0.72-0.97
From how many different villages do the herds that you meet come from	Comparison	SIMILAR	
	Mean	2.4	2.3
	Median	2	2
	IQR	1-3	1-3
Did you take your cattle to the communal dip facility?	Comparison	SIMILAR	
	Proportion	0.43	0.36
	95% CI	0.27-0.61	0.2-0.55
Did the milk collector visit your household?	Comparison	SIMILAR	
	Proportion	0.14	0.09
	95% CI	0.05-0.29	0.02-0.24
Did a livestock transport truck visit your households?	Comparison	SIMILAR	
	Proportion	0.05	0
	95% CI	0.01-0.18	0-0.11
Did s livestock worker visit your house	Comparison	SIMILAR	
	Proportion	0.11	0.15
	95% CI	0.03-0.25	0.05-0.32
Did an AI technician visit your livestock	Comparison	SIMILAR	
	Proportion	0.05	0
	95% CI	0.01-0.18	0-0.11
Did you have an FMD outbreak in your herd in the past 2 years	Comparison	SIMILAR	
	Proportion	0.84	0.76
	95% CI	0.68 - 0.94	0.58 - 0.89

4.4.11 Results from the conditional regression model

Variables that explained the odds of having an FMD outbreak

The final model explaining the probability of being a case (having an FMD outbreak) in the case-control study was:

logit (probability FMD outbreak) ~ number of cattle in herd + acquisition of animals in past 4 months

Herds with more cattle and those that acquired new animals over the risk period were more likely to suffer an FMD outbreak (Table 4.9, Figure 4.14).

Table 4.9: Results from the model explaining the likelihood of having an FMD outbreak based on data from the case-control study in Serengeti district. LRT = Likelihood ratio test, CI = Confidence interval

	LRT χ^2	p	Coefficient (95% CI)	Odds Ratio (95% CI)
Cattle in herd (per extra bovine)	12.9	< 10^-3	0.02 (0-0.03)	1.02 (1-1.03)
New animals acquired in risk period (yes versus no)	4.6	0.03	1.72 (0.01-3.431)	5.57 (1.01-30.91)

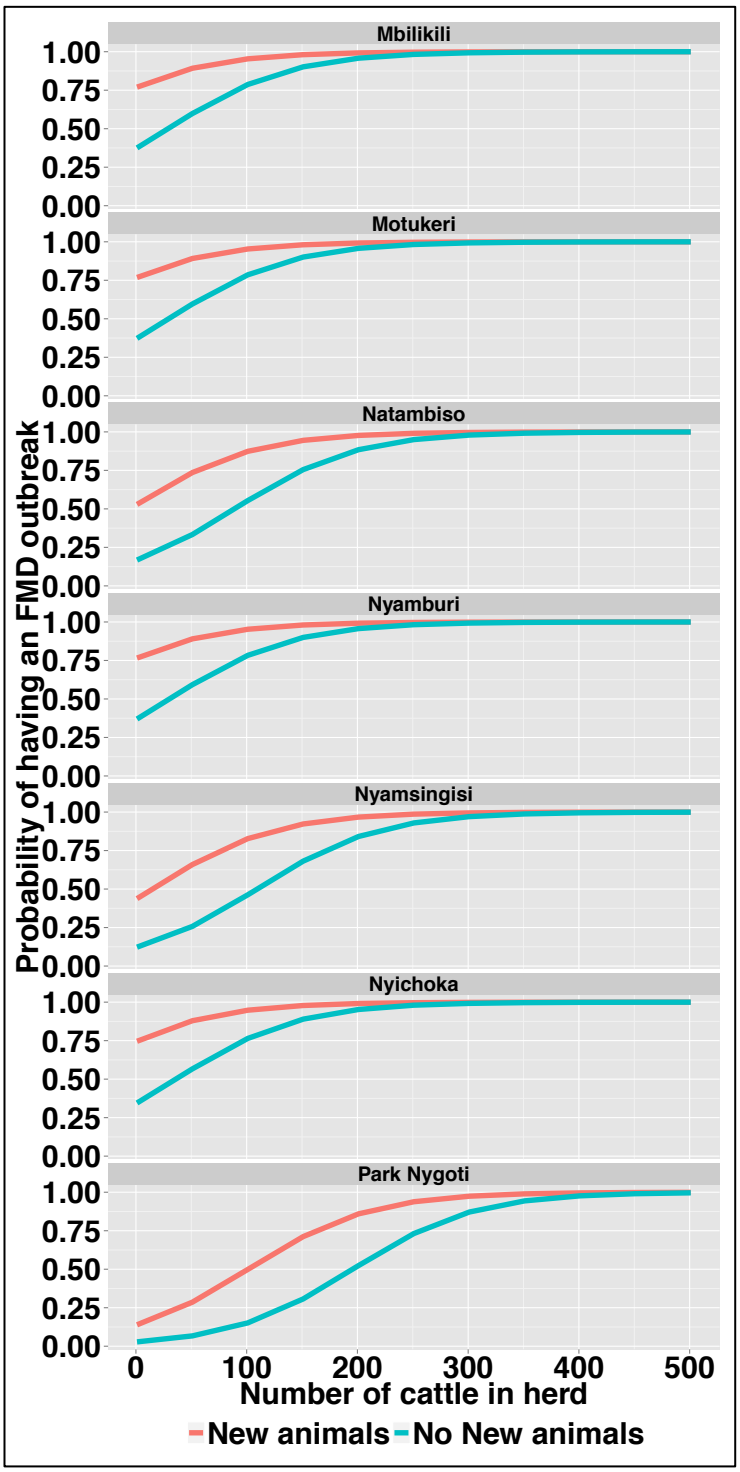


Figure 4.14: Results of the conditional logistic regression model to explain the probability of having an outbreak in the Serengeti agropastoral district. The effect of herd size (x-axis) and livestock acquisitions over the risk period (colours) on the probability of having an outbreak (y axis) is shown in each of the seven villages.

Variables that did not explain the odds of having an FMD outbreak

Reported buffalo sightings, changes in grazing or watering practices, livestock contacts during grazing/watering and sipping and people visiting the herd over the risk period did not add to the explanatory ability of the model. The coefficients and LRT results for these variables are summarised in Table 4.10.

Table 4.10: The variables in the case-control study that did not add to the model's ability to explain the probability of having an FMD outbreak.

	LRT χ^2	p	Coefficient (95% CI)	Odds Ratio (95% CI)
Buffalo sighting weekly or more often	1.26	0.26	0.8 (-0.635 - 2.227)	2.22 (0.53 - 9.27)
Grazing or watering area different to usual	1.03	0.31	-0.62 (-1.833 - 0.582)	0.54 (0.16 - 1.79)
Measure of livestock contacts during grazing and watering	1.3	0.26	0.04 (-0.03 - 0.122)	1.05 (0.97 - 1.13)
Measure of livestock contacts during dipping	0.19	0.66	-0.08 (-0.431 - 0.278)	0.92 (0.65 - 1.32)
Visitors in past month	0.03	0.87	0.11 (-1.204 - 1.418)	1.12 (0.3 - 4.13)

4.4.12 Case control model validation

Comparison between model predictions and the data

When predictions were made from study data using the final case-control model, 27 cases and 42 controls were predicted. Predictions were the same as observations 78.3% of the time, with moderate agreement between predictions and observations (Kappa = 0.57) (Figure 4.15). There were three herds predicted to be cases, although they were really controls. Two of these had greater numbers of cattle than would be expected in a control (270 and 110 head respectively) and the third had acquired new animals over the risk period. Twelve herds were predicted to be controls, although they were really cases. None of these herds had acquired new animals over the risk period and herd sizes were smaller than what would be expected in case herds (Median = 25 cattle, IQR = 15 – 33).

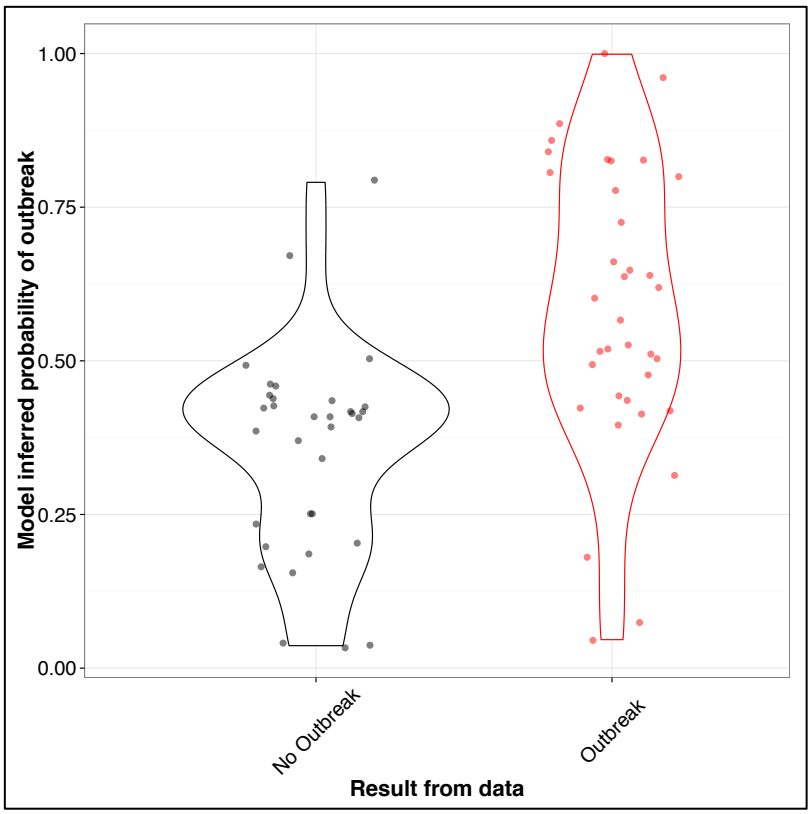


Figure 4.15: Probability of infection predicted by the case-control model compared to case or control status of each data point.

Case – control model with virus isolation confirmed cases only

There were nine case herds from five of the villages with confirmed FMD by virus isolation. These nine cases were selected out along with controls from corresponding villages and the modelling process was repeated. Number of cattle in the herd remained a significant explanatory variable (LRT: χ^2= 12.9, p= 0.0003). However, as only one out of the nine cases had acquired new animals in the past month, this did not explain the likelihood of being a case in this group (χ^2 = 0.3, p=0.87).

Power analysis for case-control study

The power of the case-control study was estimated as follows. A simulated dataset with an exposure level of 50% for buffalo sightings was generated. An odds ratio of 3 for being a case in association with weekly buffalo sightings was simulated. This simulation was repeated 10,000 times to estimate the power of the case control study. For a study with 35 cases and 35 controls, the power estimated from this calculation was 59.6% (Figure 4.16). The power estimates for sample sizes between 10 and 300 are shown in Figure 4.16. When

simulated weekly buffalo observations were reduced to 20 out of 70 households, the power was reduced to 45%.

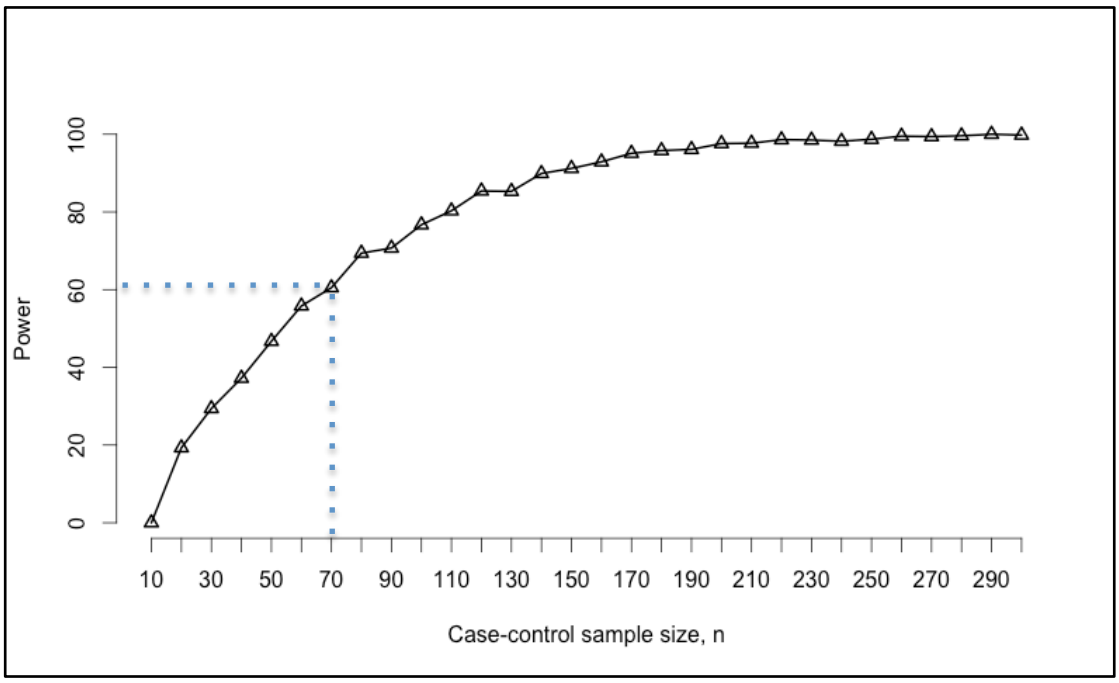


Figure 4.16: Power analysis for the case-control study.
The plot shows the power of a case-control study with a sample size between 10 and 300 to detect an effect that increased the odds of being a case by 3 and had an exposure level of 50%. The broken blue line highlights the power simulated for a study with a sample size of 70.

4.5 Discussion

Both the cross-sectional and the case-control study generated a consistent conclusion: livestock management characteristics are the most important drivers of FMD infection in northern Tanzania. In this study, there was no evidence of risk factors associated with wildlife contact. The study also demonstrated higher levels of infection in (a) pastoral and agro-pastoral livestock, (b) cattle and, (c) older animals. Herds with larger numbers of cattle and those that acquired new livestock also had increased odds of having an FMD outbreak.

Potential contact with buffalo or other FMD susceptible wildlife did not explain FMD seroprevalence patterns or FMD outbreaks in livestock in this study. This suggests that FMD drivers in our study area may be different from drivers of outbreaks in southern African livestock. In South Africa and, until recently, Zimbabwe, FMD is tightly

controlled in livestock, but endemic in buffalo populations. Multiple reports from these countries implicate buffalo as sources of infection of FMDV for livestock (Caron *et al.*, 2013; Miguel *et al.*, 2013; Thomson *et al.*, 2003; Vosloo *et al.*, 2002a, b, 2010). There are also reports of various antelope species acting as intermediary transmitters of FMDV between buffalo and livestock (Hargreaves *et al.*, 2004; Jori *et al.*, 2009; Vosloo *et al.*, 2006). Reported FMDV prevalence is as high in East African buffalo (67 – 93% NSP antibody seroprevalence (Ayebazibwe *et al.*, 2010a, 2012; Bronsvoort *et al.*, 2008), Chapter 6) as it is in southern African buffalo populations (80 – 100% SAT antibody seroprevalence (Caron *et al.*, 2013)). However the crucial difference between the two ecosystems is its prevalence in livestock. In contrast to southern Africa (Brückner *et al.*, 2002), FMD is prevalent in domestic livestock in East Africa (48 – 76.3% NSP antibody seroprevalence in cattle, Ayebazibwe *et al.*, 2010c; Kibore *et al.*, 2013; Mkama *et al.*, 2014; Namatovu *et al.*, 2013a).

There are many reasons why livestock in an FMDV endemic population pose a more significant source of FMDV for other livestock compared to potential wildlife sources. Firstly, cattle shed more infectious FMDV than buffalo (Gainaru *et al.*, 1986). FMDV transmission from buffalo to cattle is very difficult to replicate experimentally (Anderson *et al.*, 1979; Bengis *et al.*, 1986; Condy & Hedger, 1974; Dawe *et al.*, 1994; Gainaru *et al.*, 1986; Vosloo *et al.*, 1996) whereas cattle to cattle transmission is a standard procedure in vaccine testing. Secondly, buffalo are dangerous animals and people avoid them if possible and similarly buffalo avoid people. Consistently with this, the households in the current study reported more frequent reports of sightings of wildlife other than buffalo. A study investigating buffalo-livestock contacts in Zimbabwe also reported that cattle and buffalo utilize shared resources such as watering holes at different times if possible, and contacts between the two species are not common (Miguel *et al.*, 2013). Buffalo movements are predictable, facilitating avoidance by cattle herders (Caron *et al.*, 2011). In contrast to buffalo-livestock contacts, this study shows extensive opportunities for contacts between livestock from different areas through movements, acquisitions and shared resources, especially in agropastoralist and pastoralist settings. Our findings fit with increased contacts and FMDV transmission between livestock compared to buffalo and livestock. This connectivity within the livestock population, in combination with the high seroprevalence of FMD reported in this study, supports the hypothesis that livestock in our study area constitute a maintenance population for FMDV. It is likely that even if livestock

were separated from all susceptible wildlife in northern Tanzania, FMDV would persistently circulate in livestock.

The lack of evidence for buffalo to livestock FMDV transmission in this study is consistent with other studies in East African settings. A study in Kenya, albeit with low sample numbers, found no evidence that buffalo and livestock shared SAT serotype FMDV variants (Wekesa *et al.*, 2015). Wildlife contact was not perceived to be an important risk factor for FMD outbreaks by veterinary services in Uganda, and more FMD outbreaks were reported in districts with high cattle movement compared to districts adjacent to national parks (Ayebazibwe *et al.*, 2010b). A study in Cameroon in West Africa found an association between sightings of forest buffalo and reports of FMDV, but noted that this was confounded by people who travelled further afield with their cattle being more likely to see buffalo (Bronsvoort *et al.*, 2004a). Similarly, in the current study, distance walked to reach grazing and water was positively associated with buffalo sightings. However, buffalo sightings did not improve the explanatory ability of the model.

The consistency of the findings in both the cross-sectional and case-control studies adds weight to the conclusion that contact with buffalo does not play a major role in FMDV infection in livestock in northern Tanzania. The statistical power to detect an effect was interrogated in both studies and the cross-sectional study was shown to be sufficiently large to detect an effect. In isolation, the case-control study had relatively less power, but its consistent conclusions potentiate those of the overall study.

Livestock practice was an important explanatory variable for FMDV exposure. Similarly, a study in Ethiopia reported that pastoralists were more likely than settled farmers to own FMD seropositive livestock (Megersa *et al.*, 2009). Larger herds, and increased movements and potential for contacts of agropastoral and pastoral livestock compared to those of smallholders may explain this finding. Consistent with this, the case-control study in the agropastoral district highlighted herd size and livestock acquisitions as risk factors for FMD outbreaks. Each individual animal in the herd has the potential to be exposed to FMDV from an outside source and then to infect its herd mates with close contact. Therefore larger herds mean more opportunities for livestock to introduce disease into the herd, and more animals within the herd for further transmission. Herd size was also a risk factor for FMD in Ethiopia (Bayissa *et al.*, 2011; Jenbere *et al.*, 2011), and is considered

important also in the spread of other diseases, for example bovine tuberculosis and brucellosis (Cleaveland *et al.*, 2007; Makita *et al.*, 2011).

Recent acquisitions of livestock were also identified as risk factor for FMD outbreaks, which is consistent with another study in Cameroon (Bronsvoort *et al.*, 2004a). Furthermore, district veterinary officers in Uganda perceived animal movements and the introduction of sick animals to increase the risk of FMD outbreaks (Ayebazibwe *et al.*, 2010b). Such acquisitions may result in the possible introduction of new FMDV variants to naïve animals.

Our finding that cattle are more likely to be seropositive than sheep or goats is consistent with reports from Uganda (Ayebazibwe *et al.*, 2010a; Namatovu *et al.*, 2013), and makes sense in the context of the experimental literature. Cattle are recognised to be more susceptible to FMD and show longer periods of virus persistence compared to sheep and goats (reviewed by Alexandersen *et al.*, 2002 and Arzt *et al.*, 2011a, b). Furthermore, a recent study suggested that, in a mixed population, sheep played a more limited role in the transmission of FMDV than cattle (Bravo de Rueda *et al.*, 2014). However, highly variable FMD patterns of infection and clinical signs have been reported in small-ruminants, and it is likely that the role of this species varies with different breeds and virus variants (Anderson *et al.*, 1976; Barnett & Cox, 1999; Kitching & Hughes, 2002). Whilst small ruminants in our study had lower FMDV seroprevalence compared to cattle, 48.5% seropositivity in these species still represents a very high burden of infection, which could result in reduced welfare and milk production and mortality of kids and lambs (Chapter 3).

As well as innate host factors as reasons for differences in susceptibility to FMDV, species-specific management factors may explain why cattle have higher FMDV seroprevalence than small ruminants. In the dry season, agropastoralists and pastoralists commonly take their cattle far afield to locate sufficient grazing and water, whereas the small ruminants are left at home (Dr. Lugelo, personal communication). Longer distance movements may result in increased opportunities for contacts with infected livestock. Another possible risk factor is cattle movement to avoid contracting malignant catarrhal fever (MCF) during the wildebeest calving season. As cattle are more susceptible to MCF than small ruminants, they are typically taken to the hills away from the wildebeest calving zone to avoid the risk of contracting the disease (Bedelian *et al.*, 2007; Cleaveland *et al.*,

2001; Lankester *et al.*, 2015b). On the hills, cattle from many different areas mix, presenting a suitable environment for FMDV transmission. This study also shows that cattle are frequently swapped between herds for temporary care new acquisitions are more common than for small ruminants.

Our finding of age as a risk factor for FMD-NSP sero-positivity is consistent with another risk factor studies in Ethiopia (Jenbere *et al.*, 2011)(Bayissa *et al.*, 2011; Megersa *et al.*, 2009) . There are several possible explanations for age as a risk factor for seropositivity to FMDV.

a) Firstly, older animals are as likely as younger animals to succumb to FMDV infections and they have been exposed to other risk factors for longer, and therefore are more likely to be seropositive. Herd owners report FMD lesions in adult cattle at least as often as in young cattle (Figure 3.8, Chapter 3). A study in Kenya reported that FMD lesion incidence rates in an outbreak did not decline with age (Lyons et al., 2015). Older cattle may be as likely to succumb to FMDV infection as:
    i. Post-infection immunity against one serotype wanes quickly leaving the animal susceptible to further infections by that serotype.
    ii. Many different antigenic types of FMDV are circulating, so past infection with one type will not confer immunity against serial infections with different serotypes.

b) Secondly, NSP antibodies decay slowly. Therefore animals will remain seropositive for many years, as has been demonstrated by (Elnekave *et al.*, 2015). In persistently infected animals, FMDV in the oropharynx may serve as a long-term immune stimulant for NSP antibody generation (Parida *et al.*, 2005).

c) Thirdly, even if animals have less virus replication in FMDV infections subsequent to their first infection, the anamnestic antibody response will boost NSP antibody levels.

These three hypotheses will be explored further through investigations of serotype-specific FMDV infection patterns in following chapters.

The findings of this study suggest that, while FMD is circulating widely, control efforts should focus on control of livestock related risk factors for infection. There is no indication from this study that measures to separate wildlife from livestock will reduce the FMD burden in northern Tanzanian livestock in the early stages of a control programme. The conventional ranch based fencing and biosecurity measures used in Southern Africa, may not be appropriate for the East African system and alternative vaccine based strategies may be a more workable solution in these settings.

Despite the results of this study, occasional transmission events from buffalo to livestock cannot be ruled out. Whilst FMD is prevalent in livestock, any signal of wildlife-to-livestock FMDV transmission in this study is likely to be drowned out by the dominance of livestock related risk factors. An intervention study, as proposed by Haydon *et al.* (2002) and Viana *et al.* (2014), where livestock related risk factors are controlled, or a more in depth study into the rural small holder area where there are fewer livestock related risk factors may be the approach necessary to investigate wildlife-livestock transmission. Investigations into FMDV circulation in northern Tanzanian buffalo populations (as described in Chapter 6), and antigenic and genetic comparisons between the FMDVs infecting wildlife and livestock are also necessary to address patterns of cross-species transmission.

Although several robust explanatory variables for FMDV seroprevalence and outbreaks were identified, there were also data-points that the models failed to explain, meaning that not all of the variability in the system as been accounted for. The likely reason for this is that the study has addressed only some aspects of FMDV epidemiology. To understand the complex web of host, virus and environmental factors present in this system, a wider range of the epidemiological and ecological tools are necessary. Further work is required to address the dynamics of herd immunity against different variants and serotypes of FMDV, the ecology and diversity of the FMDV population in the region, and the interaction between these two elements. Investigation into which antigenic and genetic types of FMDV are circulating in livestock and wildlife populations in the study area and comparisons between them will be the first step in addressing this (as described in Chapter 6). Longitudinal studies are also necessary to capture temporal patterns rather than snapshots of virus and host ecology (Chapter 5 and 6).

The outcome variable in this study, NSP seropositivity, comes from a commercial test that was originally designed for differentiating FMDV infection from vaccination for trade purposes rather than for unraveling the epidemiology of the disease in endemic countries (Chung *et al.*, 2002; Sorensen *et al.*, 2005). Some of the challenges of maximizing the information obtained from the NSP ELISA and other diagnostic tests in the context of FMD in endemic countries are addressed in Chapter 5. The case-control study avoids some of the issues of serological test interpretation by prospectively monitoring the study area and observing clinical signs of FMD rather than diagnosing infection retrospectively. The conclusion of both study types described in this chapter are consistent; livestock management practices are the most important risk factor for FMDV and there is no evidence for wildlife contact related risk factors in the study area.

Chapter 5: Inferring foot-and-mouth disease infection history from ELISA data

5.1 Summary

To understand the epidemiology of infectious diseases, infection must be diagnosed. The use of ELISAs to test serum for antibodies produced in response to infection represents an opportunity to expand diagnostic capacity in resource-limited settings, as it requires only modest laboratory equipment. However, the interpretation of serological data is confounded by issues of cross-reactivity between different pathogens and variants, ambiguity in the interpretation of positive and negative results, and limited knowledge about antibody decay rates after infection. East African livestock may be serially infected with different serotypes of FMDV, making the interpretation of FMD serological data in this region an epitome of these challenges.

In this study, a novel Bayesian approach was used to infer the FMDV infection history of Tanzanian livestock from serological data. Longitudinal ELISA data were generated from a cattle herd that suffered serial FMD outbreaks confirmed by genotyping to be caused by different FMDV serotypes. These data were used to train a model of herd and animal FMDV infection, ELISA reactivity dynamics, and cross-reaction between FMD serotypes on the ELISAs.

The ability of the model to infer (1) whether cattle were infected, (2) which serotype they were infected most recently with, and (3) how long ago they were infected from (a) longitudinal and (b) cross-sectional ELISA data was validated using subsets of the training data and a new dataset from a second herd with known infection history. The model was applied to a cross-sectional dataset from cattle with unknown infection histories and its inferences were compared to the results of virus neutralisation testing (VNT, the conventional gold-standard diagnostic test) from the same sera.

The model correctly inferred which serotype cattle were most recently infected with from both longitudinal and cross-sectional data. Outbreak timing could be inferred from longitudinal but not from cross-sectional data. Model inferences from cross-sectional data from animals with unknown histories about which serotypes caused the most recent outbreaks were completely consistent with VNT results. Modelling approaches such as this one represent an exciting opportunity to maximise the epidemiological information available from a simple and accessible diagnostic test.

5.2 Introduction

An understanding of infection patterns and risk factors is a key component in the control of infectious diseases. To achieve this, diagnosis of who was infected when and with which disease or variant is vital. However, it is a formidable undertaking to collect samples for pathogen detection in the acute stages of infection from enough individuals to make these inferences at population level. This challenge is especially relevant in developing countries, many of which have the highest burdens of infectious disease but are the most resource limited in terms of surveillance (The World Bank, 2010, 2012).

In contrast to the isolation of pathogens or their genome from animals or people in the acute stages of disease, collection of sera to test for evidence of past exposure to pathogens is less logistically demanding. Serum antibody levels against a pathogen remain elevated after clinical signs have disappeared or in sub-clinically infected individuals, facilitating retrospective diagnosis. Enzyme linked immuno-sorbent assays (ELISAs) are available for the serological diagnosis of exposure to many pathogens and require only modest laboratory facilities and operator training. This approach to diagnosis opens up the possibility of achieving high levels of disease surveillance in resource limited settings.

However, interpretation of serology results is not always straightforward. Continuous serology results are conventionally dichotomised into positive or negative results based on a cut-off value. This approach may suffer from false positive and false negative results Several studies have addressed these issues, promoting analysis of continuous results to obtain better inferences of population level disease prevalence (Bollaerts *et al.*, 2012; Dekker *et al.*, 2008; Nielsen *et al.*, 2007). Further studies have capitalised on flexible

Bayesian modelling approaches to make inferences from serological data about the ecology of multi-host pathogens (Viana *et al.*, 2015, 2016).

As well as issues with false positives and negatives, cross-reaction problems are common in ELISAs. Antibodies produced in response to exposure to one pathogen or variant may react with serological test antigens from another. These issues with cross-reaction are documented for serological tests for a range of pathogens (Table 5.1).

Table 5.1: Examples of pathogens presenting serological diagnostic challenges.

Group	Example pathogens	Example issue with interpreting serology results	Reference
Picornaviruses	Foot-and-mouth disease virus Rhinovirus	Cross-reaction between antigenic variants	(Namatovu *et al.*, 2013a, 2015; Wekesa *et al.*, 2015)
Lyssaviruses	Rabies virus	Cross-reaction between different lyssaviruses	(Xu *et al.*, 2007)
Phleboviruses	Rift valley fever	Cross-reaction between different phleboviruses	(Wu *et al.*, 2014)
Retroviruses	Human immunodeficiency virus	Cross-reaction between HIV and other pathogens	(Böttiger *et al.*, 1990; Jacobs *et al.*, 1992)
Flaviviruses	Dengue virus, West Nile disease virus	Cross-reaction between Flaviviruses	(Mansfield *et al.*, 2011)
Brucella	B. abortus, B. miletensis	Cross-reaction between brucella and other gram negative bacteria	(Kittelberger *et al.*, 1995) (Corbel *et al.*, 1984)
Leptospira	L. hardjo, L. ictohaemorragiae	Paradoxical response against different serotype to infecting serotype	(Craig *et al.*, 2009; Levett, 2003)
Rikettsia	Murine typhus Epidemic Typhus Rocky Mountain Spotted Fever	Cross-reaction between pathogens in Spotted fever or Typhus groups of rikettsia	(Hechemy *et al.*, 1989; Wächter *et al.*, 2015)
Borellia	Lymes disease	Cross reaction between Borellia and other spirochaetes	(Magnarelli *et al.*, 1987)
Mycobacteria	Johnes disease	Difficulty differentiating infected from non infecteds	(Nielsen *et al.*, 2007)

In the case of FMD in Northern Tanzania, at least four broad groups of antigenic variants (serotypes) are circulating in livestock (Genchwere *et al.*, 2014; Kasanga *et al.*, 2012). To understand the circulation of these variants and to inform vaccine choice for future control, diagnosis of infection with specific serotypes is critical. However, cross-reaction between different FMDV serotypes on ELISAs has confounded several studies of FMDV circulation in Africa (Namatovu *et al.*, 2013a, 2015; di Nardo *et al.*, 2012; Wekesa *et al.*, 2015).

Another limitation of conventional approaches to interpreting serological results is that they do not yield information about when the infection occurred. In areas where a disease

is prevalent, information about when animals were infected can contribute to understanding disease incidence in a region. In Chapter 4, 69% of cattle tested with an FMDV non-structural protein (NSP) ELISA were diagnosed as positive according to the manufacturer's recommended cut-off value. Only 6 out of 84 herds contained no seropositive cattle. Information about timing and frequency of infection could highlight patterns of FMD circulation and risk factors in this situation. This was addressed by age-stratifying animals in another African FMD study (Bronsvoort *et al.*, 2006a). To make more complete use of the data, inferring the difference, for example, between an animal that was infected one month ago and four years ago, from a serology result, would be greatly beneficial.

In this study, the aim was to increase the inference possible from serology results, making novel use of Bayesian methodology. The example of the endemic, multiple serotype circulation of FMDV in northern Tanzania was used and a model was developed to infer 1) which animals were infected 2) with which FMDV serotype and 3) when. To conduct this study, the unique resource of two intensively studied and sampled cattle herds in northern Tanzania that suffered serial FMDV outbreaks caused by different serotypes was available. These were part of a field vaccine trial for malignant catharral fever (Lankester *et al.*, 2015a, b). Data from the first of these herds were used to train a Bayesian model to interpret FMDV ELISA data and for initial validation. Data from the second herd were used for full validation. The ability of the model to infer animals' infection histories from both cross-sectional (single sample point in time) and longitudinal (multiple sample points over time) ELISA data was interrogated using these datasets. Finally, as an example of how this methodology could be applied, we used this model to infer information about unobserved FMDV outbreaks from both longitudinal and cross-sectional data.

5.3 Materials and methods

5.3.1 Data used

Herd 1

The study herd was based on the Simanjiro plains east of Tarangire National Park in northern Tanzania (Lankester *et al.*, 2015a, b). A group of 100 cattle between one and two

years of age was purchased from a local market. There were 24 females and 76 males in the herd and the majority were a local Tanzanian breed (Tanzanian-shorthorn-zebu). They were grazed and watered on the plains during the day and held in a pen overnight. Four animals died in April 2011. In October 2012, 44 animals were sold and the remaining 52 animals were maintained. One further animal died in March 2013. None of the animals died due to FMD (Lankester *et al.*, 2015a, b).

The cattle were monitored between December 2010 and November 2013. Signs of disease were recorded by herd attendants. Serum samples were taken from each animal at intervals between two weeks and five months. When FMD outbreaks occurred, veterinarians conducted an outbreak investigation as described in Chapter 2. Animals with lesions were identified and photographed. FMD lesion material was collected shipped to the World Reference Laboratory for FMD (WRL-FMD) for virus typing. From the photographs of FMD lesions that were of sufficient quality (n = 13 cattle for August 2011, 5 cattle for July 2012 and 4 cattle for June 2013), two FMD experts (Professor David Paton and Professor Satya Parida) estimated the time since the lesion first appeared.

Between January 2011 and November 2013, sera were obtained from Herd 1 at 19 different times (Figure 5.1). Three of the five reported FMD outbreaks were serotyped by virus isolation. These were confirmed to be caused by serotype SAT2 in August 2011, SAT1 in July 2012 and serotype A in June 2013 (Figure 5.1). There were 18 cattle with confirmed lesions in August 2011, 10 in July 2012 and 7 in June 2013. The herdsmen reported an FMD outbreak in early 2011 but there was no information available on the precise dates when this outbreak occurred or what serotype caused it. Sick cattle were also reported in September 2012 in a disease outbreak possibly caused by FMD but there was no clinical or virological confirmation for this. The SAT2 outbreak in 2011 was perceived by the herd attendants to be severe. The SAT1 outbreak in 2012 and the A outbreak in 2013 were perceived to be less severe, with fewer animals showing clinical signs.

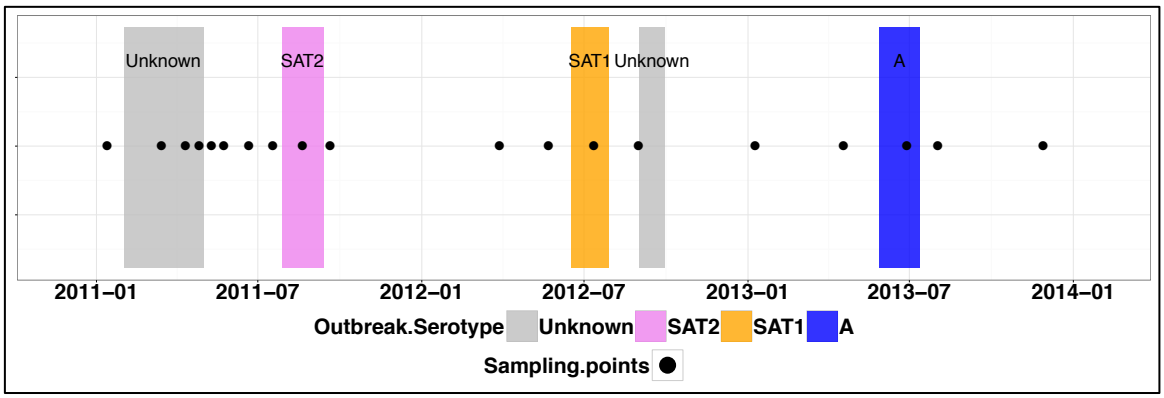


Figure 5.1: An overview of FMD outbreaks and sampling points over time (x axis) in Herd 1 – the training dataset for the model.
The grey areas mean that the herdsmen reported sick animals over this period but FMD was not confirmed. The coloured areas represent confirmed FMD outbreaks. The violet colour represents an outbreak caused by serotype SAT2, yellow represents a SAT1 outbreak and blue serotype A.

Herd 2

A second herd of 100 native breed cattle was observed and serum sampled between December 2011 and April 2013. Between these dates, they were sampled on eight occasions, at intervals between 1.4 and 4.4 months between samplings. These animals were purchased from two local markets and were aged between 1.5 and 2.5 years old. There were 26 females and 74 males and were managed similarly to Herd 1. Four of these animals died between February and July 2012. In April 2013, these animals were sold.

Between December 2011 and April 2013 there were eight serum-sampling points in Herd 2 (Figure 5.2). Herd 2 suffered an FMD outbreak in July 2012 (Figure 5.2). This was confirmed by virus isolation in the WRL to be caused by serotype SAT1. There were nine cattle in Herd 2 with confirmed lesions in this outbreak, and the experts estimated the ages of FMD lesions from eight animals.

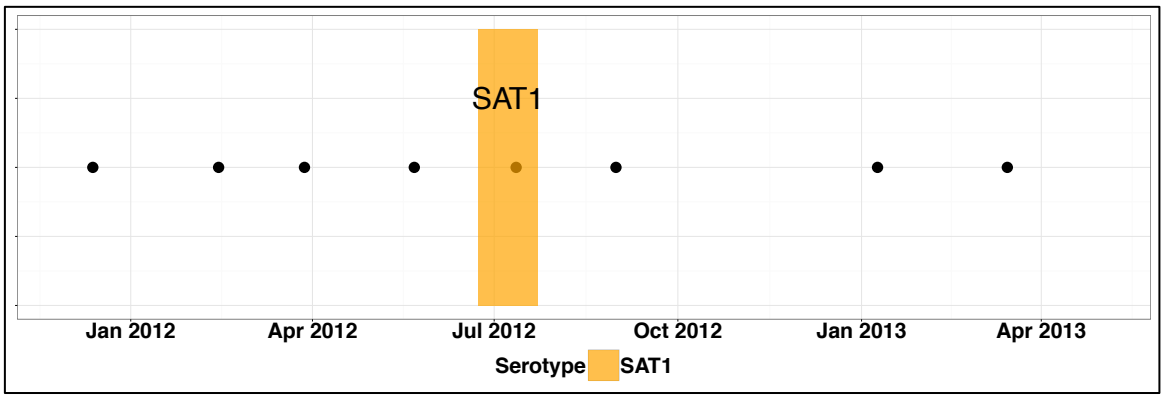


Figure 5.2: An overview of serum sampling points and the FMD outbreak caused by serotype SAT1 in Herd 2 – the test dataset.
The x-axis represents time. The yellow shaded area represents when the SAT1 outbreak occurred in this herd.

Cross-sectional study

The serological data for model application came from a subset (described in section 5.2.3) of the 2694 sera collected from 84 herds and 40 villages in the cross-sectional study as described in Chapters 2, 3 and 4.

5.3.2 Laboratory diagnostic assays

ELISAs

The sera from Herd 1, Herd 2 and the cross-sectional study were tested with the following ELISAs with the methods described in Chapter 2:

1. A commercial blocking ELISA[11] (Chung *et al.*, 2002) to detect antibodies against the non-structural proteins (NSP) of any serotype of FMDV.

2. Serotype specific solid phase competition ELISAs (SPCE) (Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004) to detect antibodies against the structural proteins of FMDV serotype O, SAT1 and SAT2. These assays were optimised to detect antibodies against the strains of these serotypes that are currently circulating in northern Tanzania.

[11] PrioCHECK FMDV NS ®, Life Technologies™, Thermo Fisher Scientific Inc., Platinastraat 33, Lelystad, Netherlands.

3. A serotype specific commercial blocking ELISA[12] was used to detect antibodies against the structural proteins of FMDV serotype A.

Virus neutralisation testing

Sera were tested in the WRL-FMD for neutralising antibodies against strains of FMDV serotype A, O, SAT1 and SAT2 that were isolated from northern Tanzanian cattle during the study period, as described in Chapter 2. These virus neutralisation testing (VNT) data were compared to model inferences from both the longitudinal and cross-sectional ELISA data.

5.3.3 Samples tested

All sera that were available from Herd 1 and Herd 2 were tested will all of the ELISAs. For Herd 1, a total of 8511 ELISA results were generated for 5304 unique animal-date-serotype combinations. For Herd 2, a total of 2844 ELISA results were generated for 2447 unique animal-date combinations. Tables 1 and 2 in the Appendix 5 show the numbers of samples from each date tested with each ELISA.

The VNT assay is time-consuming and expensive. Therefore sera from a subset of ten Herd 1 cattle sampled from between January 2011 and November 2013 were selected for VNT. This longitudinal dataset was tested to provide a standard against which to test the model's inferences, as VNT was believed to be more specific than the SPCE (Mackay *et al.*, 2001). These amounted to 332 unique animal-date-serotype combinations and 83 animal-date combinations.

All of the 2694 cross-sectional study sera were tested with the NSP ELISA. As there was a shortage of SPCE reagents, a sub-set of bovine sera was selected for both VNT and serotype specific ELISA testing. Samples were selected with the objective of identifying the most recent FMDV serotype to infect each cross sectional herd and village. Sera were

[12] PrioCHECK FMDV Type A PrioCHECK ®, Life Technologies™, Thermo Fisher Scientific Inc., Platinastraat 33, Lelystad, Netherlands.

selected at village and herd level from the youngest cattle with the highest FMDV NSP percentage inhibition (PI). Cattle with the highest NSP PIs were chosen was these were most likely to have been recently infected with FMDV and hence yield information on the most recent serotype to infect the herd. The youngest cattle (aged over six months to avoid maternally derived antibodies (Nicholls *et al.*, 1984)) were selected as they were less likely than older cattle to have suffered serial FMDV infections and therefore inference of the most recent serotype to infect the herd would be easier using their sera. The selection method is fully described in Appendix 5. A total of 96 sera from 60 herds in 36 villages were tested with the SPCE. A total of 128 sera from 77 herds in 40 villages were tested by VNT.

5.3.4 Software

Optical densities from the ELISA plates were recorded using Soft Max Pro [13] software and converted into text files. Laboratory ELISA plate-plans with animal ear-tag numbers were imported into the R Statistical environment (R version 3.1.3) (R development core team, 2008). The Bayesian models relating animal and herd FMD infection status to ELISA reactivity dynamics were written in JAGS version 3.3.0 (Plummer, 2012) and analysed using Markov Chain Monte Carlo (MCMC). The programmes Runjags version 2.0.1-4 (Denwood, 2015), Rjags version 3-13 (Plummer, 2013) and Coda version 0.17-1 (Plummer et al., 2006) were used to interface between R and JAGS and to analyse model outputs.

5.3.5 Bayesian Model structure

An autoregressive hierarchical mixture model was built. A high-level overview of the model is shown in Figure 3.

[13] 2015 Molecular Devices, LLC.

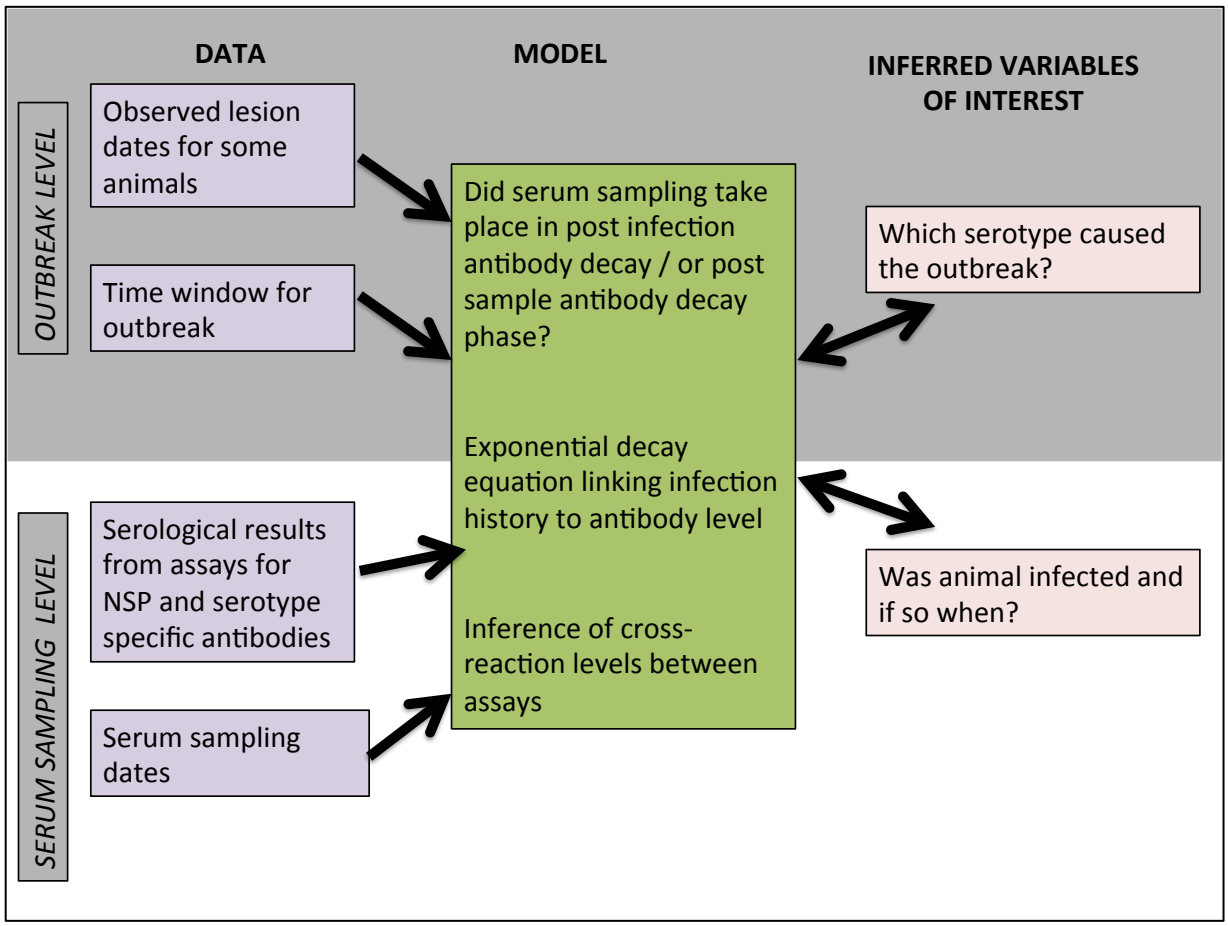


Figure 5.3: An overview of the model.

5.3.6 Assessment of MCMC convergence

A minimum of four MCMC chains was used for each model. As well as visual assessment of the MCMC traces for each parameter in the model, convergence of the different chains was summarized with the potential scale reduction factor (PSRF, ratio of between-chain variance to within-chain variance (Gelman & Rubin, 1992)). A PSRF value of 1.1 or less in combination of visual observation of convergence was considered to represent acceptable convergence between chains for each parameter.

5.3.7 Model selection

Model selection was based on biological plausibility, convergence and performance upon validation. The deviance information criterion that is commonly used for Bayesian model selection could not be used in this study due to limitations in the estimation of the effective number of parameters in mixture models such as the model in this study (Plummer, 2008).

5.4. Bayesian Model

5.4.1 Model assumptions and priors

Indices used in the model are summarised on Table 5.2. These are listed as they are used later in describing the priors and structure of the model.

Table 5.2: Indices used in the model.

Index	Explanation
i	Animal (1… number of animals in herd)
j	Serum sampling point (1 to Number of sampling points)
k	Outbreak number (1 to Number of outbreaks over study period)
n	Serotype of SPCE $n \in S = \{SAT1, SAT2, O, A\}$
h	Herd in cross-sectional study (Herd 1 to 84)
v	Village in cross-sectional study (Village 1 to 40)
d	District in cross-sectional study (District 1 to 5)

Where information was available from the literature, it was used to inform priors for the model. Where no information was available, biologically plausible uninformed priors were provided (Table 5.3).

Table 5.3: Prior assumptions and basic structure of the model. Where parameters are allowed to behave differently in the case of non-structural protein (NSP) and serotype specific structural protein (SP) ELISA reactivity, the parameters relating to SP are marked with an accent (^). Precision (the inverse of variance) is used to parameterize Normal distributions in JAGS.

Prior	Explanation	Reference	Table reference
$p_k \sim Uniform(0,1)$ $f_{i,k} \sim Bernoulli(p_k)$	Animal infection status ($f_{i,k}$) in outbreak k can be infected (1) or not infected (0), and has a probability of p_k of being infected.		1
$\alpha = (0.25, 0.25, 0.25, 0.25)$ $s_k \sim Multinomial(\alpha, 1)$	The FMDV serotype (s_k) causing each outbreak can be one of four with a flat prior (α) for which serotype.	(Genchwere *et al.*, 2014; Kasanga *et al.*, 2012)	2
$\varepsilon_{d,n} \sim Gamma(0.001, 0.001)$ $\rho_{v,n} \sim Dirichlet(\varepsilon_{d,n})$ $\varphi_h \sim Multinomial(\rho_{v,n}, 1)$	Villages in the same district share a parameter ($\varepsilon_{d,n}$) influencing the probability of animals in the village ($\rho_{v,n}$) being infected with one of four serotypes in their most recent FMD outbreak. The serotype that most recently infected herds in that village (φ_h) is influenced by the village level probability		3
$\chi_h \sim Uniform(1, 1095)$	The time since the most recent outbreak (χ_h) in each cross-sectional herd was assumed to be three years or less as NSP positive animals were selected.		4
$\tau \sim Gamma(0.001, 0.001)$ $g_{i,j} \sim Normal(a_{i,j}, \tau)$ $\hat{\tau} \sim Gamma(0.001, 0.001)$ $\hat{g}_{n,i,j} \sim Normal(\hat{a}_{n,i,j}, \hat{\tau})$	NSP ($g_{i,j}$)and SP ($\hat{g}_{n,i,j}$) ELISA results are normally distributed around the true NSP ($a_{i,j}$) and SP ($\hat{a}_{n,i,j}$) ELISA reactivity levels with uninformed priors for precision τ and $\hat{\tau}$. • $a_{i,j}$ is related to $f_{i,k}$, θ, r and $\lambda_{i,k}$, as described fully in Equation 5.1 • Similarly $\hat{a}_{n,i,j}$ is related to $f_{i,k}$, $\hat{\theta}$, r, $\lambda_{i,k}$ and also $\gamma_{n,s}$ as described fully in Equation 5.2	(Chung *et al.*, 2002; Dekker *et al.*, 2008; Sorensen *et al.*, 2005) (Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004)	5
$\gamma_{n,s} \sim \begin{cases} 1 & n = s \\ Uniform(0,1) & n \neq s \end{cases}$	Cross-reaction between assay antigen and outbreak antigen is maximal (= 1) when the serotype causing the outbreak is the same as the serotype in the SPCE. If the SPCE is for a serotype other than that causing the outbreak, cross-reaction levels can vary.	((Namatovu *et al.*, 2013a, 2015; di Nardo *et al.*, 2012; Wekesa *et al.*, 2015)	6
$\omega \sim Uniform(90, 3650)$ $\theta \sim 2^{-1/\omega}$ $\hat{\omega} \sim Uniform(90, 3650)$ $\hat{\theta} \sim 2^{-1/\hat{\omega}}$	NSP and SP ELISA reactivity levels decay with a half-life of between 3 months and ten years and corresponding daily decay rates θ and $\hat{\theta}$ for NSP and SP reactivity respectively.	(Moonen *et al.*, 2004a)	7
$\sigma \sim Uniform(1, 10)$ $\mu_k \sim Normal(m_k, \frac{1}{\sigma^2})$	Where lesion aging data were availeable, the mode for all peak reactivity times in the herd for the outbreak were normally distributed around the median first lesion time (m_k) with a standard deviation (σ) between 1 and 10 days. Where data were not available or removed, a time window (uniform distribution) was provided as a prior for μ_k. .		8
$\lambda_{i,k} \sim Gamma(c_k, q_k)$	Times of peak ELISA reactivity in each outbreak follow a gamma distribution. The shape (c) and rate (q) for each outbreak correspond to the mode (μ_k) and a standard deviation between 2 and 20 days. Please see Appendix 6 for how shape and rate were related to mode and standard deviation.		9
$r \sim Uniform(0.8, 1)$	If the animal is infected during the outbreak, ELISA reactivity peaks at a maximum threshold (r) that occurs at time $\lambda_{i,k}$. Peak reactivity is constrained with a fixed range.	(Dekker *et al.*, 2008)	10

5.4.2 Terminology

The model describes the relationship between ELISA reactivity and animals' infection histories. The term "ELISA reactivity" (ER) was used to represent measure of the ability of the sera to bind to the antigens on the ELISA plate, rather than the term "antibody levels," as not enough is known about antibody dynamics in serially infected cattle, or about their relationship to ELISA results, to accurately model them with the experiment described here.

5.4.3 Assumptions made in the model

1. **Assumption 1: ELISA reactivity levels and assay results:** The FMDV non-structural protein (NSP) ELISA test result (PI, percentage inhibition) is related to levels of antibodies against non-structural proteins in the test serum (Chung *et al.*, 2002; Dekker *et al.*, 2008; Sorensen *et al.*, 2005). The FMDV solid phase competition ELISA (SPCE) PI is related to levels of antibodies against serotype-specific structural proteins (SP) (Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004). The reactivity levels reflected by the ELISA results ($g_{i,j}$ and $\hat{g}_{n,i,j}$) are normally distributed around the true ELISA reactivity levels ($a_{i,j}$ and $\hat{a}_{n,i,j}$). (Reference 5 on Table 5.3).

2. **Assumption 2: ELISA reactivity dynamics after infection:** ELISA reactivity levels at each sampling point were modelled as a piecewise continuous function with two possible states related to outbreak timing and animal infection status (based on references 1, 8 and 9 on Table 5.3 and equation 5.1).

   State 1: No FMDV infection since the last sampling point. ELISA reactivity levels at the time of the current sampling point (t_j) have decayed from levels at the last sampling point (t_{j-1}). All decay was assumed to be exponential with a half-life of ω and corresponding decay rate of θ (Dekker *et al.*, 2008; Moonen *et al.*, 2004a).

   State 2: FMDV infection since the last sampling point. ELISA reactivity levels at the time of the current sampling point (t_j) have decayed from a maximum level at the time of maximum antibodies subsequent to the infection ($\lambda_{i,j}$) with the same decay rate θ. Figure 5.4 illustrates an example of these dynamics.

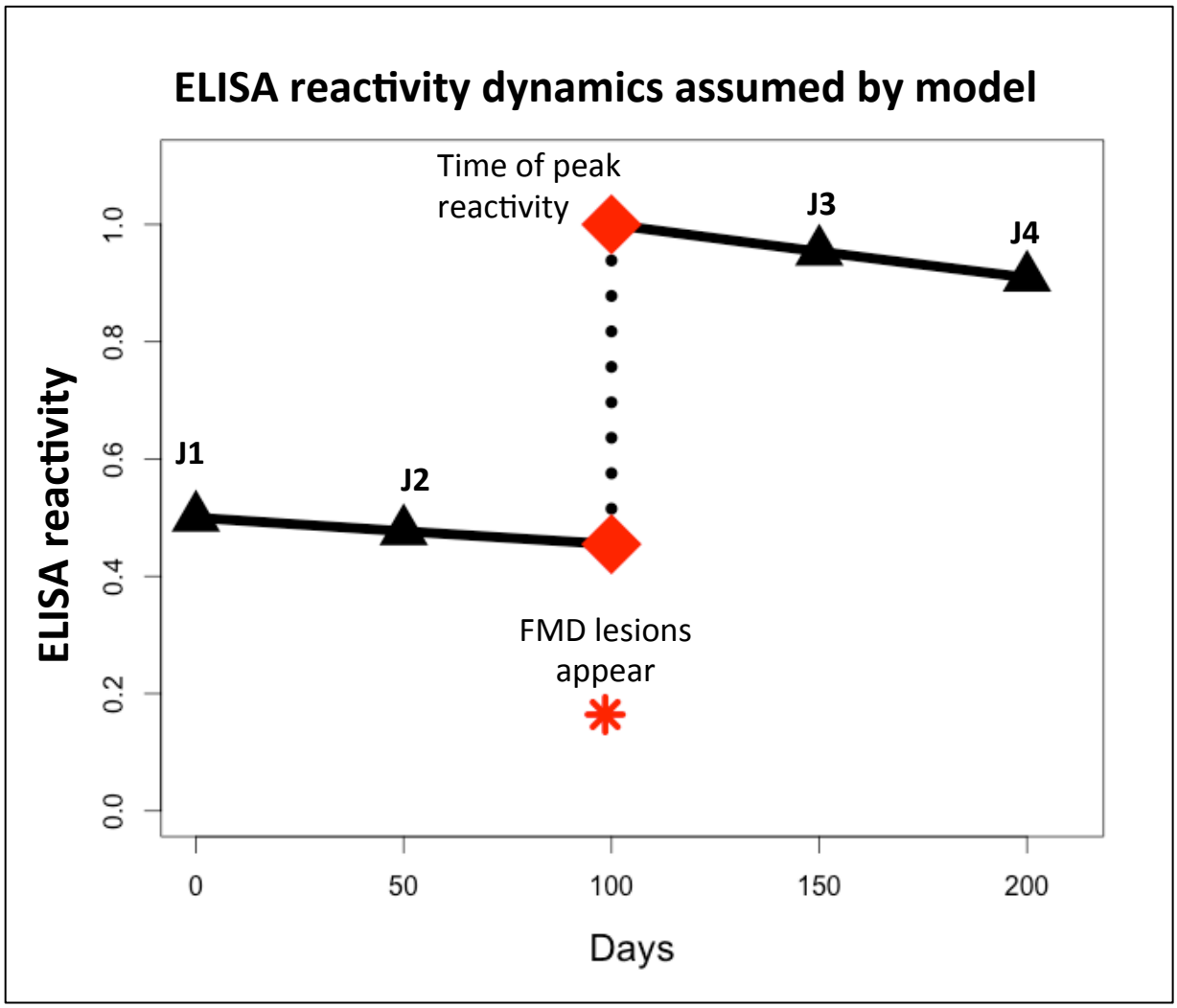


Figure 5.4: Assumed dynamics of ELISA reactivity against foot-and-mouth disease virus non-structural proteins with half-life set (as an example) at two years in an individual animal.
The black triangles represent serum-sampling points (J1-J4). The red diamonds at 100 days represent when the animal's antibodies reached the peak level subsequent to infection. The animal's state switches from decay after the last sample directly to maximum ELISA reactivity levels without a growth phase. This is indicated by the dotted line. This animal was not infected between J1 and J2 and between J3 and J4 respectively. Therefore the ELISA reactivity levels at J2 and J4 have decayed from what they were at J1 and J3. In contrast, the animal was infected between J2 and J3. Therefore the ELISA reactivity levels at J3 have decayed from the maximum ELISA reactivity level.

Equation 5.1 describes the decay of NSP ELISA reactivity, depending on whether the animal is in State 1 or State 2 at a particular time-point, as shown in Figure 5.2.

$$a_{i,j} = \begin{cases} a_{i,\ j-1} \times \theta^{t_j - t_{j-1}} & \textit{if no FMDV infection } (\textit{State } 1) \\ r \times \theta^{t_j - \lambda_{i,j}} & \textit{otherwise } (\textit{State } 2) \end{cases} \qquad \text{Equation 5.1}$$

3. **Assumption 3: Cross-reaction between different serotypes:** Antibodies that are elevated subsequent to an infection with one serotype of FMDV may bind to the structural proteins of another serotype and influence ELISA reactivity. A measure of this cross-reaction was included as the parameter $\gamma_{n,s}$ in the model where the minimum value was zero (no cross-reaction between assay serotype and infection serotype) and the maximum value was one. (Reference 6, Table 5.3).

   If the animal has not been infected with FMDV since the last sampling point (State 1), SP ELISA reactivity decays exponentially from levels at the last sampling point with a half-life of $\widehat{\omega}$ and corresponding decay rate of $\widehat{\theta}$. If the animal has been infected (State 2), SP ELISA reactivity decays from a level determined by cross-reaction ($\gamma_{n,s}$) between SPCE serotype (n) and outbreak serotype (s_k) and the window over which this increase can happen. If the outbreak serotype and SPCE serotype are the same, the maximum cross-reaction value (1) applies. A possible example of these dynamics is shown in Figure 5.5. (Based on references 1, 6, 8 and 9 on Table 5.3 and equation 5.2).

   Equation 5.2 describes SP ELISA reactivity dynamics in either State 1 or State 2, taking cross-reaction on assays against different serotypes into account.

$$\hat{a}_{n,i,j} = \begin{cases} \hat{a}_{n,i,j} \times \hat{\theta}^{t_j - t_{j-1}} & if\ not\ infected\ (State\ 1) \\ \left((\hat{a}_{n,i,j-1} \times \hat{\theta}^{\lambda_{i,j} - t_{j-1}}) + \gamma_{n,s}\left(h - (\hat{a}_{n,i,j-1} \times \hat{\theta}^{\lambda_{i,j} - t_{j-1}})\right)\right) \times \hat{\theta}^{t_j - \lambda_{i,j}} & otherwise\ (State\ 2) \end{cases} \quad \textbf{Equation 5.2}$$

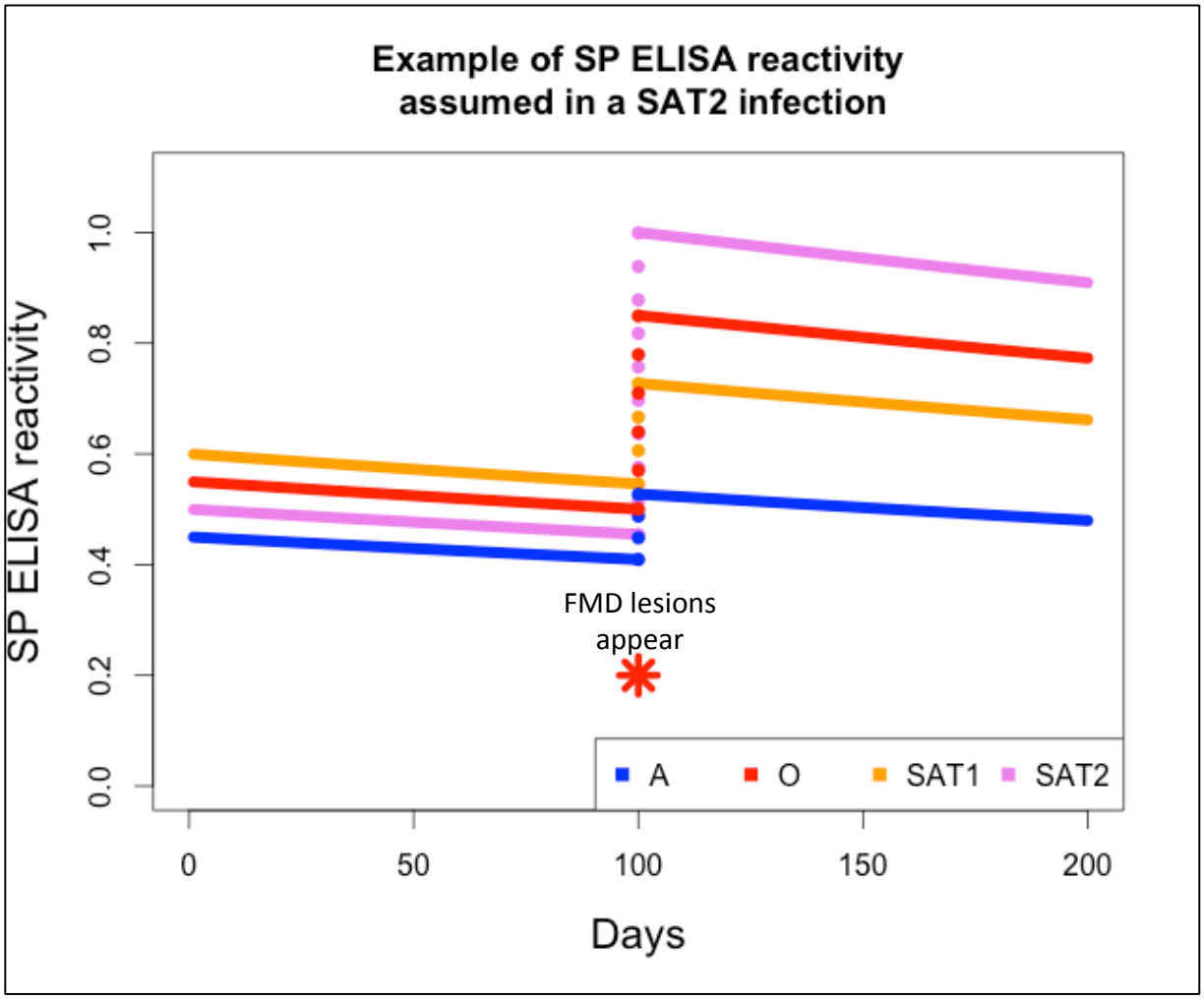


Figure 5.5: An example of how cross-reaction between antibodies against different serotypes of foot-and-mouth disease virus (FMDV) could be inferred within the assumptions of the model for an individual animal.
The different coloured lines represent antibody levels against the four circulating serotypes of FMDV (A=blue, O=red, SAT1=yellow, SAT2=violet). The antibody levels shown at day zero are arbitrary. If the animal is infected with SAT2 serotype FMDV, antibodies against SAT2 will increase to a maximum level (violet line). The levels of antibodies against the other serotypes increase to varying degrees depending on their level at the point of infection and on the degree of cross-reaction between them and the serotype (SAT2 in this case) that is causing the outbreak. After the time of maximum antibodies subsequent to infection, antibody levels against all serotypes will decay.

4. **Assumption 4: Time of peak antibodies after infection:** Given the temporal scale of the model spanning multiple years, for the purposes of this study, time of peak ELISA reactivity ($\boldsymbol{\lambda_{i,k}}$) was assumed to be the same time as when lesions first appeared on infected animals.

5. **Assumption 5: Outbreak time and individual animal infection times**
    a. In the simplest versions of the model, all animals infected in a particular outbreak were assumed to reach their ELISA reactivity peak on the same day (Group 1 models in Table 5.4).
    b. In a more complex version of the model, each animals infected in a single outbreak were assumed to reach their peak ELISA reactivity levels at a time ($\lambda_{i,k}$) distributed around a mode of μ_k and within a 90-day period (Group 2 models in Table 5.4, References 8 and 9 Table 5.3). Where available, lesion-dating information could be incorporated as training data into the model as $m_{k,}$ (around which μ_k was normally distributed), and as data-points for $\boldsymbol{\lambda_{i,k}}$ (References 8 and 9, Table 5.3). Where lesion-timing data were not available or removed for validation, a time window (uniform distribution) was provided as a prior for μ_k**.** The width of the time window allowed varied between five months and ten years, depending on the validation or application. (Details in Sections 5.3.6 and 5.3.7).

6. **Maximum ELISA reactivity thresholds:** The model included a maximum threshold for ELISA reactivity levels (r). In Models 1A and 2A (on Table 5.4 below), this threshold was fixed. Models "1B" and "2B" allowed the model to converge on a maximum reactivity threshold (rather than fixing it) and models "1C" and "2C" allowed this threshold to vary for each individual animal (Reference 10, Table 5.3).

7. **Outbreak serotype:** For any outbreak, the dominant virus causing the outbreak could be one of the four serotypes that are currently circulating amongst livestock in Northern Tanzania (Genchwere *et al.*, 2014; Kasanga *et al.*, 2012). For the study herd, this is s_k and for the cross-sectional herds it is s_h (Reference 2, Table 5.3).

8. **Common hyper-parameters for meta-populations:** When the model was used to

infer infection histories from the cross-sectional study data, herds from the same village, and villages from the same districts were assumed to share hyper-parameters influencing which serotype of FMDV caused the most recent FMD outbreak. That is s_h (herd serotype) depends on $\rho_{v,n}$ (village serotype probability), which depends on $\varepsilon_{d,n}$ (a prior parameter common to all villages in the same district). (Reference 3, Table 5.3).

A summary of the assumptions made in different models is presented on Table 5.4.

Table 5.4: The assumptions made in different model versions. The numbers in the top row are cross-references to model assumptions 1 – 7 described above and the test on the second row is a brief description of these assumptions.

	1	2	3	4	5	6			7
Model	**Assay and antibody levels**	**ELISA reactivity dynamics after infection**	**Cross-reaction between serotypes**	**Time of peak reactivity**	**Time of peak reactivity allowed to vary for individual animals**	**Fixed maximum reactivity threshold**	**One maximum reactivity threshold**	**Individual maximum reactivity threshold**	**Outbreak serotype**
1A	✔	✔	✔	✔		✔			✔
1B	✔	✔	✔	✔			✔		✔
1C	✔	✔	✔	✔				✔	✔
2A	✔	✔	✔	✔	✔	✔			✔
2B	✔	✔	✔	✔	✔		✔		✔
2C	✔	✔	✔	✔	✔			✔	✔

5.4.4 Model validation

Validation of longitudinal inferences

Validation of longitudinal inferences using training data

All models were assessed for convergence with the training data. They were validated by omitting some or all information about outbreak serotype, timing and infected animals and leaving only the longitudinal ELISA data. The ability of the models to converge on the correct serotype, outbreak time and infected animals using only longitudinal ELISA data was tested. As there were sequential outbreaks in Herd 1, when all data for one outbreak

was removed for validation, data for the other outbreaks were left in place as training data. Time windows of 13 months, 18 months and 12 months, the widest possible given the sequence of outbreaks, were provided as uniform priors from which the model selected outbreak times for the SAT2, SAT1 and A outbreaks respectively.

Validation of longitudinal inferences using test data

The model that performed best upon validation with the longitudinal training data from Herd 1 was tested with a new dataset from study Herd 2 (test data) that had not been used for model training. The model was adapted to include a second longitudinal dataset with independent inference for outbreak time, serotype and infected animals.

The full training dataset including outbreak serotypes, timing and known clinically affected animal data from Herd 1 was fed to the model in addition to test dataset ELISA data only from Herd 2. Both sections of the model shared parameters for ELISA reactivity decay rate and ELISA cross-reactivity. The ability of the model to infer outbreak serotype, timing and infected animals from the test dataset was validated. A maximal time window of 15 months (the period over which the herd was serum sampled) was provided.

Validation of cross-sectional inferences

The cattle with confirmed infection histories were selected from the Herd 1 and Herd 2. Results from NSP ELISAs and SPCEs from one point in time from each of these animals were fed to the model as cross-sectional data and the model's ability to infer when they were infected and with which serotype was tested. Different points in time after the animals' confirmed infections were selected to test the model's ability to infer infections at different durations of time since the infection. Where animals had the same confirmed infection histories, they were grouped as "herds" within the model structure. This validation was testing the model more severely than the application dataset, which incorporated a hierarchical structure to assist inference at village and district level. In contrast, the validation was testing the model's ability to make inferences from small groups of animals in isolation. A window for the time since the most recent outbreak of up to ten years was allowed.

5.4.5 Model application

Inference of the serotype of an uncharacterised FMD outbreak

The herd managers noted that an FMD outbreak occurred in study Herd 1 in Spring 2011 but lesion material was not collected from the animals and the dates that animals presented with lesions were unknown. The model was used to infer the serotype and timing of this outbreak. Sick animals were also reported in Herd 1 in September 2012, but no lesion material was available to confirm FMD or identify the serotype. The model was used to interrogate whether or not FMD caused the animals to be sick during this period. Five-month time-window priors were allowed for each uncharacterised outbreak.

Inference of infection history at village and district level from cross-sectional data

The cross-sectional data from animals with unknown infection history were incorporated into the model adapted for cross-sectional data. As these data came from NSP seropositive animals (Appendix 5), a window of 3 years was allowed from which to select time since the most recent outbreak. Model inferences were then used to add evidence to patterns of serotype dominance over time in East Africa as described in Chapter 6.

5.5 Results

5.5.1 ELISA Data

Using a cut-off value of 50% percentage inhibition (Chung *et al.*, 2002; Li *et al.*, 2012; Paiba *et al.*, 2004), the proportion of positive and negative ELISA results at each sampling point in Herd 1 and Herd 2 over the course of the study are shown in Figures 5.6 and 5.7 respectively. It is not possible to tell which serotype caused which outbreak from the ELISA results interpreted in the conventional way. This represents the motivation for the development of the Bayesian model described in this chapter to infer animals' infection histories from ELISA data.

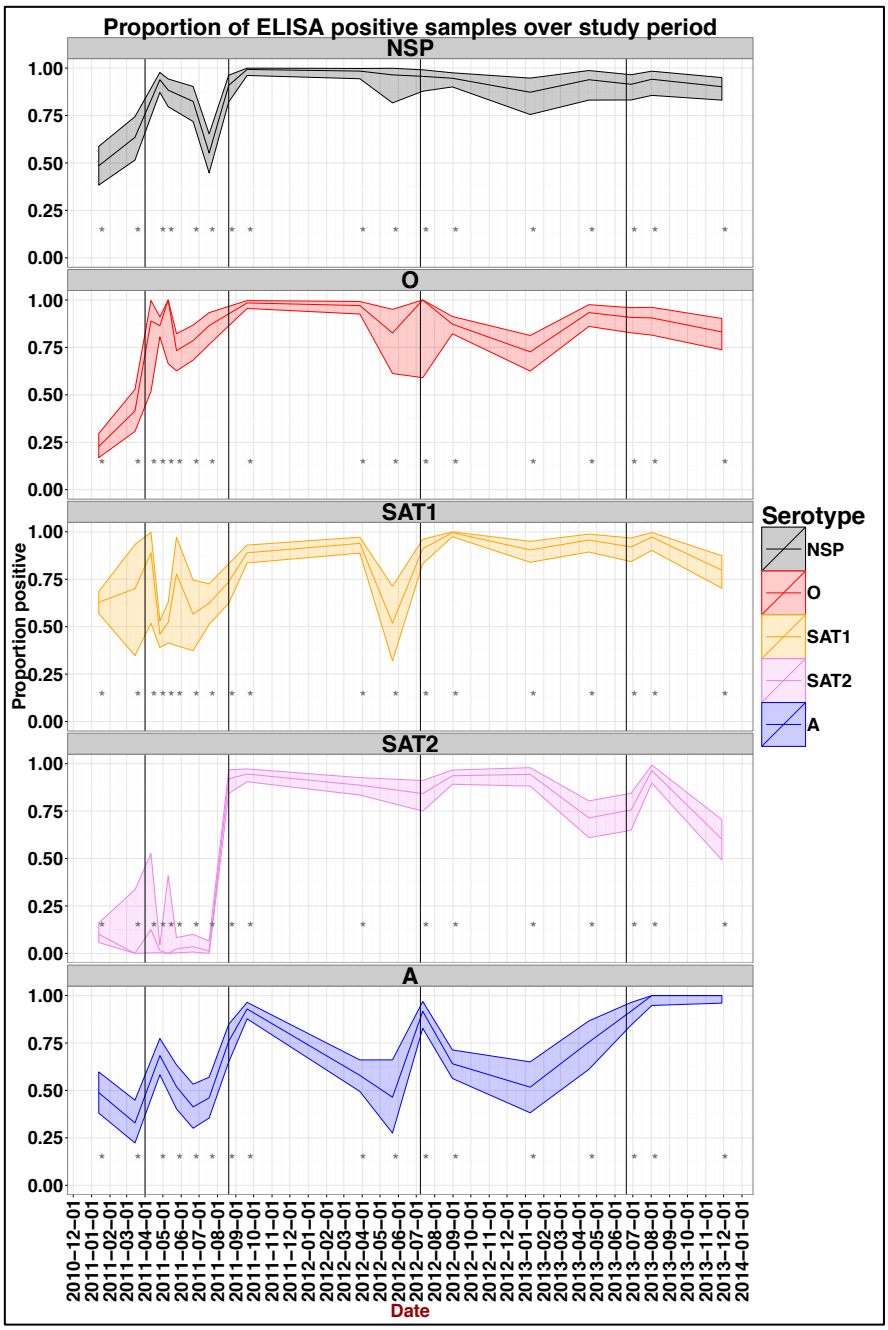


Figure 5.6: A line plot showing the proportion of positive ELISA results over the study period in Herd 1.
The shaded areas indicate binomial 95% confidence intervals for proportions. The vertical lines represent when FMD outbreaks occurred. (The initial vertical line in Spring 2011 is based on model inferences described later. The remaining vertical lines represent confirmed SAT2, SAT1 and A outbreak times). The stars represent serum sampling time-points.

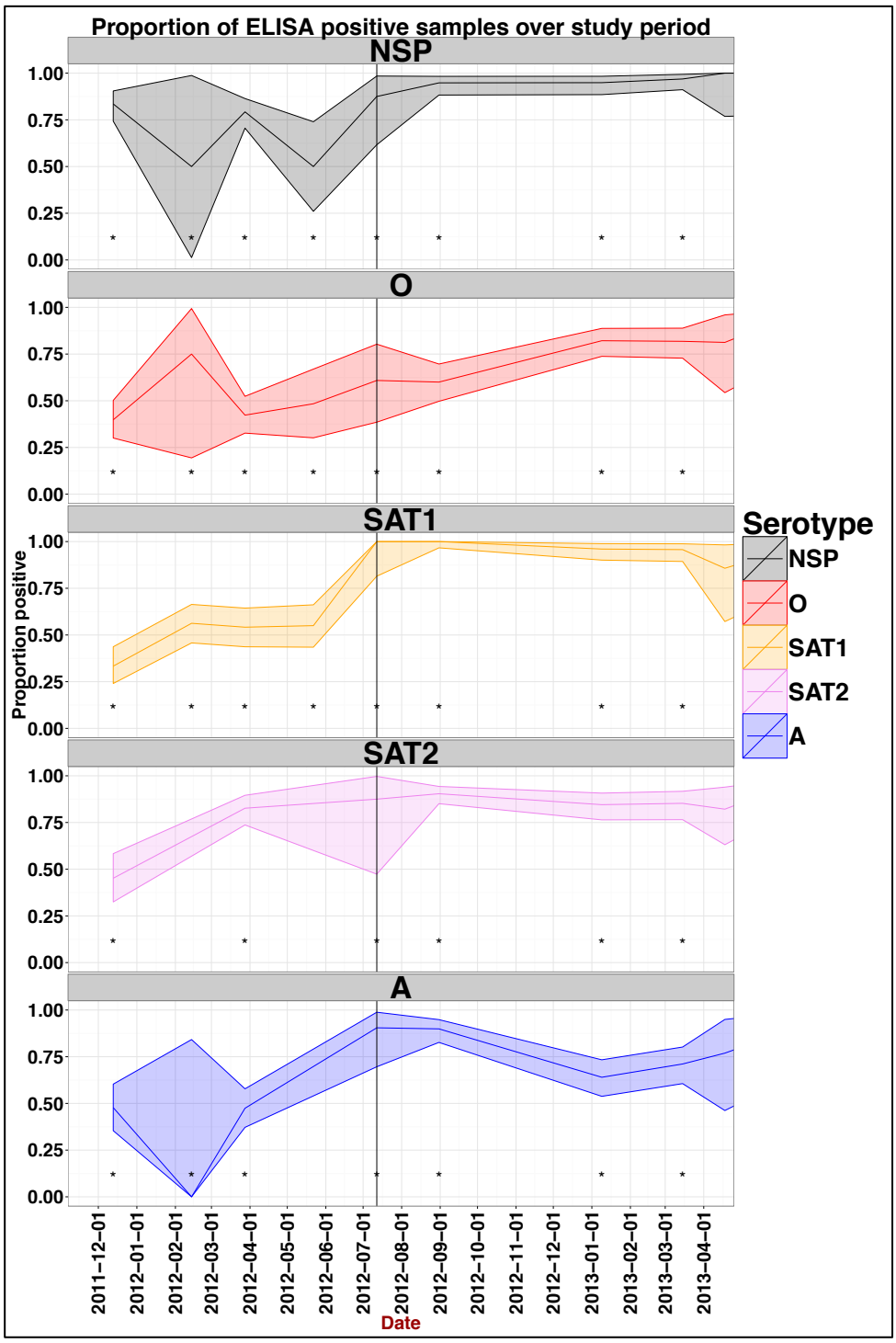


Figure 5.7: A line plot showing the proportion of positive ELISA results over the study period in Herd 2.
The shaded areas indicate binomial 95% confidence intervals for proportions. The vertical line represents when the SAT1 FMD outbreak occurred. The stars represent serum sampling time-points.

Figure 5.8 summarises the SPCE results from the 96 cross-sectional sera dichotomised in the conventional way. In three out of the five districts, more than 50% of the young cattle

are positive for three out of four serotypes, making it difficult to tell which serotype passed through the district most recently.

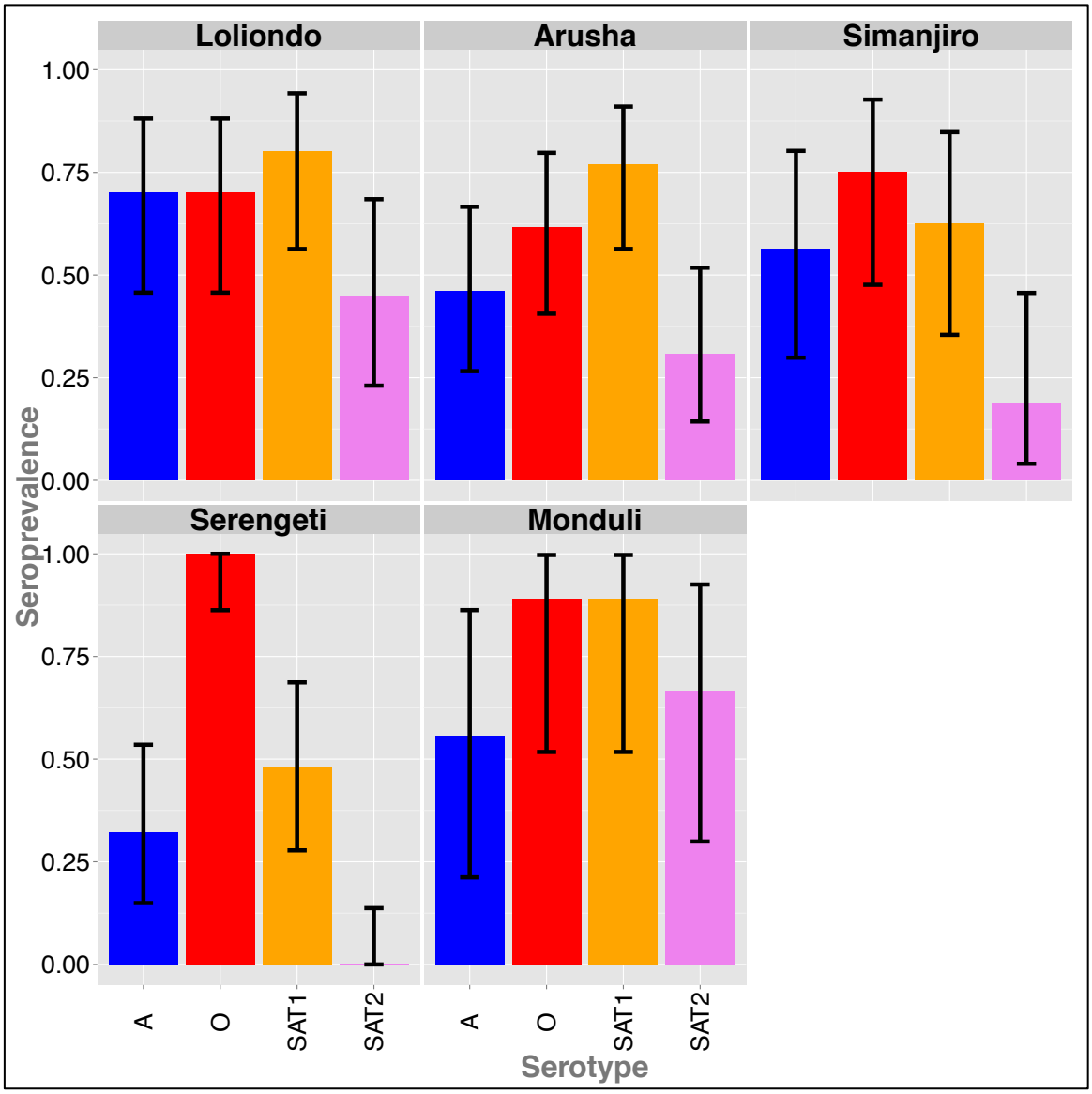


Figure 5.8: Seroprevalence of serotypes A (blue), O (red), SAT1 (yellow) and SAT2 (violet) detected by solid-phase competition ELISA in the five districts in the cross-sectional study. The bars indicate binomial 95% confidence intervals for proportions.

5.5.2 Model selection

The fixed outbreak date models (Model 1 group, Table 2) were built using the known dates of the SAT2 (Aug 2011), SAT1 (July 2012) and A (June 2013) outbreaks. For the

unknown outbreak in Spring 2011, dates every month from February 1st to May 1st were trialled. Initial model selection was based on (a) convergence of the MCMC chains and (b) correct selection of outbreak serotype for all three known outbreaks when these data were not fed to the model. The models using April 1st 2011 for the date of the unknown outbreak converged on the correct serotypes for the known outbreaks. 100% of hyper-parameters also converged, giving the best convergence and validation performance out of fixed date the models trialling four different months. Figure 5.9 shows these convergence results for the simplest fixed time model (Model 1A). On Figure 5.9, convergence was reported for 22 hyper-parameters; ELISA cross-reaction for each combination of serotypes, decay rates for ELISA reactivity for NSP and SP ELISAs, outbreak serotype and outbreak probability of infection. The model converged on serotype O as the causative virus of this April 2011 outbreak.

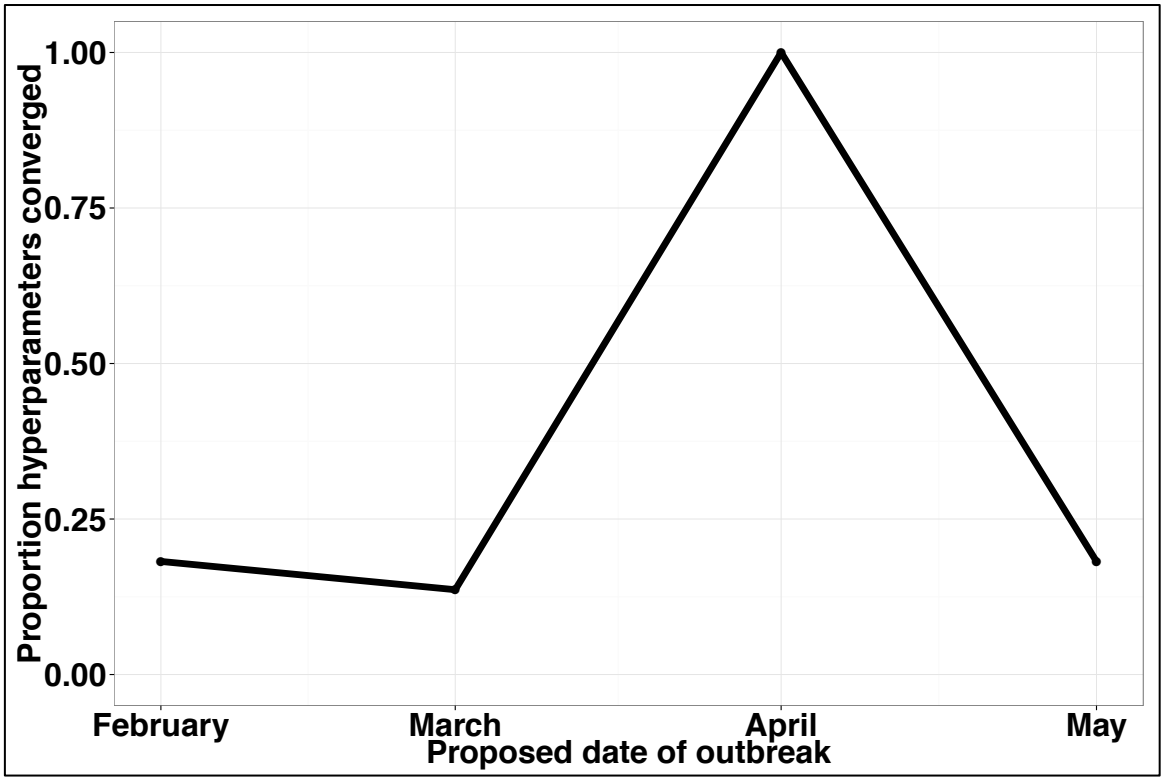


Figure 5.9: The proportions of converged hyper-parameter variables (with the potential scale reduction factor below 1.1) in fixed date models trialling different dates for the unknown outbreak in Spring 2011.
There was a 1,000-sample adaptation period and a 10,000 sample burn-in period before the convergence for the chains began to be monitored. Thereafter the four MCMC chains ran for 200,000 samples, which were thinned by 100. No outbreak serotype information was provided to the models. Output from the simplest mode, model 1A, is shown. Convergence was reported for 22 hyperparameters; ELISA cross-reaction for each combination of serotypes, decay rates for ELISA reactivity for NSP and SP ELISAs, outbreak serotype and outbreak probability of infection.

To investigate whether an FMD outbreak caused cattle to be sick in September 2012 as the herdsmen described, a potential September 2012 outbreak was incorporated into the simplest fixed date model (Model 1A, Table 2). However, the model failed to converge on the correct sequence of serotypes of the known outbreaks and on many other variables. This suggested that reported disease in September 2012 was not caused by FMDV. These inferences supporting the occurrence on a serotype O outbreak in April 2011 and rejecting an FMD outbreak in September 2012 highlight the utility of the model in gleaning information about unknown FMD infection histories from serological data.

Models that allowed variable dates for outbreaks (Models 2A, B and C) were built since variable outbreak durations and variable individual animal infection times were more biologically plausible than single outbreak dates. Further, the Model 1 group converged when fed with either infected animal data or outbreak serotype data, but could not do so when both sets of data were omitted (Table 5.5, Figure 5.10).

Table 5.5: Validation of model inferences form longitudinal data about the FMDV serotype causing the outbreak and which animals were infected.
The colour codes for the different models shown on the second row are to correspond with Figure 5.10. Convergence failures are highlighted in red.

		MODELS					
	Outbreak	**MODEL 1A**	**MODEL 1B**	**MODEL 1C**	**MODEL 2A**	**MODEL 2B**	**MODEL 2C**
Infected animal validation	**Aug-11**	18 out of 18	18 out of 18	18 out of 18	18 out of 18	18 out of 18	18 out of 18
	Jul-12	9 out of 11	10 out of 11	11 out of 11	8 out of 11	8 out of 11	9 out of 11
	Jun-13	6 out of 7	6 out of 7	6 out of 7	6 out of 7	7 out of 7	6 out of 7
Outbreak serotype validation	**Aug-11**	SAT2	SAT2	SAT2	Not converged	SAT2	Not converged
	Jul-11	SAT1	SAT1	SAT1	Not converged	SAT1	Not converged
	Jun-13	A	A	A	Not converged	A	Not converged
Infected animal Validation and serotype validation together	**Aug-11**	18 out of 18	18 out of 18	18 out of 18	18 out of 18	18 out of 18	18 out of 18
		Not converged	Not converged	Not converged	Not converged	SAT2	SAT2
	Jul-12	8 out of 11	10 out of 11	11 out of 11	8 out of 11	8 out of 11	9 out of 11
		Not converged	Not converged	Not converged	Not converged	SAT1	SAT1
	Jun-13	6 out of 7	6 out of 7	6 out of 7	6 out of 7	7 out of 7	6 out of 7
		Not converged	Not converged	Not converged	Not converged	A	A

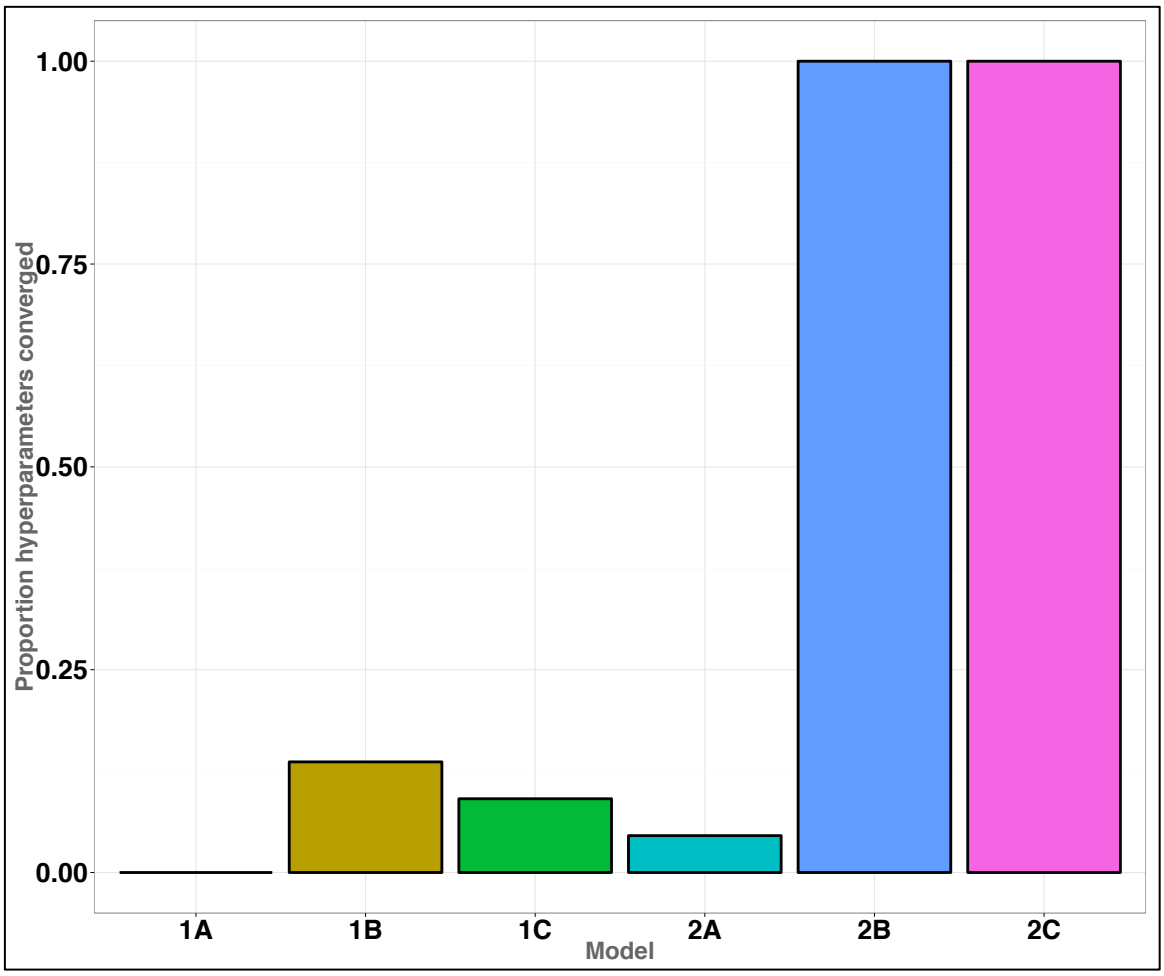


Figure 5.10: Convergence summaries for models 1A, 1B, 1C, 2A, 2B and 2C with the training dataset when all outbreak serotype and infected animal data were withheld.
The colour codes for the different models correspond with Table 5.5. Four MCMC chains were run with a 1,000-sample adaptation period and a 10,000 sample burn-in period before averaged across chains convergence was monitored. Thereafter the MCMC chains ran for 400,000 samples, which were thinned by 100. Models 2B (blue) and 2C (pink) converged on the correct sequence of serotypes as well 95% of the other variables. Convergence was reported for 22 hyperparameters; ELISA cross-reaction for each combination of serotypes, decay rates for ELISA reactivity for NSP and SP ELISAs, outbreak serotype and outbreak probability of infection.

Giving the model a free maximum ELISA reactivity threshold rather than fixing this value also afforded increased model flexibility. Models 1B and 2B allowed the model to select a single threshold for the whole herd, while Models 1C and 2C allowed a maximum threshold parameter for each individual animal. The models that allowed both individual animal infection times and upper ELISA reactivity thresholds (Models 2B and 2C) performed best upon validation omitting both outbreak serotype and infected animal data (Table 5.5, Figure 5.10).

However Model 2C failed to converge when only data about outbreak serotype were omitted (Table 5.5). In addition, the individual animal threshold of Model 2C could not be used for cross-sectional data because individual animal thresholds could not be modelled from single data-points. For this reason, we chose to bring Model 2B forward for further validation.

As well as inferring serotype O as the cause the outbreak in Spring 2011, Model 2B inferred the time of peak ELISA reactivity to be 20th of March 2011, and the 95% highest posterior density (HPD) was the 6th of March to the 14th of April (Figure 5.11). This was consistent with an earlier estimate from the simpler model (1st April, Figure 5.10). The two models also converged upon the same serotype (O) for this outbreak, and both similarly failed to converge when we attempted to introduce an extra outbreak in September 2012. This consistency in results increases confidence in our inferences about the unknown outbreaks.

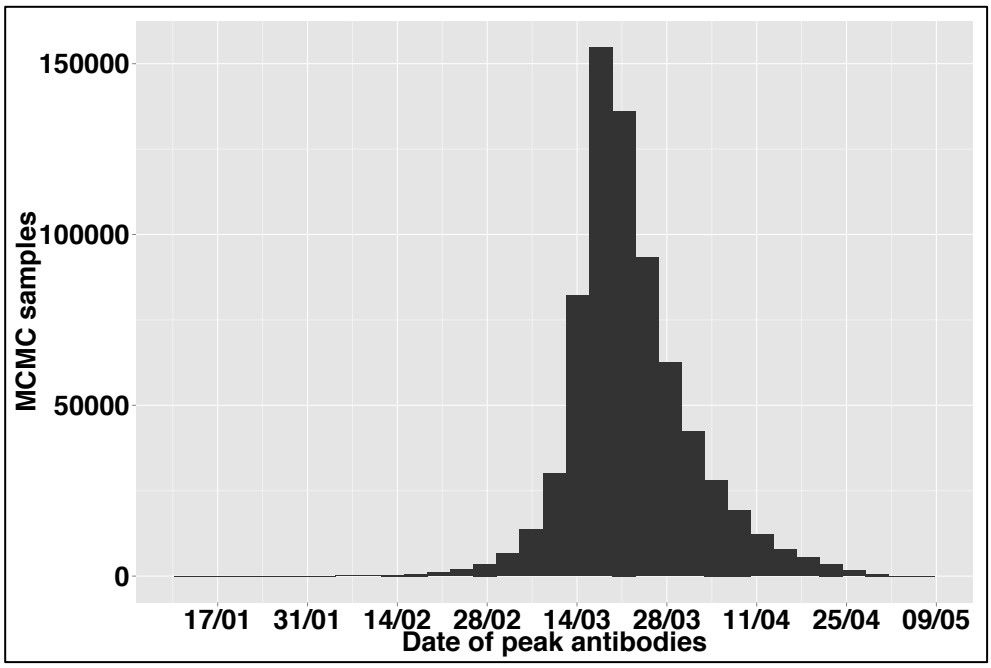


Figure 5.11: A histogram summarising Model 2B inferred times of peak ELISA reactivity in cattle inferred to be infected in the Spring 2011 outbreak.

5.5.3 Validation from field observations

Model 2B performed well in identifying the infected animals from the longitudinal ELISA dataset. Of the 36 cattle that were known from FMD lesion photographs to be infected, 33 were identified from model inferences. The three animals that remained unidentified were infected in the SAT1 outbreak. Issues with the SAT1 assay may explain why the model failed to identify these three infected animals (Chapter 2).

The model correctly identified the two animals with clear VNT negative results after the SAT2 outbreak as uninfected. Furthermore, the model inferred a higher probability of infection for the SAT2 outbreak (Mean = 0.93, HPD=0.87-0.97) compared to the SAT1 outbreak (Mean=0.74, HPD = 0.63 – 0.84), which is consistent with the herdsmen's estimates on the severity (meaning how many animals were sick with FMD lesions) of these two outbreaks.

5.5.4 Validation of inferences about outbreak timing from longitudinal data

Model 2B was next tested for further correct inference of when the FMD outbreaks happened as well as the serotypes causing them and which animals were infected. Initially, the Herd 1 dataset was used for this validation. For each of the three known outbreaks, a twelve-month window was supplied to the as a uniform prior for the outbreak time, and lesion timing information was removed.

The model converged upon the correct time-range and selected the correct serotype for all three outbreaks. It correctly selected all animals known to be infected in the case of the SAT2 and A outbreaks. For the SAT1 outbreak, it selected 8 out of the 11 infected animals. Therefore, it performed well with the longitudinal dataset in absence of information about timing, serotype or infected animals in each outbreak. These results are summarised on Table 5.6.

Table 5.6: Validation of model inferences from longitudinal data about when the outbreak happened.
HPD = Highest posterior density.

		Median time of first lesion appearance (from data)	95% HPD for peak ELISA reactivity times (from model with do lesion data)	Serotype	Infected animals
Aug-11	Data (13 animals)	20/08/2011	17/08/2011 - 24/08/2011	SAT2	18 out of 18
	Inference (13 animals)	17/08/2011	25/07/2011 - 21/09/2011		
	Inference (whole herd)	10/08/2011	23/07/2011 - 30/08/2011		
Jul-12	Data (5) animals)	08/07/2012	07/07/2012 - 08/07/2012	SAT1	8 out of 11
	Inference (5) animals)	06/07/2012	12/06/2012 - 01/08/2012		
	Inference (whole herd)	08/07/2012	12/06/2012 - 07/08/2012		
Jun-13	Data (7 animals)	20/06/2013	18/06/2013 - 22/06/2013	A	7 out of 7
	Inference (7 animals)	20/06/2013	30/05/2013 - 27/06/2013		
	Inference (whole herd)	23/06/2013	01/06/2013 - 06/07/2013		

The model performed similarly well in inferring outbreak timing from a new longitudinal dataset from Herd 2. ELISA data from the 96 animals from between December 2011 and April 2013 (Figure 5.6) was provided to the model but information on when the outbreak happened, which FMDV serotype caused it and which animals were infected was withheld. The correct serotype (SAT1) was inferred. The model inferred infection in the nine animals with lesions. The inferred time of the outbreak included the known lesion appearance times (Table 5.7).

Table 5.7: Comparison between FMD lesion appearance times and times of peak antibodies inferred by the model based on data from a new longitudinal dataset.

		Median time of peak ELISA reactivity	95% Credible Interval for peak ELISA reactivity times
Jul-12	Data (9 animals with lesions)	08/07/2012	05/07/2012 - 09/07/2012
	Inference (9 animals with lesions)	06/07/2012	20/06/2012 - 10/09/2012
	Inference (whole herd)	01/08/2012	25/06/2012 - 09/09/2012

5.5.5 Cross-reactivity parameters

Table 5.8 and Figure 5.12 show the cross-reactivity parameters between assay and outbreak serotypes that model 2B converged upon. The highest levels of cross reaction were between the serotype O assay and sera from animals with serotype A and serotype SAT2 causing the most recent infections. The next highest cross-reaction levels were between the serotype SAT1 assay and sera from animals with serotype SAT2 and serotype A causing the most recent infections.

Table 5.8: The cross reaction parameters that Model 2B converged upon.

Assay	Outbreak	Mean cross-reaction (SD)
O	A	0.97 (0.02)
O	SAT2	0.62 (0.04)
SAT1	SAT2	0.58 (0.02)
SAT1	A	0.41 (0.09)
A	SAT2	0.38 (0.02)
A	SAT1	0.34 (0.04)
A	O	0.32 (0.03)
SAT2	A	0.3 (0.08)
O	SAT1	0.27 (0.08)
SAT1	O	0.2 (0.03)
SAT2	SAT1	0.18 (0.07)
SAT2	O	0.12 (0.02)

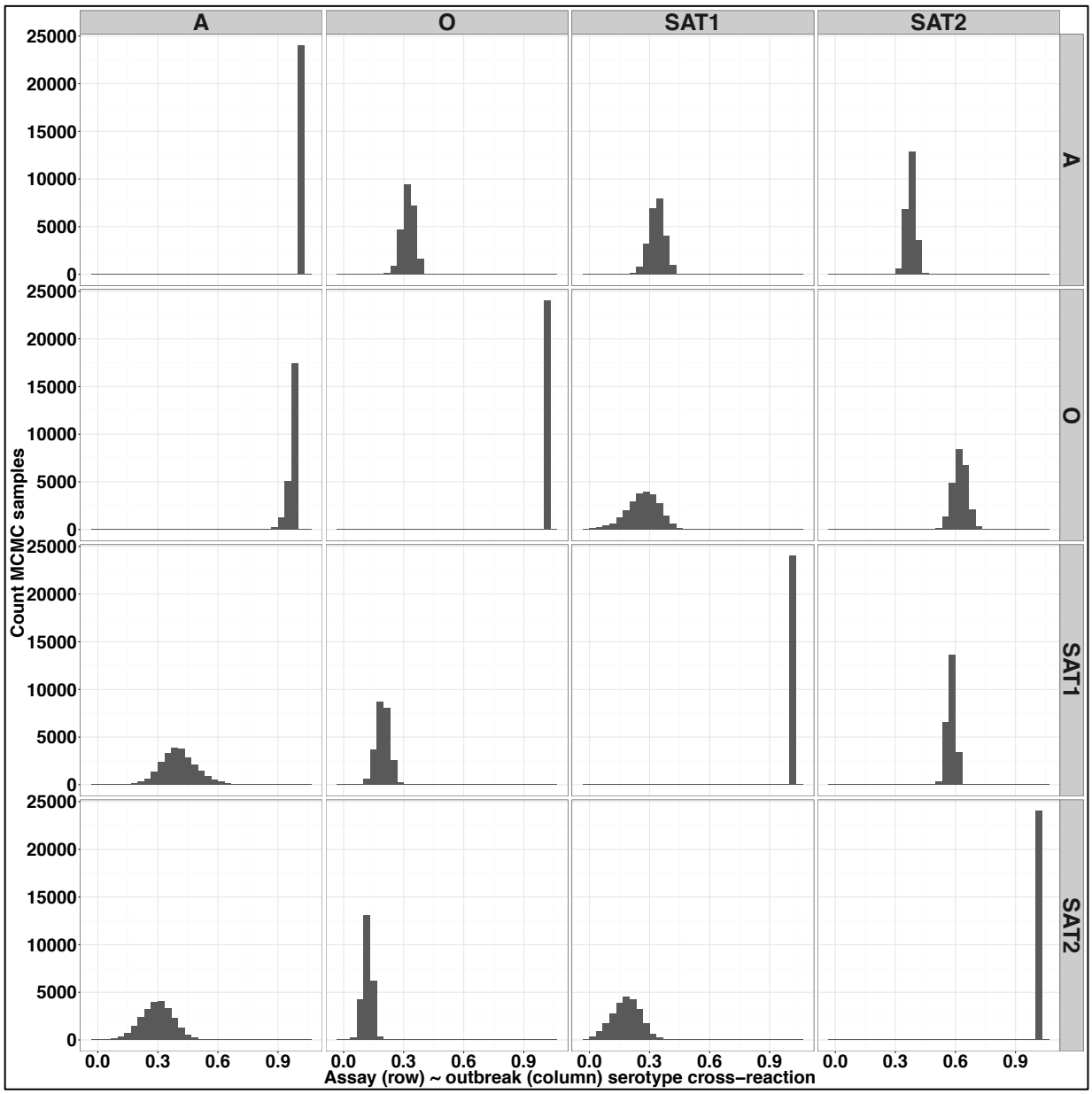


Figure 5.12: Histograms of the MCMC parameter space sampled for cross reaction between assay and outbreak serotypes in Model 2B.
The labels on the right hand side ("rows") represent the ELSIA serotype and the labels on the top ("columns") represent the most recent outbreak serotype. Samples were thinned by 100.

5.5.5 VNT results in Herd 1

The VNT results from the subset of ten Herd 1 cattle are presented in Figure 5.13. Of the 332 VNT results generated, 87 (26.2%) were negative, 87 (26.2%) were inconclusive and 158 (47.6%) were positive according to OIE recommended cut-off values (OIE, 2012a), Chapter 2.

Contrary to the serotype specificity that we expected from VNT, we observed increases in neutralising titres against FMDV against serotypes after outbreaks caused by different serotypes. This meant that VNT could not provide specific results against which to test model inferences. As the majority of VNT results were either positive or inconclusive according to the conventional titre cut-off values (OIE, 2012a), an alternative approach was developed for tracking changes in antibody titres after outbreaks compared to before them. For the purposes of comparison to model results, VNT results after each FMD outbreak were classified into positive (VNT titre increased after the outbreak compared to before the outbreak and reciprocal titre after the outbreak ≥ 32), negative (VNT titre not increased after the outbreak compared to before the outbreak and reciprocal titre after the outbreak < 32) and inconclusive (reciprocal titre after outbreak ≥32 but not increased compared to before the outbreak or titre increased after outbreak but <32). Even with this approach, only two animals were VNT negative for the SAT2 outbreak, and none were negative for the SAT1 or A outbreaks (Table 5.9).

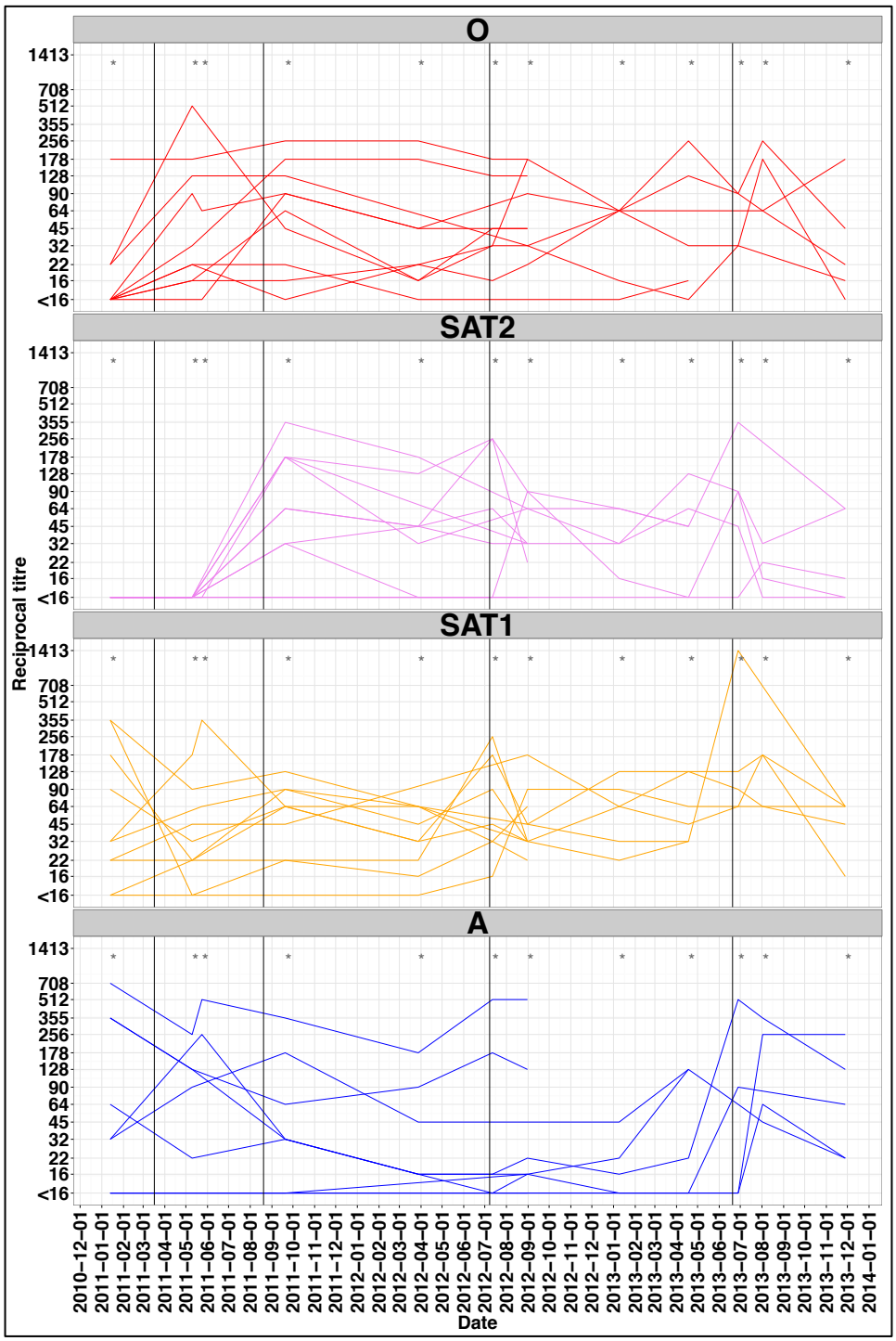


Figure 5.13: Virus neutralisation testing titres against serotypes O, SAT2, SAT1 and A for a subset of ten animals from the study herd between January 2011 and November 2013.
The y axis represents reciprocal titre and the x axis represents time.

Table 5.9: A summary of VNT results from Herd 1.
*** Positive = VNT titre increased after the outbreak compared to before the outbreak and reciprocal titre after the outbreak ≥ 32, Negative = VNT titre not increased after the outbreak compared to before the outbreak and reciprocal titre after the outbreak < 32, and inconclusive = reciprocal titre after outbreak ≥32 but not increased compared to before the outbreak or titre increased after outbreak <32.**

Preceding outbreak	VNT Serotype	N Positive * (Percentage)	N Negative * (Percentage)	N Inconclusive * (Percentage)
SAT2	O	7 (70%)	1 (10%)	2 (20%)
	SAT2	8 (80%)	2 (20%)	0 (0%)
	SAT1	4 (40%)	2 (20%)	4 (40%)
	A	1 (10%)	4 (40%)	5 (50%)
SAT1	O	4 (40%)	2 (20%)	4 (40%)
	SAT2	5 (50%)	2 (20%)	3 (30%)
	SAT1	7 (70%)	0 (0%)	3 (30%)
	A	2 (20%)	5 (50%)	3 (30%)
A	O	3 (60%)	0 (0%)	2 (40%)
	SAT2	2 (40%)	0 (0%)	3 (60%)
	SAT1	4 (80%)	0 (0%)	1 (20%)
	A	4 (80%)	0 (0%)	1 (20%)

5.5.6 Inference from cross-sectional data

Model 2B performed well with inference of outbreak serotype, timing and infected animals based on longitudinal ELISA data from the training dataset. The next step was to test its ability to infer infection histories from cross-sectional data-points, as would be the requirement if using it to infer infection histories from field cross-sectional studies.

To perform, this validation, cross-sectional data points were generated by selecting single serum sampling points from known infected animals at variable intervals after their FMDV lesions were recorded. Serum points for these confirmed clinically infected animals for which full ELISA datasets (NSP and the four serotypes) were identified. These points are shown on Figures 5.14-5.16 for the SAT2, SAT1 and A outbreaks respectively. Figure 5.17 shows cross-sectional points from the new Herd 2 dataset.

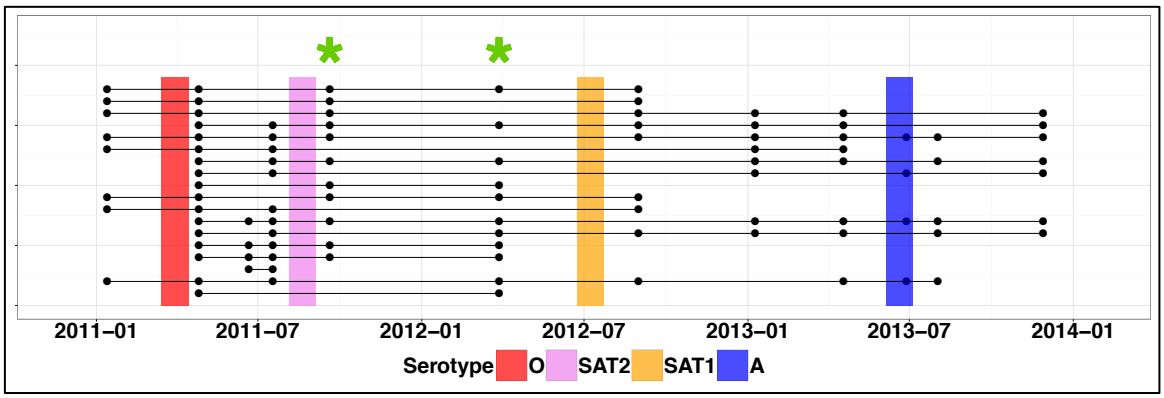


Figure 5.14: Sampling points (black dots) where the 18 cattle with confirmed lesions in the August 2011 SAT2 FMD outbreak that had a full ELISA dataset available (NSP ELISA and the four serotypes).
The green stars highlight suitable sample points to use as virtual cross-sectional data to test the model with for inferences about when the SAT2 outbreak occurred and which serotypes caused it. There are eleven suitable data points available from September 2011 and eleven from March 2012.

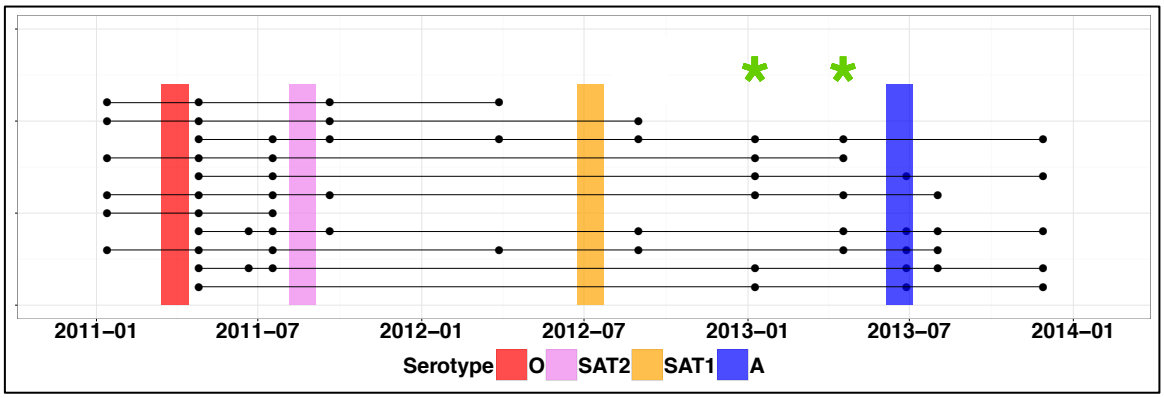


Figure 5.15: Sampling points (black dots) where the eleven cattle with confirmed lesions in the July 2012 SAT1 outbreak had a full ELISA dataset available (NSP ELISA and the four serotypes).
The green stars highlight suitable sample points to use as virtual cross-sectional data to test the model's inferences about when the SAT1 outbreak occurred and which serotype caused it. The data point with four animals available immediately after the outbreak was not utilised as the VNT data showed that some animals in the herd had not yet seroconverted at this point.

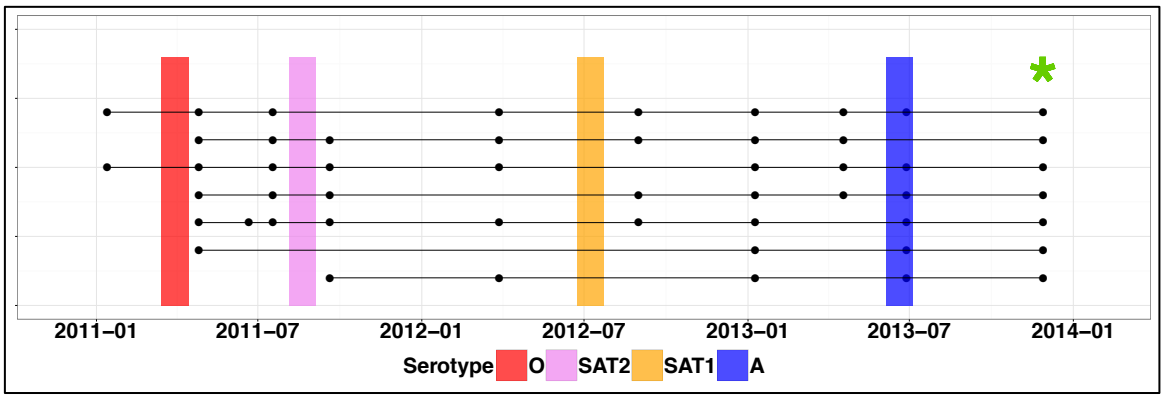


Figure 5.16: Sampling points (black dots) where the seven cattle with confirmed lesions in the June 2013 serotype A outbreak had a full ELISA dataset available (NSP ELISA and the four serotypes).
The green star highlights the suitable sample point to use as virtual cross-sectional data to test the model's inferences about when the A outbreak occurred and which serotype caused it.

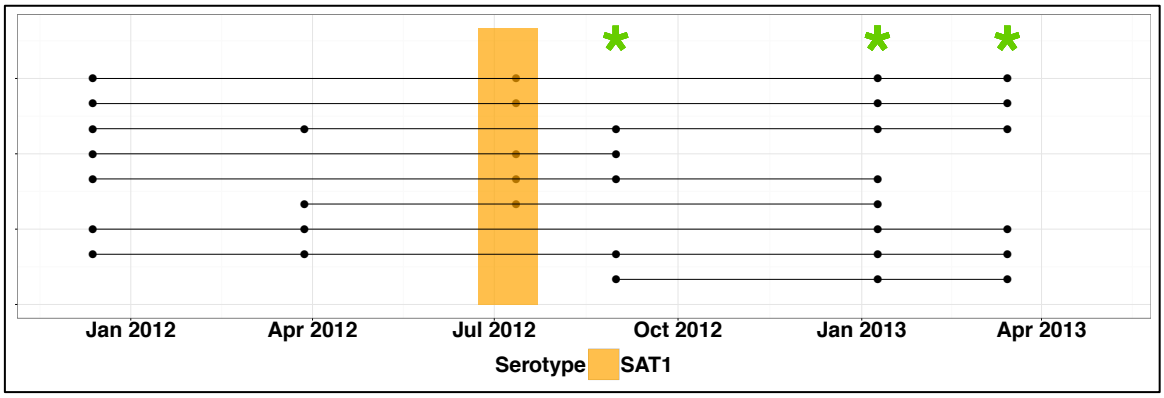


Figure 5.17: Sampling points (black dots) where the nine cattle with lesions in the July 2012 SAT1 outbreak (test dataset) had a full ELISA dataset available (NSP ELISA and the four serotypes).
The green stars highlight the suitable sample points to use as virtual cross-sectional data to test the model's inferences about when the SAT1 outbreak occurred and which serotype caused it. Full ELISA datasets were available from five of these animals in August 2012, eight in January 2013 and six in March 2013.

Data from the virtual cross-sectional data-points were fed to the model as from separate cross-sectional herds. Initially, the maximum number of data-points available from each date was used (Figures 5.14-5.17 above). To further challenge the model's capacity for inference from cross-sectional data, smaller groups from each date were used. Model 2B performed well in inferring the correct serotype of the most recent outbreaks. It converged in 21 out of 23 trials with different data and always chose the correct serotype when it did converge (Table 5.10). However, with sampling points farther away in time from the outbreaks, it underestimated the time elapsed since the outbreaks (Table 5.10).

Table 5.10: Validation of Model 2B inferences about the date and serotype of the most recent outbreak using Herd 1 and Herd 2 data from single time points. Convergence failures are highlighted with red shading.

Sampling date	Number of animals	Observed time since outbreak (days)	Highest Posterior Density for inferred time since outbreak (days)	Inferred serotype
20-Sep-11 (after SAT2 outbreak in Herd 1)	2	31	42 (10-131) days	SAT2
	3		36 (10-105) days	SAT2
	4		28 (10-70) days	SAT2
	4		29 (10-73) days	SAT2
	8		23 (10-50) days	SAT2
	11		22 (10-48) days	SAT2
28-Mar-12 (after SAT2 outbreak in Herd 1)	2	221	NA	Not converged
	3		42 (10-126) days	SAT2
	3		59 (10-159) days	SAT2
	4		47 (10-136) days	SAT2
	4		55 (10-161) days	SAT2
	5		43 (10-120) days	SAT2
	11		41 (10-110) days	SAT2
9-Jan-13 (after SAT1 outbreak in Herd 1)	6	181	70 (10-207)	SAT1
18-Apr-13 (after SAT1 outbreak in Herd 1))	5	280	NA	Not converged
28-Nov-13 (after serotype A outbreak in Herd 1))	3	153	85(10-265)	A
	4		78(10-249)	A
	7		80(10-220)	A
31-Aug-12 (after SAT1 outbreak in Herd 2)	2	50	115(10-386)	SAT1
	5		29(10-72)	SAT1
9-Jan-13 (after SAT1 outbreak in Herd 2)	4	181	48(10-133)	SAT1
	8		26(10-60)	SAT1
15-Mar-13 (after SAT1 outbreak in Herd 2)	6	246	44(10-140)	SAT1

5.4.7 Application of the model

During validation for inference from cross-sectional ELISA data from animals with known infection history, the model performed well in inferring the serotype that caused the most recent outbreak. Therefore, we took it forward to apply to a dataset from animals with unknown infection histories. When applied to the cross-sectional SPCE data, Model 2B converged after 400 thousand MCMC samples on the most recent serotype for 56 out of the 60 cross-sectional study herds that we fed it data from, and on the hyper-parameter for serotype probability in all 36 villages. Village level parameters were summarised at district level to highlight a pattern of serotypic dominance at district level (Figure 5.18). Inferences

from villages in Arusha and Loliondo districts that were sampled earlier in 2011 suggested recent SAT1 outbreaks. In Serengeti and Simanjiro districts, which were sampled later in 2011, inferences suggested recent serotype O outbreaks. Inferences from Monduli district, which was sampled at the end of 2011, indicated SAT2 dominance (Figure 5.18). When VNT titres were ranked as described in Chapter 6, and compared to Bayesian model inferences, the patterns of serotypic dominance inferred from both approaches were completely in agreement (Chapter 6). Further, the inferred sequence of serotypic dominance was consistent with virus isolation data from neighbouring southern Kenya over the same time period (Chapter 6).

The consistency between the models, VNT analysis and virus isolation in a neighbouring region adds confidence in the model's inferences. These inferences formed an integral part of the detection of a structure of FMDV antigenic dominance over time in East Africa, as is described in more detail in Chapter 6.

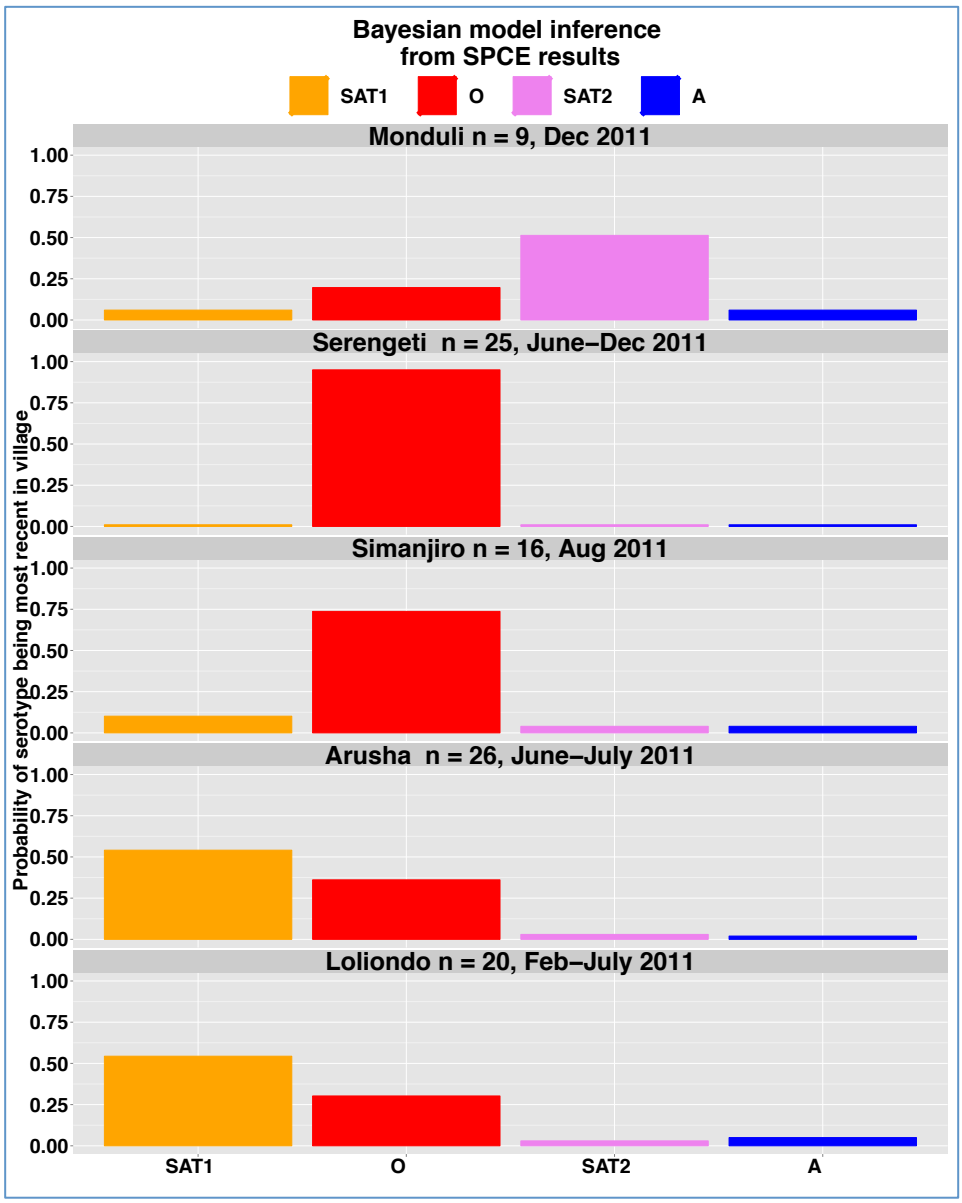


Figure 5.18: Results of Bayesian analysis of solid phase competition ELISA results (SPCE, right) in each district.
District and sampling dates are shown on the tabs above each bar plot. The y axis on the The y axis on the plot represents the mean village level probability that each serotype was most recent averaged for each district.

5.6 Discussion

This work represents an exciting advance in our ability to interpret a simple and accessible serological test. We have developed a method to interpret serology results specific to different serotypes of FMD in the face of massive cross-reaction between these serotypes in ELISA tests. This issue has posed a challenge for previous FMD research in Africa (Namatovu *et al.*, 2013a; Di Nardo *et al.*, 2015).

The modelling approach described here addresses the need for increased understanding of infectious disease dynamics in the countries that require this insight the most. It is vital to get the very most out of any investment in surveillance and diagnostics in developing countries. The approach described here capitalises on a flexible Bayesian modelling platform and increased computer efficiency to provide a tool to overcome many of the logistical and technical challenges in understanding how FMD circulates in East Africa. This has been achieved through inferring animals' infection histories from cross-sectional serological data. These data are currently easier to generate in resource limited settings compared to other serotype specific diagnostics such as virus isolation and genotyping.

We had access to sera, virus isolation data and infection histories from two large, longitudinally tracked herds of cattle that suffered serial FMD outbreaks. This unique dataset enabled us to develop a new but relatively simple model of FMDV ELISA reactivity and validate it. We had the facility to fully validate the model with a completely new dataset from the second herd, and with virtual cross-sectional data from animals with known infection histories.

The model performed well upon longitudinal validation, correctly inferring the serotypes that caused the outbreaks and estimating outbreak time-windows close to when the cattle were observed to have FMD lesions. We applied the model to infer the serotype O outbreak in Spring 2011 and also to infer the absence on an outbreak when sick cattle were reported in September 2012. This highlights the utility of our model for inferences from longitudinal datasets. The early host-virus dynamics of FMDV have been studied in detail under experimental conditions in bio secure facilities (for example (Charleston *et al.*, 2011)). However, once experimental cattle show clinical signs of FMD, these experiments

can rarely continue beyond a few days due to regulatory issues. Furthermore, the numbers of animals allowed in these experiments are very limited and the conditions are far removed from the African field situation. There is a great need to better understand the long-term host-virus dynamics of FMDV in the endemic multi-serotype setting. This will entail capitalising upon flexible modelling techniques, such as the approach presented here, to extract a meaningful signal from large longitudinal datasets amidst a cacophony of variability in field conditions and limitations in diagnostic assays.

The validation of inferences from cross-sectional data presented a much greater challenge to the model compared to the longitudinal validation. We validated the model's ability to infer infection histories from small groups of animals in isolation and it performed well in inferring the most recent FMDV serotype to infect the animals. The application model had the advantage of grouping herds from the same village and villages with common hyper-parameters, increasing the information available to facilitate inference on the most recent serotype circulating. When we applied the model to a true cross-sectional dataset, its inferences were consistent with trends observed in the VNT data from the same sera and with nearby virus isolation results from the same time period (Chapter 6). The utility of this application to the relatively small number of cross-sectional samples in this study highlights the exciting possibility to tap the information available from further cross-sectional datasets.

In contrast to the longitudinal validation, the model performed less well at estimating the time of the most recent FMD outbreak from cross-sectional data. This limitation highlights that we have not captured all of the underlying biology of host and virus dynamics with the model. One aspect of biology we failed to cover was persistent FMDV infection. For example, a number of animals had PCR positive results for SAT2 from oropharyngeal samples taken in March 2012, seven months after the SAT2 outbreak (Kasia Bankowska, Pirbright Institute, personal communication). Similarly, there were PCR positives for serotype A evident five months after the A outbreak. A previous abattoir study in Uganda reported the presence of SAT2 FMDV in the oropharynx of cattle three months after FMD outbreak restrictions were lifted in the area (Balinda *et al.*, 2010a). Infectious FMDV has been retrieved from the oropharynx of a proportion of cattle for up to 3.5 years (reviewed by Alexandersen *et al.* (2002)). Exposure of the immune system to persistent virus in the oropharynx could alter the decay rates of antibodies against the virus in a manner that our

model has not fully captured. As well as increased efforts to interpret combined experimental serological and virological findings that are currently available (Zhang & Alexandersen, 2004; Zhang *et al.*, 2004), further longitudinal studies in endemic countries are required to fully understand this aspect of chronic FMDV infection and its relationship with antibody dynamics. Another aspect that was not taken into account in this study was the possibility for mixed FMDV infections in a single outbreak and for anamnestic antibody responses in serial FMD outbreaks. Mixed persistent FMDV infections have been reported in buffalo (Bengis *et al.*, 1986), and discussed as a possibility in addition to sample contamination artefacts in cattle (Abubakar *et al.*, 2012). Virus isolation work in other aspects of the current project has shown that two different serotypes can be isolated from different cattle with acute FMDV lesions at the same time in the same herd (Chapter 6). Early molecular work points towards RNA from two different FMDV serotypes being present in the same lesion (Veronica Fowler and Kasia Bankowska, Pirbright Institute, personal communication). However, given the WRL-FMD virus isolation results for the study herds and the decades of single serotype outbreak reports, our assumption that one serotype is dominant in a given outbreak is reasonable, even if another may be present as a persistent infection.

A subset of samples from our study was VN tested with the objective of providing a specific dataset against which to compare our modelling results. The VN test is expensive and time-consuming, but was reported to be more specific than the ELISA testing that we used (Mackay *et al.*, 2001). Based on experimental evidence, it is widely accepted that there is no cross-protection between different VNT serotypes (Grubman & Baxt, 2004). Therefore it was unexpected that antibodies that neutralised heterologous serotypes increased subsequent to each outbreak. The work here, in combination with previous findings in serially experimentally infected animals (Cottral & Gailunas, 1971) and observations in other parts of Africa (Hedger *et al.*, 1982; Ludi *et al.*, 2014b) indicate that the neutralising responses of serially infected bovids to FMDV are not straightforward. Discovering the reasons for these unexpected neutralising responses is an important avenue of FMDV and immunological research, but for this study, it meant a dearth of negative controls for our model inferences. There were two clear negative VNT samples available for the SAT2 outbreak in herd 1, and the model inferences were in agreement with this. Furthermore, the models predictions on the probability of infection in the SAT2 and SAT1

outbreaks in Herd 1 fitted with the herdsmen's descriptions of the severity of the outbreaks.

Had the longitudinal VNT study yielded more specific results, it could have been assumed that cross-reactivity on the SPCE was due to conserved non-neutralising antigens or due to contamination of the plates or reagent production process with FMDV NSP. However, the increases in VNT titres subsequent to outbreaks caused by heterologous serotypes increases the spectrum of explanations for the cross- reaction. These possibilities are shown in Figure 5.19.

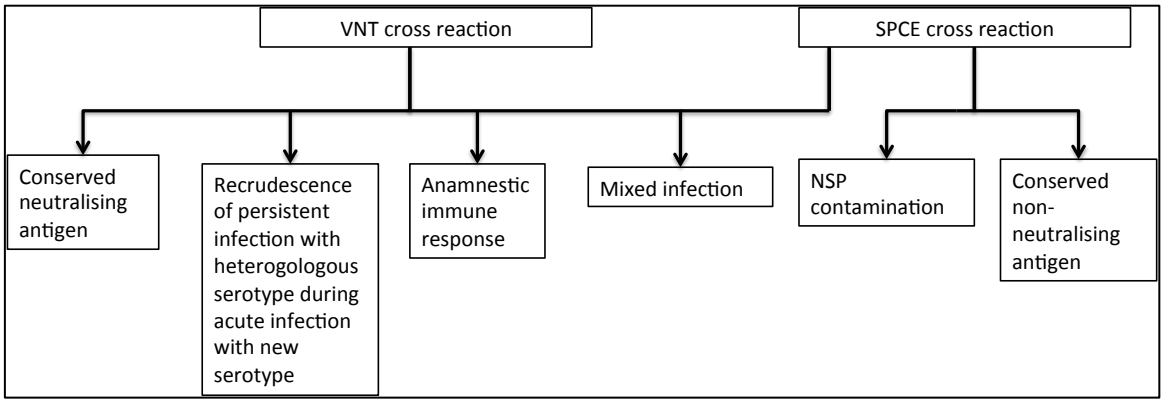


Figure 5.19: Possible explanations for cross-reaction in both virus neutralisation (VNT) and solid phase competition ELISA (SPCE) assays.

Many of the potential drivers of VNT and SPCE cross-reactivity warrant further research, and understanding them may be highly relevant to FMD control, especially in the case of potential conserved neutralising antigens or recrudescence of persistent infections. East Africa, where serial FMD outbreaks in the same herds are common, is the ideal arena in which to collect data on these aspects of FMD epidemiology. Flexible modelling approaches such as the one described here could be adapted to help understand data from further studies

Many of the limitations of the present work are because we are at the frontier of understanding FMDV dynamics in East Africa. There was little prior knowledge of FMDV dynamics in a multiple serotype environment where herds become serially infected. The information was simply not there with which to inform the priors for our model. In spite of these challenges, our model proved highly applicable to a cross-sectional dataset and fed into our understanding of patterns of FMDV circulation at regional level. In combination

with the emerging "ready-to-go" ELISA kits in development for African purposes (Brocchi, 2012a), our model represents a potent tool utilise in making an inroad into endemic multiple serotype FMDV epidemiology.

Chapter 6: Spatial and temporal patterns of serotype-specific FMDV infection in Tanzanian cattle and buffalo

6.1 Summary

Multiple serotypes of FMDV circulate in East Africa, creating a major challenge for control by serotype-specific FMD vaccines. Yet, little is known about the drivers of infection with different serotypes. As buffalo are recognised to have high infection levels with the SAT serotypes, investigation of the role of this species as a potential source of SAT serotypes for livestock is relevant to targeting control policies.

Model inferences from serology data and longitudinal virus isolation results were combined to describe serotype-specific patterns of infection in East African livestock over space and time. Potential risk-factors for infection of cattle with specific FMDV serotypes were examined. Finally, FMDV infection patterns in northern Tanzanian buffalo were investigated and compared to those in cattle in the same ecosystems.

Virus typing data available from northern Tanzania and southern Kenya suggested sequential waves of serotype SAT1, O, SAT2, and A outbreaks in cattle populations between 2009 and 2014. Model inferences from 2011 serology results and virus typing results from longitudinally tracked herds were consistent with this temporal sequence. More limited virus typing data from after 2014 show outbreaks caused by serotypes SAT1 and O.

In combination with the pattern of sequential antigenic dominance and the same herds suffering outbreaks caused by different serotypes, the absence of serotype-specific risk factors in the available sample of cattle support the interpretation that factors other than contact with wildlife currently drive FMD transmission in cattle in the study area. In contrast to cattle, only two serotypes, SAT1 and SAT2, were dominant in a cross-section

of buffalo in the same ecosystems and at the same time as the serotype O wave in cattle. In addition, unlike cattle a decrease in the likelihood of FMDV infection in buffalo with age was observed. Combined, these findings suggest that, in contrast to southern Africa, FMDV circulation patterns in East African cattle and buffalo are not currently tightly linked. Further work is warranted to investigate the potential predictability of waves of distinct FMDV antigenic variants sweeping across the northern Tanzanian and southern Kenyan region.

6.2 Introduction

Foot-and-mouth disease virus (FMDV) represents a diverse group of pathogens that wreak havoc on livestock productivity in the world's poorest countries. This is despite being one of the longest studied viruses (Loeffler & Frosch, 1898). Over the twentieth century, many developed countries have eradicated and prevented recurrence of FMD through aggressive and resource demanding control policies, initially involving intensive vaccination and, when FMD incidence dropped sufficiently, through a culling policy for infected animals and their contacts (OIE, 2012a)

There are major epidemiological, ecological, and logistical impediments to implementing similar control policies in most African countries. Yet, these are settings where the rural poor need livestock disease control the most due to their reliance on livestock for their livelihoods (Knight-Jones & Rushton, 2013).

East Africa hosts one of the diverse ranges of FMDV antigenic variants globally, with five of the seven serotypes (A, O SAT1, SAT2 and SAT3) circulating (Dhikusooka *et al.*, 2015; Kasanga *et al.*, 2012; Namatovu *et al.*, 2015). Within each of these serotypes, there is further antigenic divergence, and therefore a single vaccine may not confer protection even within the same serotype (Paton *et al.*, 2009).

The majority of sampled cattle in East Africa have serological evidence of FMD infection (Mkama *et al.*, 2014; Namatovu *et al.*, 2013a), and herds have been reported to suffer up to three outbreaks per year (Chapter 3). The presence of FMD susceptible wildlife in these areas, especially the African buffalo (*Syncerus caffer)* that has been consistently reported

to have high levels of infection with SAT serotypes (Ayebazibwe *et al.*, 2010a; Bronsvoort *et al.*, 2008; di Nardo *et al.*, 2012), further complicates FMD epidemiology.

In more developed livestock industries, such as Latin America, FMD control and elimination has required high levels of veterinary surveillance and management, vaccine cold chain maintenance and six-monthly vaccination, which are not currently realistic in many African settings. Furthermore, conventional animal movement controls and wildlife separation from livestock may be inappropriate in the rangeland ecosystems of East Africa where free movement of wildlife, cattle and people are integral to livestock management, conservation and income from wildlife tourism (Ferguson *et al.*, 2013).

There is a need to update our armoury of knowledge of multi-serotype FMDV ecology if FMD control is to be implemented effectively under such challenging conditions. Northern Tanzania, the focus of this study, presents the ideal opportunity to study FMDV dynamics in an ecosystem where the disease is almost completely uncontrolled. Tanzania has the highest buffalo population in Africa (East, 1999; TAWIRI, 2014), the third highest cattle population (FAO, 2014), as well as vast wildlife and livestock movements. At least four serotypes of FMDV are circulating, O, A, SAT1 and SAT2 (Kasanga *et al.*, 2014a). This study focused on the wildlife – livestock interface in Northern Tanzania. Such settings provide an opportunity to add evidence towards confirming or refuting the hypothesis that buffalo are important sources of SAT serotypes for livestock in East Africa, as is the accepted case in southern Africa (Brückner *et al.*, 2002; Vosloo *et al.*, 2002a, 2010).

In this chapter, I aim to address the following questions:

(a) Which serotypes of FMDV circulate in cattle and buffalo in northern Tanzania and are there any patterns of serotypic dominance over space and time?
(b) Are there different risk factors for infections caused by specific serotypes of FMDV in cattle?
(c) Are patterns of FMDV serotypic dominance in cattle and buffalo similar?

6.2 Methods

6.2.1 Description of FMDV serotypes in the study area and in East Africa over space and time

Selection of sera from cross-sectional study for serotype specific assays

Sera (n= 2694) were collected during a cross-sectional study in 2011 as described in Chapter 2. All of these sera were tested by NSP ELISA (Chapter 2), but this only measured antibodies against FMDV non-structural protein (NSP), an antigen that is exposed upon infection with FMDV of any serotype. To diagnose from serology which serotypes the animal was infected with, virus neutralisation testing (VNT, OIE, 2012a) or serotype-specific ELISAs (Hamblin *et al.*, 1986b; Li *et al.*, 2012; Mackay *et al.*, 2001; Paiba *et al.*, 2004) are necessary. The VNT assay is time-consuming and expensive, and there was a shortage of serotype-specific ELISA reagents over the course of this study. For this reason, only a sub-set of samples was selected for VNT and serotype-specific ELISA testing. Samples were selected with the objective of identifying the most recent FMDV serotype to infect the herd and village. Sera were selected at village- and herd-level from the youngest cattle with the highest FMDV NSP percentage inhibition (PI). Cattle with the highest NSP PIs were chosen as these were most likely to have been recently infected with FMDV and subsequently yield information on the most recent serotype to infect the herd. The youngest cattle (aged over six months to avoid maternally derived antibodies (Nicholls *et al.*, 1984)) were selected, as they were less likely than older cattle to have suffered serial FMDV infections, which may result in a broader reactivity profile. Inference of the most recent serotype to infect the herd would therefore be easier using these sera. The selection method is fully described in Appendix 5 The selected sera were tested with both VNT and serotype-specific ELISAs as described in Chapter 2.

Analysis of serological results

The VNT results were initially summarised by district and by animal age from dentition, using both VNT titres and VNT positive or negative results. Titres were adjusted to take into account variation in avidity between the sera and test viruses (Appendix 7). As the majority of animals were VNT positive for multiple serotypes, a pairwise ranking algorithm was developed to compare titres across animals and serotypes to determine district level patterns of FMDV antigenic deominance. The algorithm is described as

follows. For VNT serotypes *k* and *j* in serum from animal *i*, titre j was subtracted from titre k. The difference was divided by the minimum unit by which two titres can differ (0.15 for logged titres) to give δkj_i.

$$\boldsymbol{\delta kj_i = \frac{Titre\ k_i - Titre\ j_i}{0.15}}$$

k = serotype A, O, SAT1 or SAT2

j = serotype A, O, SAT1 or SAT2

The cumulative probability ($p_{k,j,i}$) of this difference on a normal distribution was calculated. The standard deviation used (1) was based on the reported variability in VNT titres (Hingley & Pay, 1987).

$$\boldsymbol{X \sim Normal(0, 1)}$$

$$\boldsymbol{p_{k,j,i} = p(\delta kj_i \geq X)}$$

For each district, the average $p_{k,j,i}$ per comparison (v_k) was calculated to highlight which serotypes were most dominant.

$$v_k = \frac{\sum p_{k,j,i}}{n_k}$$

n_k = Number of pairwise comparisons between titre k and titres against the other serotypes

The serotype specific ELISA results were interpreted by the specifically designed Bayesian model as described in Chapter 5.

Collection of lesion material from the study area for FMDV isolation

Lesion material was collected during an outbreak study between 2011 and 2015 as described in Chapter 2. For each outbreak visited, the GPS coordinates of the herd, the date of the visit and the date of initial observation of clinical signs in the livestock were

recorded. Lesion material was sent to the WRL for virus isolation and molecular genotyping using OIE manual methods (OIE, 2012a). Antigen typing was also conducted as described by Roeder & Le Blanc Smith (1987) (Chapter 2).

Review of serotypes causing FMD outbreaks in East Africa

To investigate the consistency of FMDV serotype circulation patterns and serotypic dominance over a broader geographic scale, virus isolation results from the eastern African region were examined. The "Pubmed" database (http://www.ncbi.nlm.nih.gov/pubmed/) was reviewed using the search terms "Foot-and-mouth disease," "cattle" and each of "Kenya," "Tanzania," and "Uganda." Articles from this search that reported virus isolation or virus typing results after 2008 were selected and summarised. Where sample collection dates and locations were available in association with virus typing results, these were collated for comparison to the results from the present study.

In addition, the WRL database (http://www.wrlfmd.org/fmd_genotyping/) was searched for results from Kenya, Tanzania and Uganda. The WRL records from 2010 onwards had location data readily available and therefore these were also included.

Analysis of virus isolation and typing results

The null hypothesis that the occurrence of different FMDV serotypes was randomly distributed over time was tested. A contingency table with the frequency of each leader-follower serotype combination over time was created and Pearson's chi-squared test for count data was used to test whether this distribution was random.

6.2.2 Investigation of serotype specific risk-factors for FMDV in cattle

Study design

Potential livestock management and wildlife contact related risk factors for infection with FMDV were extracted from cross-sectional questionnaire and GPS data as described in Chapter 4. Cross-sectional herd infection with specific serotypes was inferred as described in Table 6.1.

Table 6.1: Summary of the sample, outcome and explanatory variables for the serotype-specific generalised linear mixed model.
NSP = foot-and-mouth disease virus non structural protein ELISA VNT = virus neutralisation test

Model of virus neutralisation results at herd level	
Sample	Herds with NSP positive cattle from cross-sectional study
Outcome variable	Herd VNT positive or negative for each serotype. If one or more animals in a herd had positive VNT results for a serotype, the herd was considered positive. If no animals tested for that serotype were positive, the herd was considered negative
Potential explanatory variables	District
	Max hours walked to reach grazing and water
	Number acquired livestock in last 4 months
	Number of cattle/small ruminants in herd
	Max weekly frequency of buffalo/wildlife sightings
	Distance to buffalo area
Random effect	Tribe

Generalised linear mixed model to explain patterns of FMDV seroprevalence

A generalised linear mixed effects model (GLMM) was used to investigate the effects of explanatory variables on the likelihood of a positive VNT result for each serotype. A separate model was built for each serotype. Six potential explanatory variables were selected for the initial trial model based on the strongest biological rationale and avoiding extreme colinearity between variables.

Positive or negative serological results for each serotype ($y_{h,l}$) from herd *h* and tribe *l* were assumed to follow a Bernoulli distribution based on a probability of $p_{h,l}$ of being seropositive.

$$\boldsymbol{y_{h,l} \sim Bernoulli(p_{h,l})}$$

A logit function was used to link $p_{h,l}$ to the GLMM as $\eta_{h,l}$.

$$\boldsymbol{\eta_{h,l} = \log\left(\frac{p_{h,l}}{1 - p_{h,l}}\right)}$$

The initial model included coefficients for maximum time walked to reach grazing and water (β_1), herd size (β_2), proximity to wildlife area (β_3), livestock acquired over the past

four months (β_4), district (α_s), and wildlife sightings ω_q. To account for local similarities in exposure to FMDV serotypes, a tribe level random effect (γ_l) was added to the model. The intercept was termed β_0. (Model 6.1)

$$\eta_{h,l} = \beta_0 + \beta_1 x_{1,h} + \beta_2 x_{2,h} + \beta_3 x_{3,h} + \beta_4 x_{4,h} + \alpha_s + \omega_q + \gamma_l \qquad \text{Model 6.1}$$

h= herd

l = tribe

x_1 = max time walked to reach grazing and water

x_2 = number of cattle

x_3 = km to wildlife area

x_4 = number of livestock acquired in the last four months

s =district- Serengeti, Simanjiro, Loliondo, Monduli or Arusha

q = wildlife sightings weekly or less often

Model selection

For model selection, variables were dropped in a stepwise fashion with the least significant variable (Neyman & Pearson, 1928) being dropped first upon likelihood ratio testing (LRT). For each step, the LRT was repeated for the remaining variables. The LRT is explained in Chapter 3.

Model validation

Where effects on the likelihood of VNT seropositive herds were found, final model predictions based on these effects were compared to the data to assess how much variation was accounted for with the model.

Retrospective power analysis for serotype specific risk factor study

Power analysis for the serotype-specific risk factor study was performed by simulation as described by (Johnson *et al.*, 2015). Simulations of 77 herds were made and buffalo sighting data were randomly generated based on a Bernoulli distribution and with a probability of 0.5 of a buffalo sighting weekly or more often. Simulated tribe levels were generated based on eleven herds per tribe. Simulated effects of buffalo sightings were created where weekly buffalo sightings by the household increased the probability their livestock being seropositive by between 0 and 0.4 (or buffalo sightings increased the odds

of being seropositive by a ratio between 1 and 9). A variance of 1 was assumed for the tribe level random effects. A GLMM was run with the simulated data:

$$\eta_{h,l} = \beta_0 + \omega_q + \gamma_l$$

h = herd

l = tribe

q = wildlife sightings weekly or less often

$\boldsymbol{h}$ = **herd**

The p value from a Wald test was recorded. This procedure was repeated with 1000 simulated responses for each size of buffalo sighting effect. The proportion of times that the p value was less than 0.05 was calculated.

6.2.3 Investigation of FMDV infection patterns in buffalo

Buffalo sampling numbers, locations and times

Serum samples were collected from buffalo in four different ecosystems: Arusha National Park (NP), Ngorongoro Conservation Area (NCA), Serengeti NP and Tarangire NP, as described in Chapter 2. In total, 199 sera were sampled between July 2010 and April 2012.

The buffalo were anaesthetised for sampling, their age was estimated from their dentition and horn size (Sinclair, 1977), and their sex, location (GPS coordinates) and the size of the group that they were with were also recorded. Chapter 2 details ethical permission for this sampling. Figure 6.1 shows the locations of buffalo and livestock sampling. The field team was given a quota of 25 buffalo for each of the four ecosystems. In NCA additional buffalo sera (Table 6.2) were available as part of serological surveillance operations performed by the local veterinary unit to investigate other pathogens.

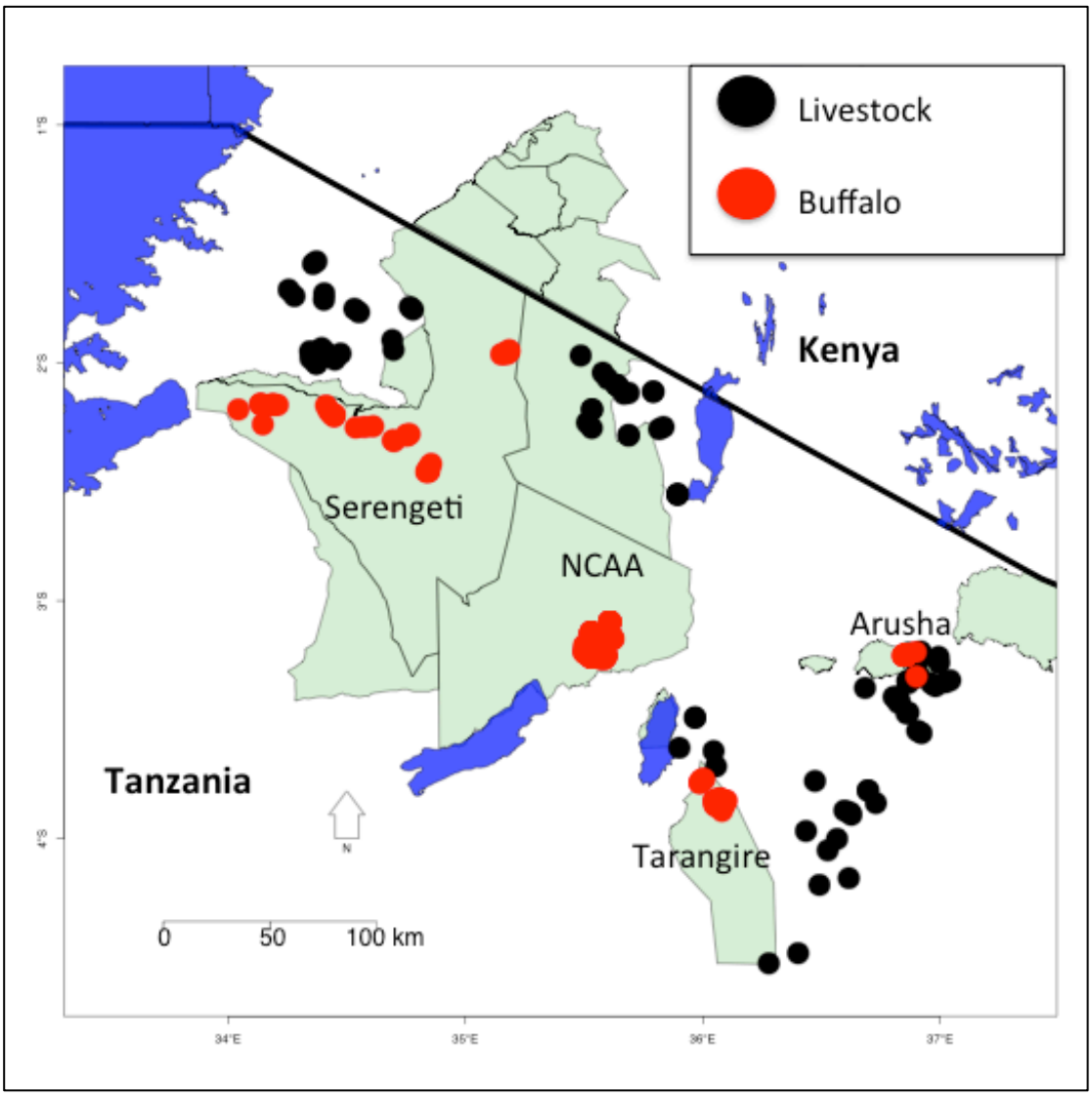


Figure 6.1: A map showing the locations where buffalo and livestock were sampled in the cross sectional study.

Table 6.2 summarises the numbers of buffalo sera from each ecosystem that were tested by NSP ELISA and VNT. While all available buffalo sera were NSP tested, samples for VNT were selected based on high NSP PIs, the availability of information about the buffalo from which the sample came, and sufficient serum volume.

Table 6.2: The numbers of buffalo sera that were tested by NSP ELISA and VNT. NCA = Ngorongoro Conservation Area, NSP = foot-and-mouth disease non structural protein, VNT = virus neutralisation testing

Ecosystem and date	Sera tested by NSP ELISA	Sera tested by VNT
Arusha March 2012	23	11
NCA August 2011	27	2
NCA April 2012	89	16
Serengeti July 2011	36	8
Tarangire July 2011	11	6
Tarangire November 2011	13	12
Total	199	55

Laboratory assays for buffalo sera

NSP-ELISA and VNT were performed on buffalo sera following the same procedure as per livestock (Chapter 2). In addition to serotypes A, O, SAT1 and SAT2, unlike livestock, buffalo sera were also tested for neutralising antibodies against SAT3. Due to lack of a SAT3 virus isolated from our study area, the standard WRL virus for SAT3 VNT was used. This was SAT309, isolated from Zimbabwe in 1983 (Fargeaud, 1995).

Analysis of buffalo serology results

Seroprevalence patterns for buffalo antibodies against NSP and against each serotype of FMDV in each protected area were summarised. Buffalo VNT titres were ranked at ecosystem level as was done for the cattle titres at district level. The relationship between a buffalo's age, sex, herd size and the likelihood of it being seropositive for FMDV NSP was investigated. A buffalo, b, from ecosystem j, had a probability of $p_{b,j}$ of being seropositive. This was linked to the GLMM as $\eta_{b,j}$ using a logit function. Coefficients for the buffalo's age (β_1), herd size (β_2) and sex (α_s) were included in the initial model. Ecosystem was taken into account as a random effect (γ_j). The GLMM is shown as Model 6.2 below. Variables from the initial model were dropped according to stepwise model selection using LRT.

$$\eta_{b,j} = \beta_0 + \beta_1 x_{1,b} + \beta_2 x_{2,b} + \alpha_s + \gamma_j \qquad \text{Model 6.2}$$

b = buffalo

j = Ecosystem (Serengeti, Ngorongoro, Tarangire or Arusha)

x_1 = Buffalo age in years

x_2 = Buffalo herd size (number of buffalo)

s = Gender

6.3 Results

6.3.1 FMDV serotypes in cattle

Cattle serotype-specific serology results

Of the 128 cattle sera tested by VNT, 10 (7.8%) lacked positive results for any of the four serotypes, despite being NSP ELISA positive, 45 (35.2%) were positive for a single serotype, 41 (32.0%) were positive for two serotypes, 23 (18.0%) were positive for three, and 9 (7.0%) for all four serotypes. Of the ten non-positive sera, five had inconclusive results for serotype O and five were negative for all four serotypes. Seven of the ten non-positives came from Arusha area, one came from Loliondo and two came from Serengeti. Of the five VNT O inconclusive sera, four were positive on the SPCE for serotype O and the fifth did not have an SPCE result. In the five VNT negative sera, variable SPCE patterns of positivity were seen (all serotypes positive (n=1), SAT1 positive (n=2), all but SAT1 positive (n=1), and not tested (n=1)).

Figure 6.2 shows the diversity of VNT seropositivity in each district and Appendix 8 relates VNT seroprevalence of specific serotypes to sampled cattle ages in each district.

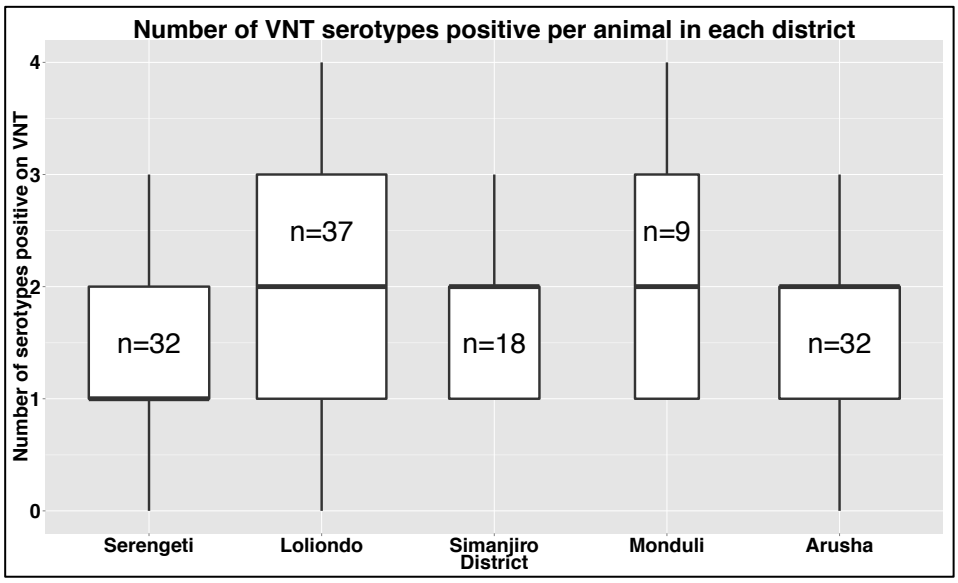


Figure 6.2: A boxplot summarising by district the number of serotypes with positive virus neutralisation test (VNT) results per serum sample.

Of the 96 sera tested by SPCE, 2 (2.1%) were negative for all serotypes, whereas 29 (30.2%) were positive for one serotype, 26 (27.1%) for two, 22 (22.9%) for three and 17 (17.2%) for four. Of the two SPCE negative but NSP ELISA positive sera, one was positive for SAT1 and one was positive for A on the VNT.

The serology results from VNT and SPCE showed the same pattern of serotype dominance in each district (Figure 6.3). Figure 6.4 highlights the clear temporal pattern of serotype dominance evidenced by inferences from the Bayesian model (described in Chapter 5) of SPCE results.

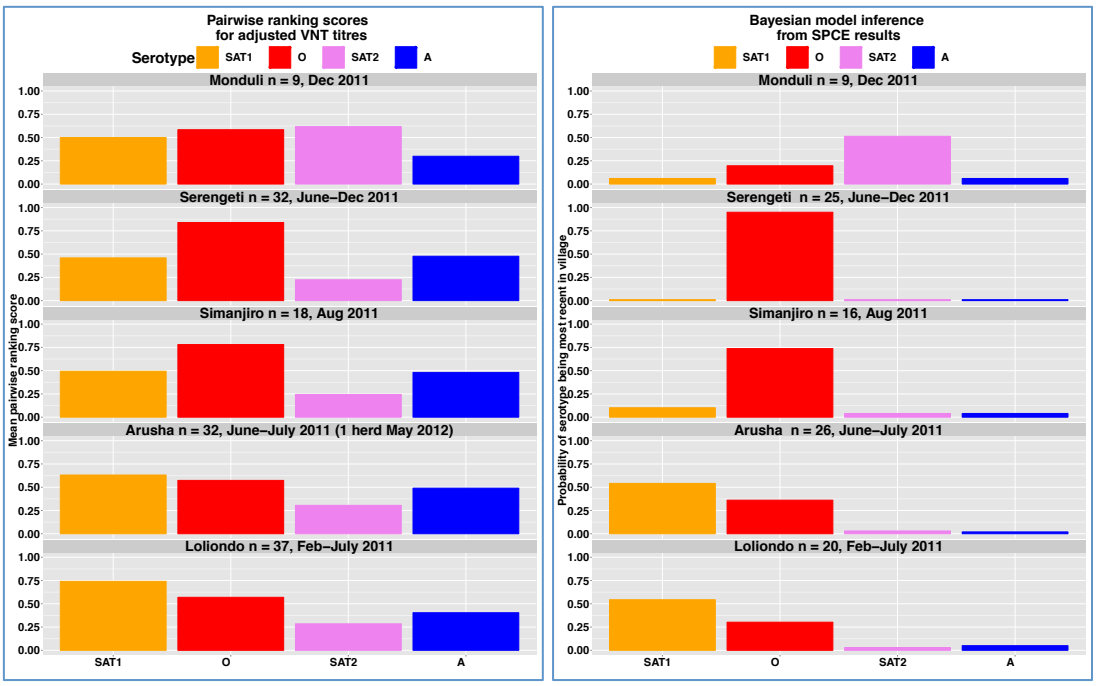


Figure 6.3: Results of virus neutralisation titre ranking results (VNT, left) and Bayesian analysis of solid phase competition ELISA results (SPCE, right). District and sampling dates are shown on the tabs above each bar plot. The y axis on the left plot represents the mean pairwise ranking score for cattle VNT titres in the district. The y axis on the plot on the right represents the village-level probability that each serotype was most recent averaged for each district, as inferred from SPCE results by the Bayesian model described in chapter 5.

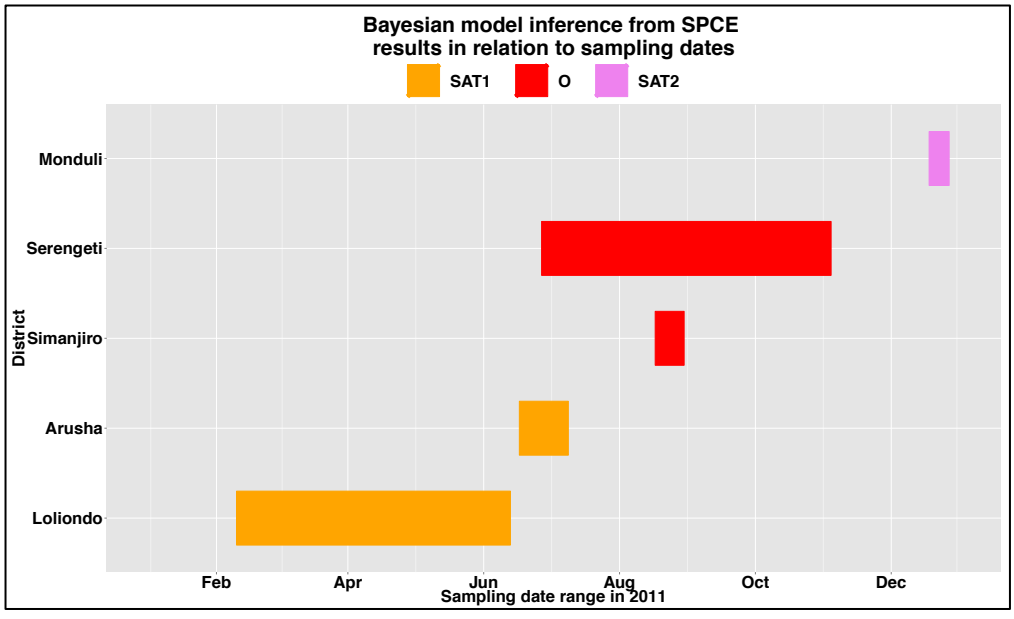


Figure 6.4: The results of Bayesian inference (Chapter 5) from solid phase competition ELISA results showing the serotype with the highest probability of most recently occurring in each district, plotted according to serum sampling periods in each district.

Cattle virus isolation results from the study area

Virus isolation results in each district

Virus isolation results from each district in the study area are summarised in Table 6.3. Virus isolation and typing was successful for 110 samples from cattle collected in the study area between July 2011 and December 2014. Fifteen herds were tracked through serial FMD outbreaks and eight of these had virus-typing results available for more than one of their outbreaks.

Table 6.3: Summary of virus isolation results available from each district in the study area.
Herds from the same village were considered to be in the same outbreak if they had outbreaks caused by the same serotype within 50 days of each other. WRL = World Reference Laboratory for foot-and-mouth disease, Pirbright.

District	N herd out-break visits	N herds sampled	N herds with serial outbreaks sampled	N samples sent to WRL	N samples with successful virus typing	N herd outbreaks with successful virus typing	N village outbreaks with successful virus typing	Date range for successful virus typing	Sequence of serotypes over time
Bunda	2	2	0	4	2	2	1	Feb-12	SAT2
Longido	1	1	0	2	2	1	1	Jul-12	SAT2
Loliondo	6	6	0	15	5	3	3	July 11 - June 12	SAT2 - A/O (A and O occurred in same village on same date)
Serengeti	41	31	7	105	85	37	23	Feb 12 - Nov 14	SAT2 - A - SAT1
Simanjiro	12	10	1	33	16	10	8	Aug 11 - June 13	SAT2/SAT 1 - SAT1 - A
TOTAL	62	50	8	159	110	53	36		

Virus isolation results from Serengeti district in relation to space and time

Serengeti district had the greatest number of FMDV isolates that covered the broadest range of space and time. Therefore, a detailed investigation of spatio-temporal patterns of FMDV serotype occurrences was conducted in this district. A pattern of different serotypes over time was evident. Prior to lesion collection, inference from serological data from late 2011 showed that serotype O was the last serotype to occur in Serengeti and VNT titres were lowest against serotype SAT2. Virus isolation showed that these O outbreaks in 2011 were followed by SAT2 outbreaks in early 2012, serotype A outbreaks in late 2012 / early

2013 and SAT1 outbreaks from late 2013 to 2015. As the serotype A outbreaks were ending and SAT1 outbreaks beginning, one herd had both serotype A and SAT1 isolated from different cattle in the same herd outbreak (Figure 6.5). One village suffered an O outbreak in May 2014. From the virus typing data available from Serengeti district, each new serotype appears to cause outbreaks initially in the South and West, and progress to cause further outbreaks in the North-Eastern direction. These data are shown in Figure 6.5.

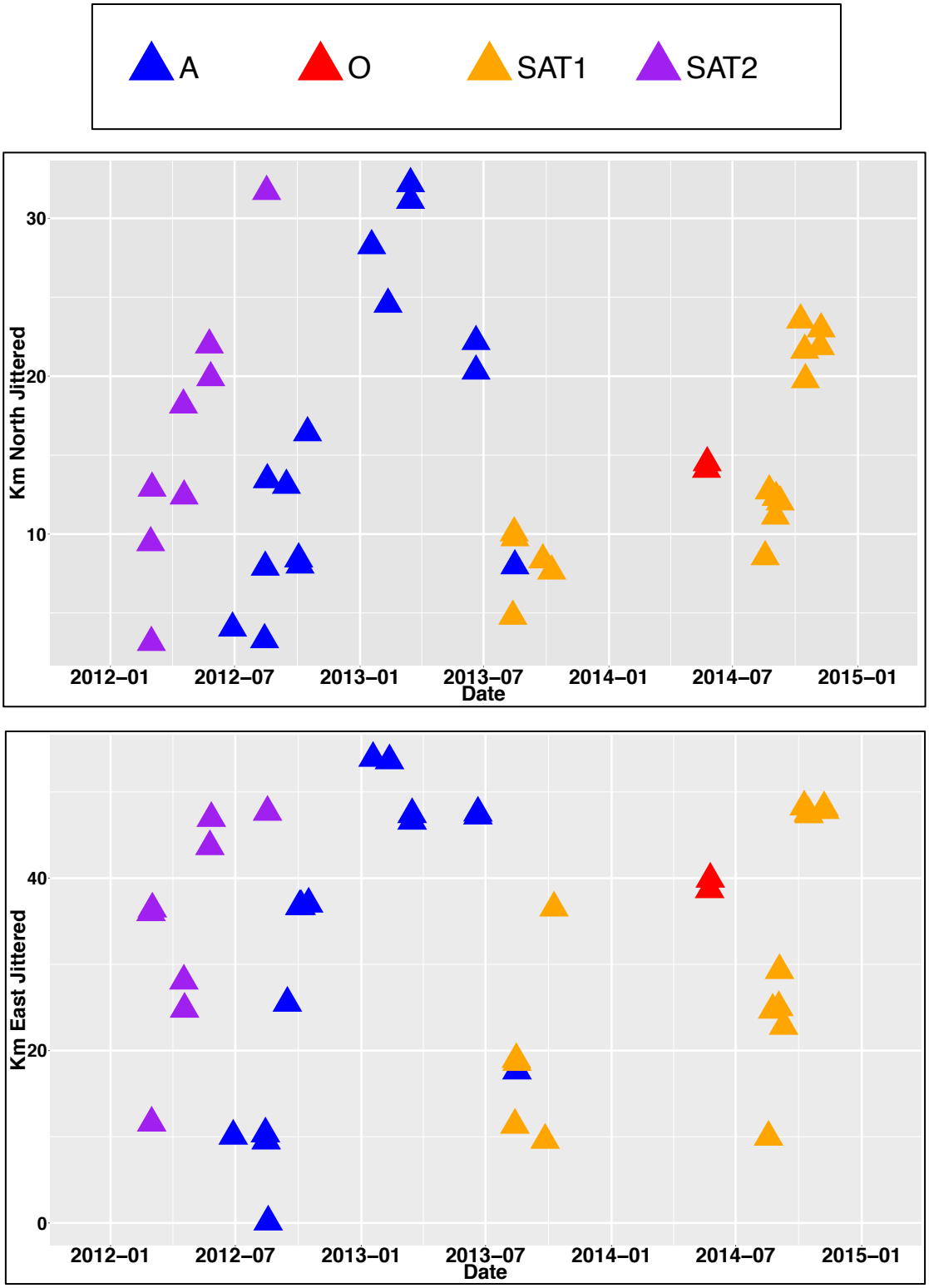


Figure 6.5: Virus isolation results from Serengeti district between 2012 and 2015. The x-axis shows time, and the y-axes show Kilometers, either northwards or eastwards.

In Serengeti district, fourteen herds were observed to suffer serial outbreaks over the study period. Lesion material was collected and virus isolation was successful for serial outbreaks in seven herds. The sequential pattern of serotypes in each herd fitted with the overall pattern in the district (SAT2 – A – SAT1) (Figure 6.6).

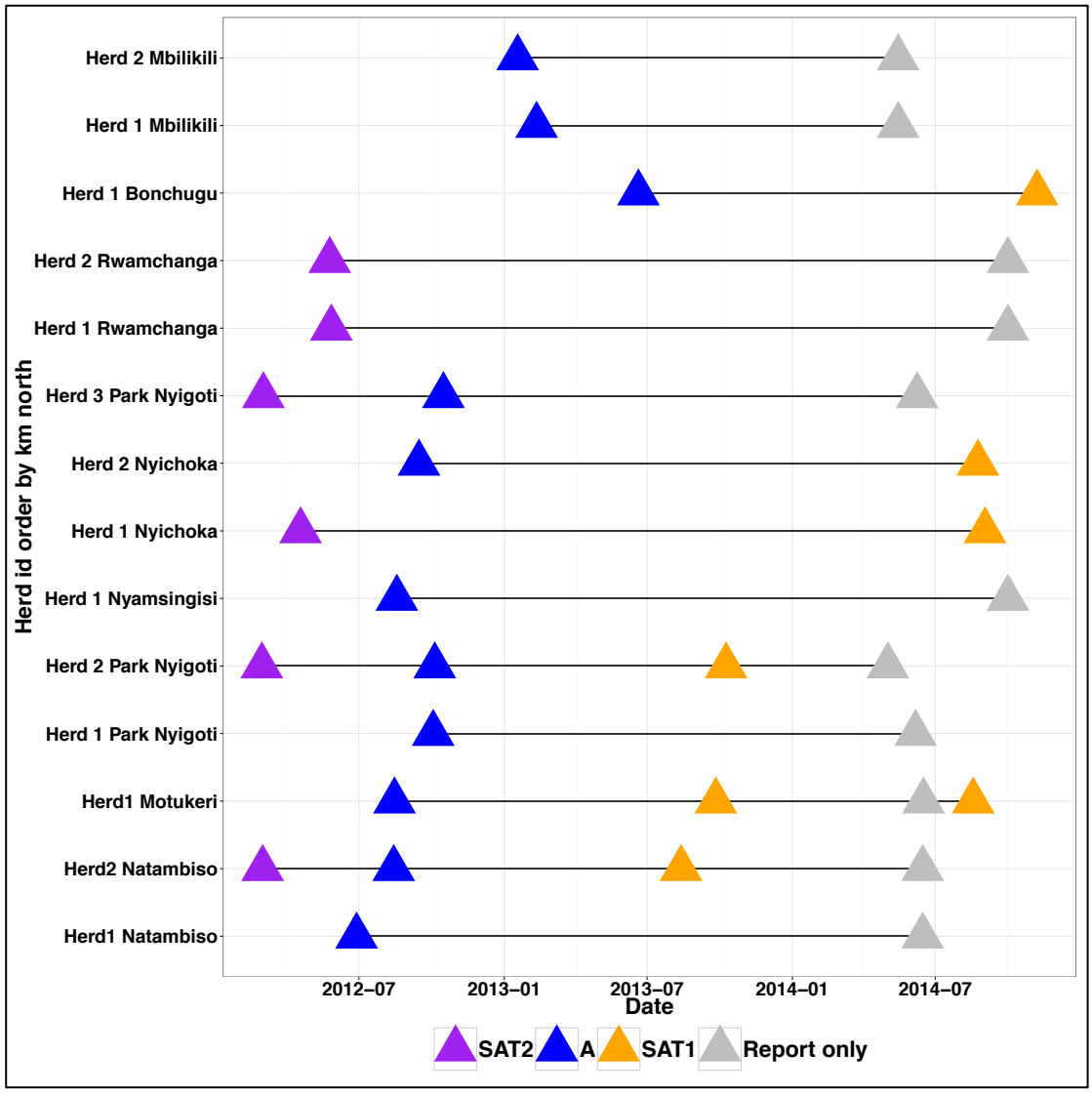


Figure 6.6: Outbreak reports and virus-typing results in fourteen herds followed through serial FMD outbreaks in Serengeti district.

Serological and virus isolation results from this study in the context of findings across Southern Kenya and Tanzania between 2010 and 2014

Results of review of FMDV types circulating in East Africa

The review of the literature and WRL database (Table 6.4) indicated that four serotypes (A, O, SAT1 and SAT2) caused FMD outbreaks in livestock in East Africa, with a fifth, SAT3, being isolated from buffalo and from a single Ankole calf in absence of clinical signs in Uganda (Dhikusooka *et al.*, 2015; WRL, 2015). Within the serotypes, multiple topotypes (variants with differences of 15% nucleotides for O and A and 20% for SAT1 and SAT2 (Knowles & Samuel, 2003)) were circulating. Amongst recent serotype A isolates from Kenya, Tanzania and Uganda, genotype 1 (based on the classification system of Mohapatra *et al.* (2011) dominated, but genotype 7 was also present. The East Africa 2 topotype was most common amongst serotype O viruses isolated, but East Africa 3 and 4 were also present. Amongst the SAT1 viruses isolated, the "North West Zimbabwe 1" (NWZ1) Topotype predominated, but Topotype IV (EA-1) was also detected in Uganda in 2013. For SAT2, Topotype 4 (as classified by Bastos, (2003)) was the most common but Topotype 1 was also reported.

Table 6.4: Results of a review of foot-and-mouth disease virus typing results in Ethiopia, Kenya, Tanzania and Uganda from 2008 onwards. World Reference Laboratory results from 2010 onwards were reviewed as these had information about sampling locations available.

Country	Study period	Virus ID method	Serotype	N viruses	Message	Reference	Availability for integration into this study
Kenya	1948 -2007	VP1 sequencing	SAT2	31	Topotype 5 not evident after the 1990s. Two diverse clades of topotype 1 circulating.	(Sangula *et al.*, 2010)	Outside of time-window required
Kenya	1964 - 2013	VP1 sequencing	A	38	High genetic diversity, widespread distribution and trans-boundary spread of serotype A in East Africa	(Wekesa *et al.*, 2013b)	Dates and locations available
Kenya	2010-2011	VP1 sequencing	O	35	Many outbreaks caused by serotype O in 2010 and 2011. Three independent lineages of EA2 circulating	(Wekesa *et al.*, 2013a)	Dates and locations available
Kenya	2008 - 2012	Sequencing (n=2 buffalo, 21 cattle) Antigen typing (n=26 cattle)	Cattle: A (n=20), O (n=1), SAT1 (n=7), SAT2 (n=19) Buffalo: SAT 1 (n=1), SAT2 (n=1)	49	Cattle sequences were distinct from buffalo sequences.	(Wekesa *et al.*, 2015)	Dates and locations available
Kenya	2010 - 2013	VP1 sequencing	A (n=2), O (n=18), SAT1 (n=67), SAT2 (n=8)	95		WRL reports 2010-2013 (WRL, 2015)	Dates and locations available
Kenya and Uganda	1964 - 2008	VP1 sequencing	O	46 (Kenya), 8 (Uganda)	Cross-border transmission of serotype O. Topotype EA 1 not common any more. Topotypes EA 2-4 present	(Balinda *et al.*, 2010b)	Dates and locations available
Uganda	2008-2009	VP1 sequencing	O	27	Sequences from six different districts were similar (EA2), indicating a single introduction.	(Kasambula *et al.*, 2012)	Dates and locations available
Uganda	2012 - 2013	VP1 sequencing	A (n=2) and SAT2 (n=6)	8	Multiple serotypes circulating in Uganda	(Namatovu *et al.*, 2015)	Dates and locations available
Uganda	2013	VP1 sequencing	SAT1 (n=2 cattle)	2	Undetected SAT1 infection in two cattle. Distinct lineage from previous cattle and buffalo samples.	Dhikusooka et al., 2015	Dates and locations available
Tanzania	2003-2008	Antigen typing	A (n=7), O (n=37), SAT1 (n=45), SAT2 (n=79)	167	Four serotypes circulating in Tanzania	(Kasanga *et al.*, 2012)	Dates and locations summarised but not directly available
Tanzania	2008-2009	VP1 sequencing	A (n=7), O (n=3), SAT2 (n=1)	11		WRL reports (WRL,	Dates and locations available

Country	Study period	Virus ID method	Serotype	N viruses	Message	Reference	Availability for integration into this study
						2015)	
Tanzania	2009 - 2013	VP1 sequencing	A (n=9), O (n=19), SAT1 (n=5), SAT2 (n=1)	35	Four serotypes circulating in Tanzania	(Sallu *et al.*, 2014)	Dates and locations summarised but not directly available
Tanzania	2011-2014	VP1 sequencing	A (n=26), O (n=11), SAT1 (n=50), SAT2 (n=23)	110		This study	Dates and locations available

Identification of northern Tanzania – southern Kenya region with most information for further analyses

The majority of virus isolation results as part of this study came from the northern part of the study area (Serengeti district), and the majority of results in the literature with readily available spatio-temporal information came from southern Kenya. Given the connectivity between these two regions in terms of livestock movements (GFRA, 2013; ILRI, 2014), patterns of serotype occurrence between 2008 and 2015 within this entire area (Latitude 3 S to 1.5 N and Longitude 33.5 – 39 E) were characterised. The results from Simanjiro further south in our study district and from the rest of Tanzania were sparser, and less was known about the movements of livestock in these areas. Therefore these results were omitted from further analyses. Figure 6.7 shows all virus typing results for Kenya and Tanzania between 2008 and 2015 with readily available spatio-temporal data and also the selected region for further analyses.

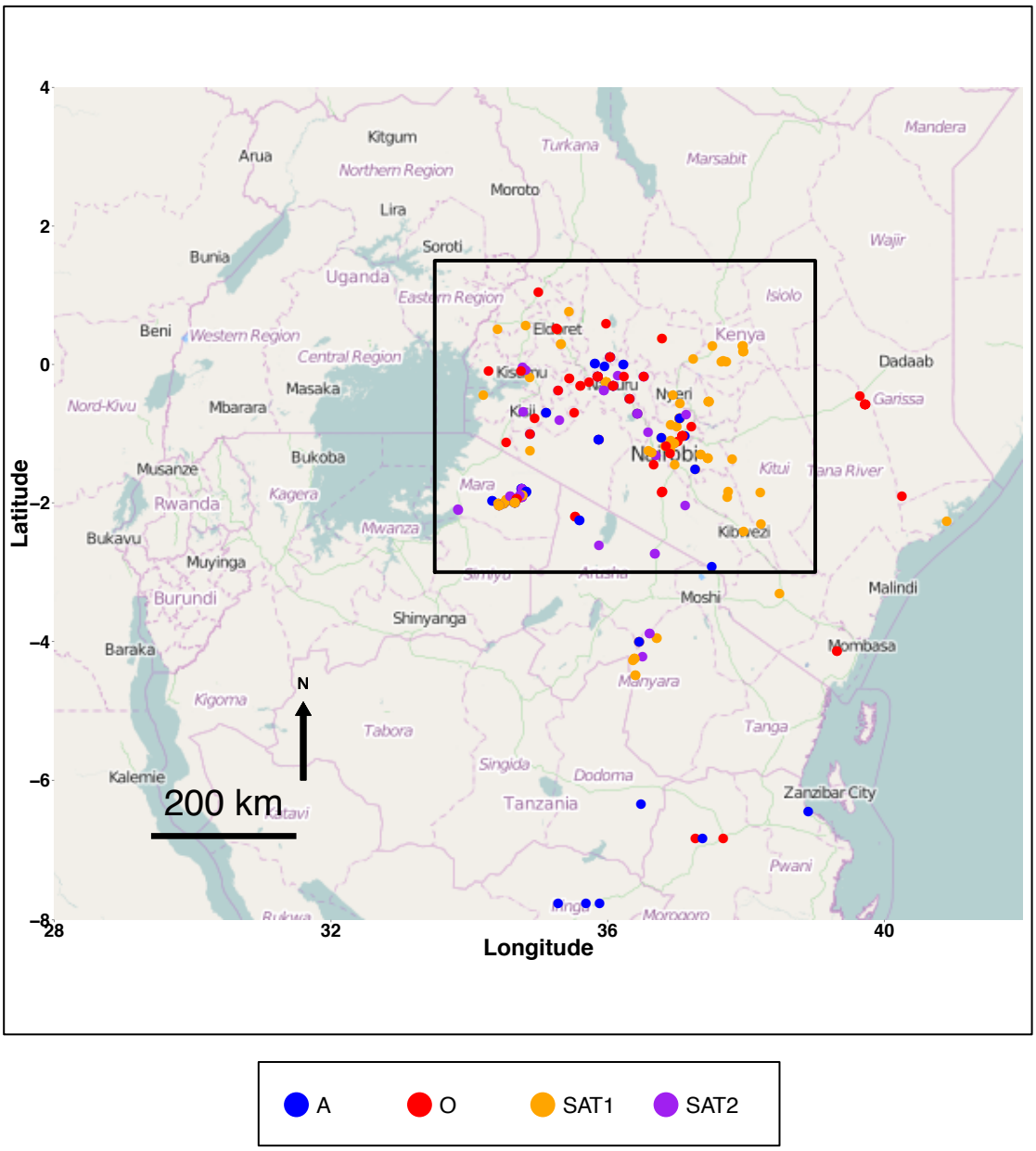


Figure 6.7: Virus isolation results from Kenya and Tanzania between 2008 and 2015 with spatio-temporal information available.
The box outlines the area in northern Tanzania and southern Kenya selected for further analyses. The background map was obtained from https://www.openstreetmap.org

Description of FMDV serotypes in the study area and in Northern Tanzania – Southern Kenya region over space and time

Figure 6.8 shows the virus typing results from the selected northern Tanzania – southern Kenya area plotted over time.

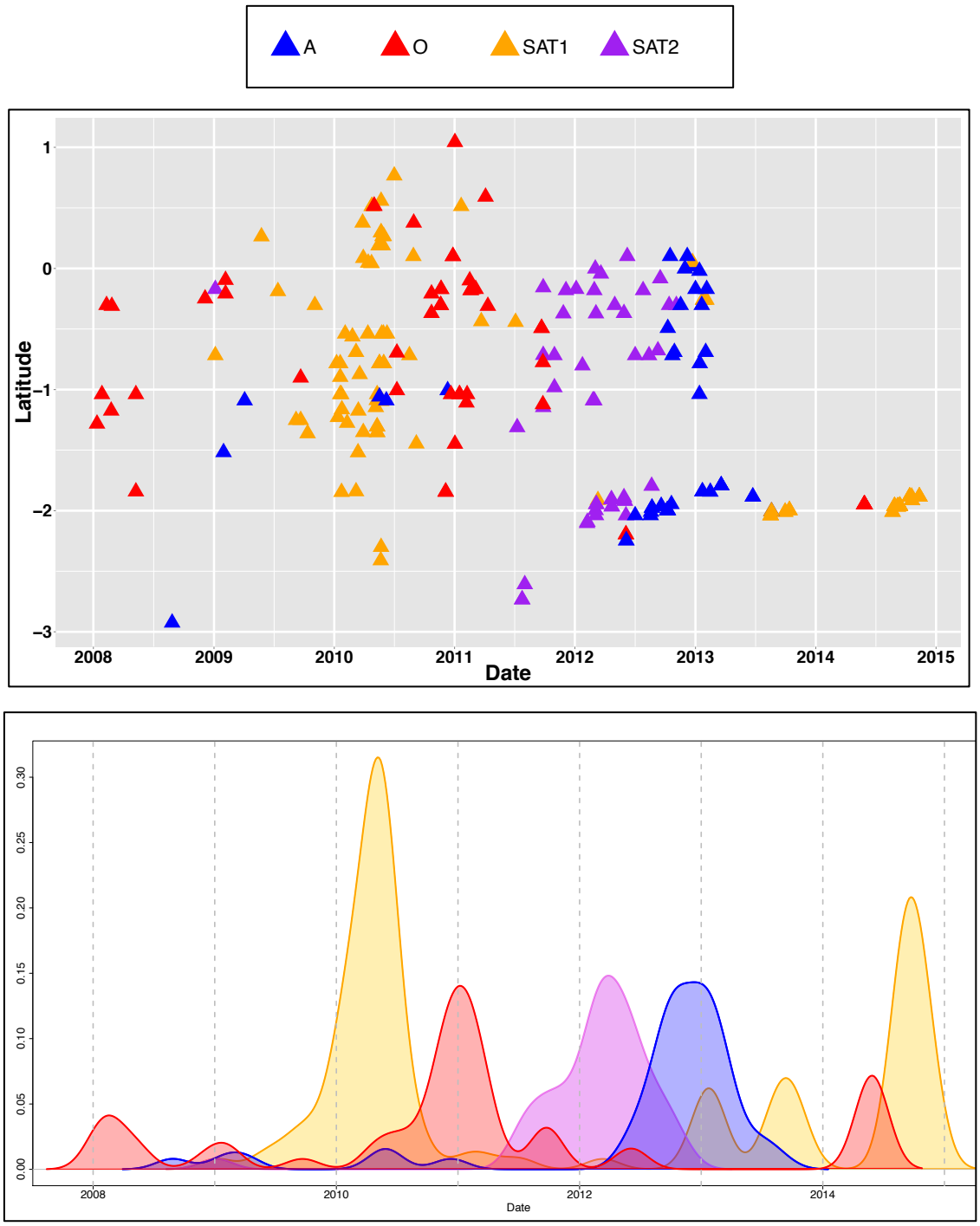


Figure 6.8: Virus isolation results from Serengeti district, Loliondo and Longido in the study area from 2011 – 2015 and results from southern Kenya from 2008 – 2013 (Latitude, -3S to 1.5 N and Longitude, 33.5W to 39 E).
The upper plot shows the latitude (y axis) and date of collection (x-axis) for FMDV isolates. The colours represent the serotype (red = O, blue = A, yellow = SAT1, violet = SAT2). For the lower plot, kernel density estimates for each serotype were generated using a smoothing bandwidth of 50 units. Note that virus typing data available after 2014 were from a limited geographical area.

Observations from Figure 6.8 were:

1. Four serotypes of FMDV are circulating in the northern Tanzania – southern Kenya area.
2. Serotype SAT1 dominated amongst Kenyan isolates in early 2010 and serotype O dominated in late 2010 and early 2011. This is consistent with inferences from serology results in the study area in 2011 with serotype SAT1 followed by serotype O.
3. Serotype SAT2 emerged in mid-2011 and remained dominant until mid-2012 both in the study area and in southern Kenya.
4. Serotype A dominated between mid-2012 and mid-2013.
5. Only results from the present study are available from after 2013. These indicated that serotype SAT1 emerged in mid-2013 and continued to be prevalent in Serengeti until the end of 2014.
6. The combination of inference from serology, and virus isolation results from the northern Tanzanian study and southern Kenya suggests a temporal pattern to FMDV serotype dominance in this area, with a SAT1 – O – SAT2 – A sequence between 2010 and 2013.

Chi-squared test to show that serotype distribution over time is not random

The virus typing results of the northern Tanzania – southern Kenya area were ordered over time. The serotype of each virus and that of the virus following it over time was recorded and the frequency of each combination was recorded (Table 6.5). The distribution of serotypes was not random, with a clear pattern of leader and follower serotypes being the same (Chi-squared test: $X^2 = 369.82$, p-value $< 10^{-16}$).

Table 6.5: Count of which serotype followed which when all serotypes from the northern Tanzania – southern Kenya area between 2008 and 2015 were ordered over time.

		Leader serotype over time			
		A	**O**	**SAT1**	**SAT2**
Follower serotype over time	**A**	30	3	8	6
	O	4	42	8	2
	SAT1	7	8	98	2
	SAT2	6	2	2	36

6.3.2 Investigation of serotype-specific risk factors for FMDV in cattle

Generalised linear mixed model results

LRT did not highlight any wildlife contact or livestock management related variables in the GLMM as being useful for explaining infection with serotypes A, O or SAT1 (Appendix 9). Non-significant variables included buffalo sightings non-buffalo FMD susceptible wildlife sightings, proximity to buffalo area, herd size, maximum distance walked for grazing or water, district and livestock practice (Appendix 9).

Given that SAT2 had very low seroprevalence in the study area until Monduli district was sampled in December 2011, district was the only variable that contributed towards explaining SAT2 infection (LRT: χ^2= 19.87, p =0.0005). Similarly to the other serotypes, no other variable helped explain SAT2 infection (Appendix 9).

Serial outbreaks caused by different serotypes in the same herds

Seven herds in Serengeti district as well as a herd in Simanjiro were observed to suffer serial outbreaks caused by different serotypes. The herd in Simanjiro district was shown to suffer serial serotype O, SAT2, SAT1 and A outbreaks over three years. Two herds in Serengeti district suffered serial SAT2, A, and SAT1 outbreaks and a further five had serial outbreaks caused by two different serotypes. The same herds (with the same risk-factors) succumbed to outbreaks caused by different FMDV serotypes. This is consistent with the lack of evidence for serotype-specific risk factors.

Retrospective power analysis for serotype-specific risk factor study

The results of the power calculation for the GLMM investigating risk factors for serotype-specific FMDV infection in cattle herds are summarised in Figure 6.9. For 77 herds, when buffalo sightings had no effect, Wald p values were less than 0.05 for 4.9% of simulations. When buffalo sightings increased the probability of livestock in the herd being seropositive by 0.2, Wald p values were less than 0.05 for 35.2% of simulations. When the probability was increased by 0.35 due to weekly buffalo sightings, p values were less than 0.05 for 86.6% of simulations. This meant that the numbers of herds covered by VNT in our study gave a power of >80% only for detection of large effects, and more subtle effects may have been missed (Figure 6.9).

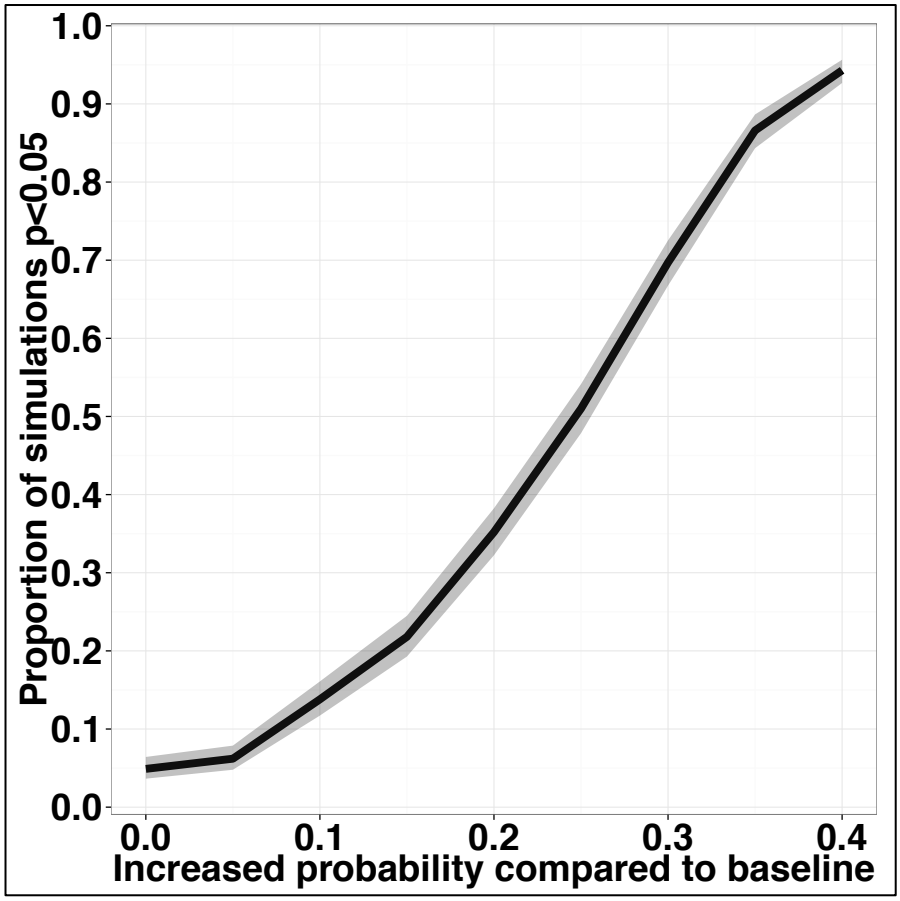


Figure 6.9: Power analysis based on 77 herds in the serotype-specific risk factor study.
The shaded areas represent binomial 95% confidence intervals for proportions.

6.3.3 FMDV infection patterns in buffalo

Buffalo NSP ELISA results

Of the 199 buffalo sera tested by NSP ELISA, 161 (80.9%, CI: 74.7-86.1%) were positive for NSP antibodies. Of the four ecosystems, buffalo in Arusha NP had the lowest NSP antibody levels. Tarangire NP and NCA buffalo had the highest levels (Table 6.6, Figure 6.10).

Table 6.6: Seroprevalence of antibodies against foot-and-mouth disease virus NSP in buffalo in the four ecosystems.

Ecosystem	NSP seroprevalence (95% CI)
Arusha	47.8% (26.8-69.4%)
NCA	86.2% (78.6-91.9%)
Serengeti	75% (57.8-87.9%)
Tarangire	95.8% (78.9-99.9%)

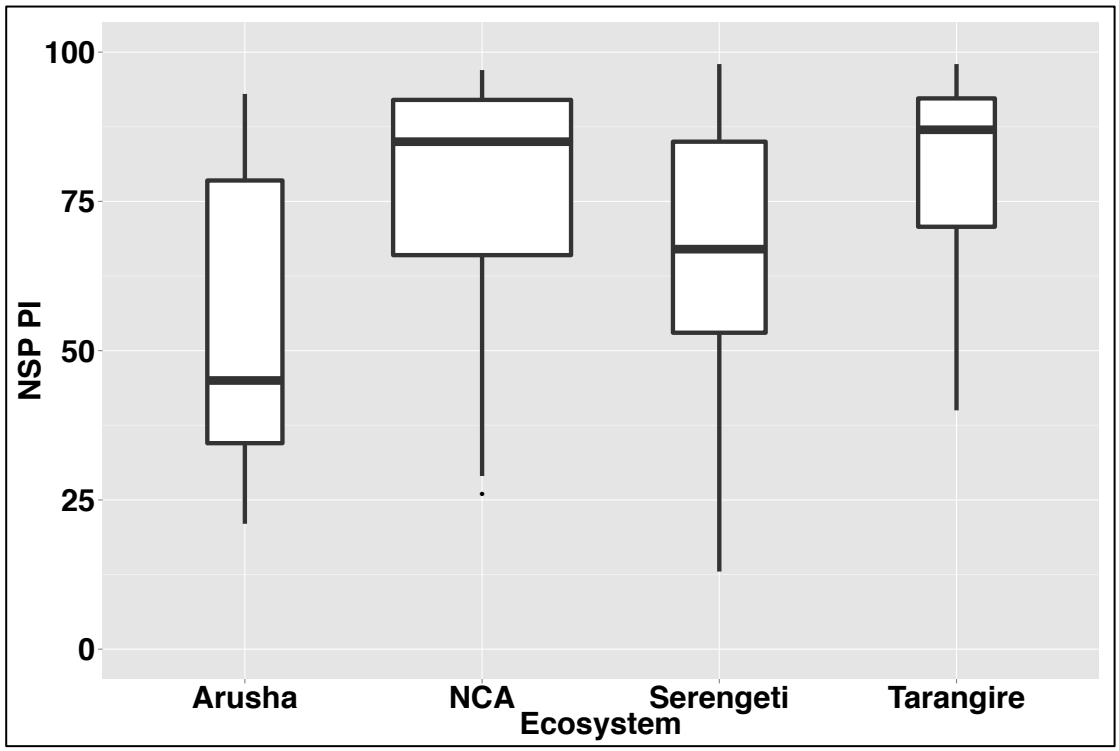


Figure 6.10: NSP ELISA results for buffalo.
Boxplots summarising foot-and-mouth disease virus non-structural protein (NSP) ELISA percentage inhibition (PI) correlating with antibodies against NSP in Arusha National Park, Ngorongoro Crater Conservation Area (NCA), Serengeti National Park and Taran.

Results of GLMM investigating whether age, sex or herd size explained the likelihood of a buffalo being NSP seropositive positive

The GLMM showed that increasing age had a negative effect on the likelihood of buffalo being seropositive (Table 6.7, Figure 6.11). Buffalo sex or group size did not have an effect. The proportions of seropositive buffalo in different age groups are compared to those in domestic livestock in Appendix 10.

Table 6.7: The effect of buffalo age on the likelihood of being NSP seropositive.

	LRT Chi	p	Estimate (95% CI)	Odds Ratio (95% CI)
Buffalo age per extra year	11.03	0.0009	-0.121 (-0.195--0.048)	0.886 (0.823-0.953)

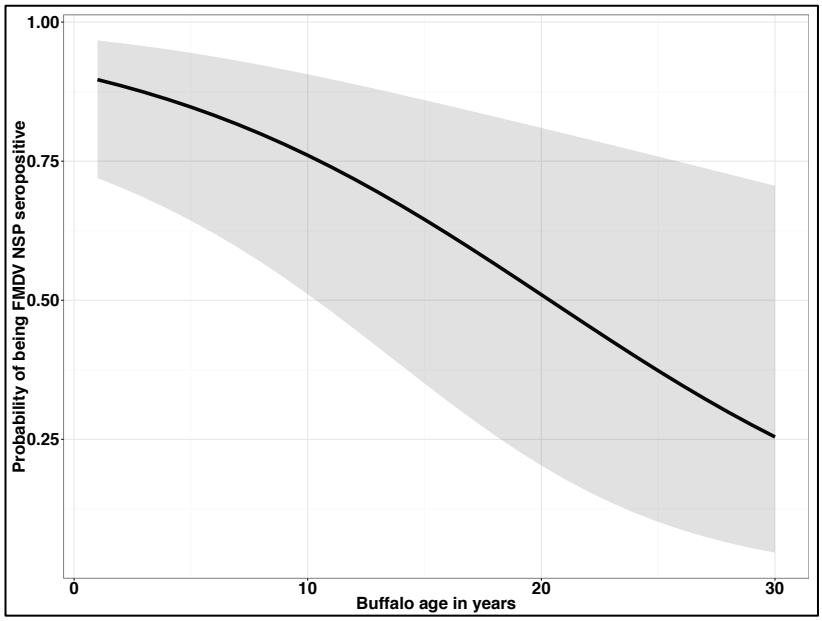


Figure 6.11: The effect of buffalo age on the probability of being NSP seropositive. The shaded area represents 95% confidence intervals.

Buffalo serotype-specific seroprevalence

All of the VN tested sera were from buffalo aged seven years old or less. Serotype SAT1 had the highest seroprevalence in all four ecosystems, followed by SAT2. There were buffalo with positive VNT titres against serotype O in NCA (n = 4 out of 14 conclusive results for serotype O), Serengeti (n = 1 out of 5) and Tarangire (n = 2 out of 13). Only a single buffalo in Serengeti NP had a positive titre for serotype A and there were no positive titres against SAT3 (Figure 6.12).

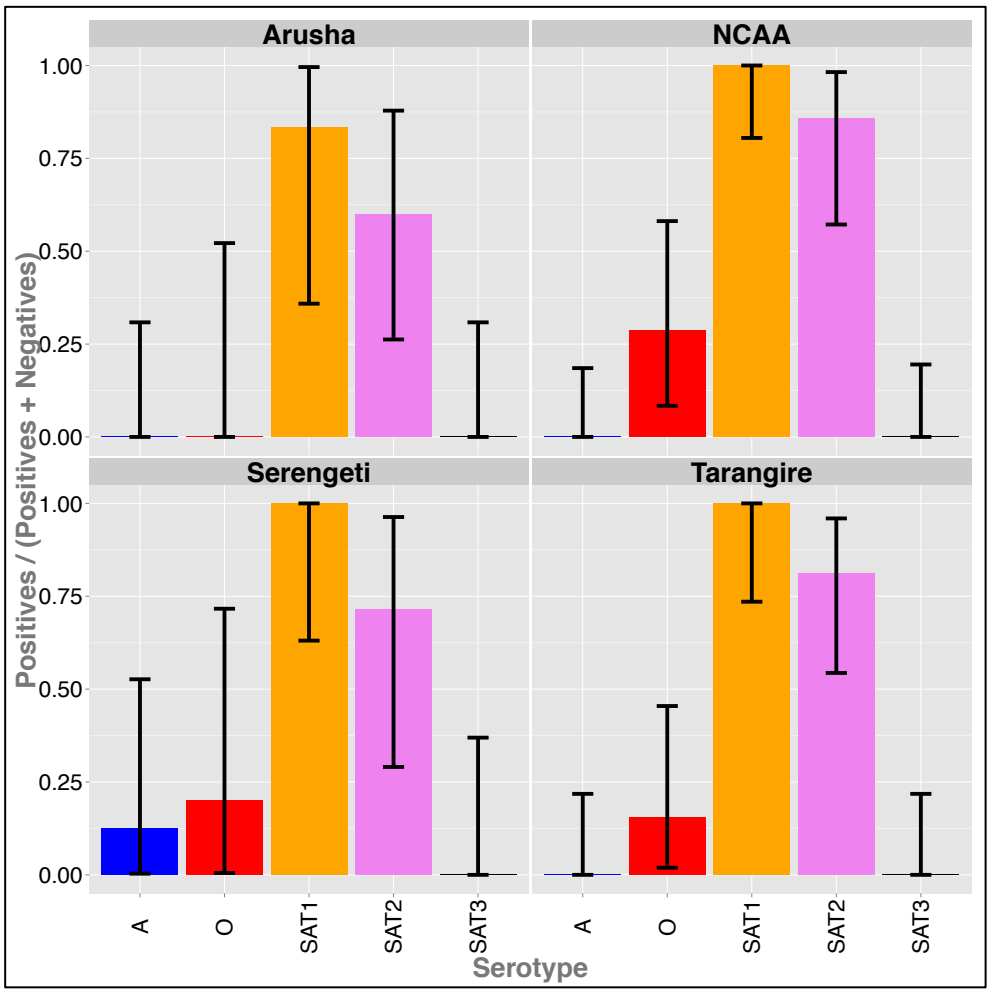


Figure 6.12: Seroprevalence of neutralising antibodies against serotypes A, O, SAT1, SAT2 and SAT3 in buffalo
The buffalo were sampled in Arusha National Park, Ngorongoro Conservation Area (NCAA), Serengeti National Park and Tarangire National Park.
The bars represent 95% confidence intervals

Buffalo VN titre ranking results

Patterns of serotype dominance in buffalo VNT titres (Figure 6.13) and pairwise ranking results (Figure 6.14) were consistent with seroprevalence patterns for each serotype, with SAT1 being most dominant, followed by SAT2. Two female buffalo in NCA (sampled in August 2011 and April 2012, aged over five and four years old, respectively) had higher neutralising titres against serotype O compared to any other serotype. Both of them had also positive titres against SAT1 and SAT2. Sera from two five-year-old male buffalo from Tarangire NP lacked positive VN titres against any serotype. These buffalo were both NSP ELISA positive (NSP PI = 51% and 83%, respectively).

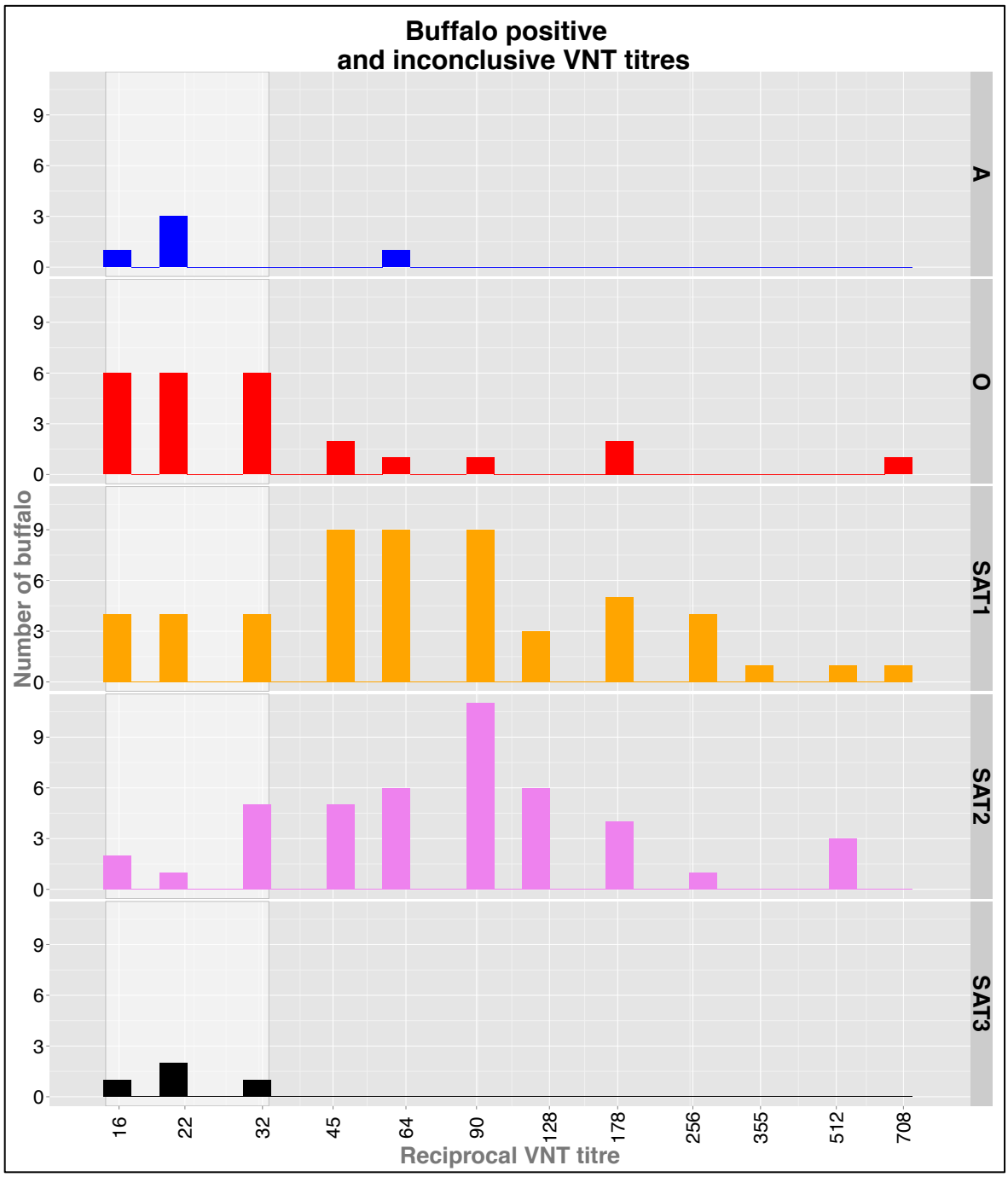


Figure 6.13: Reciprocal VN titres of 16 and above in buffalo sera against serotypes A, O, SAT1, SAT2 and SAT3.
The lighter grey background indicated inconclusive recripocal VNT titres between 16 and 32. Recriprocal titres above 32 were considered positive.

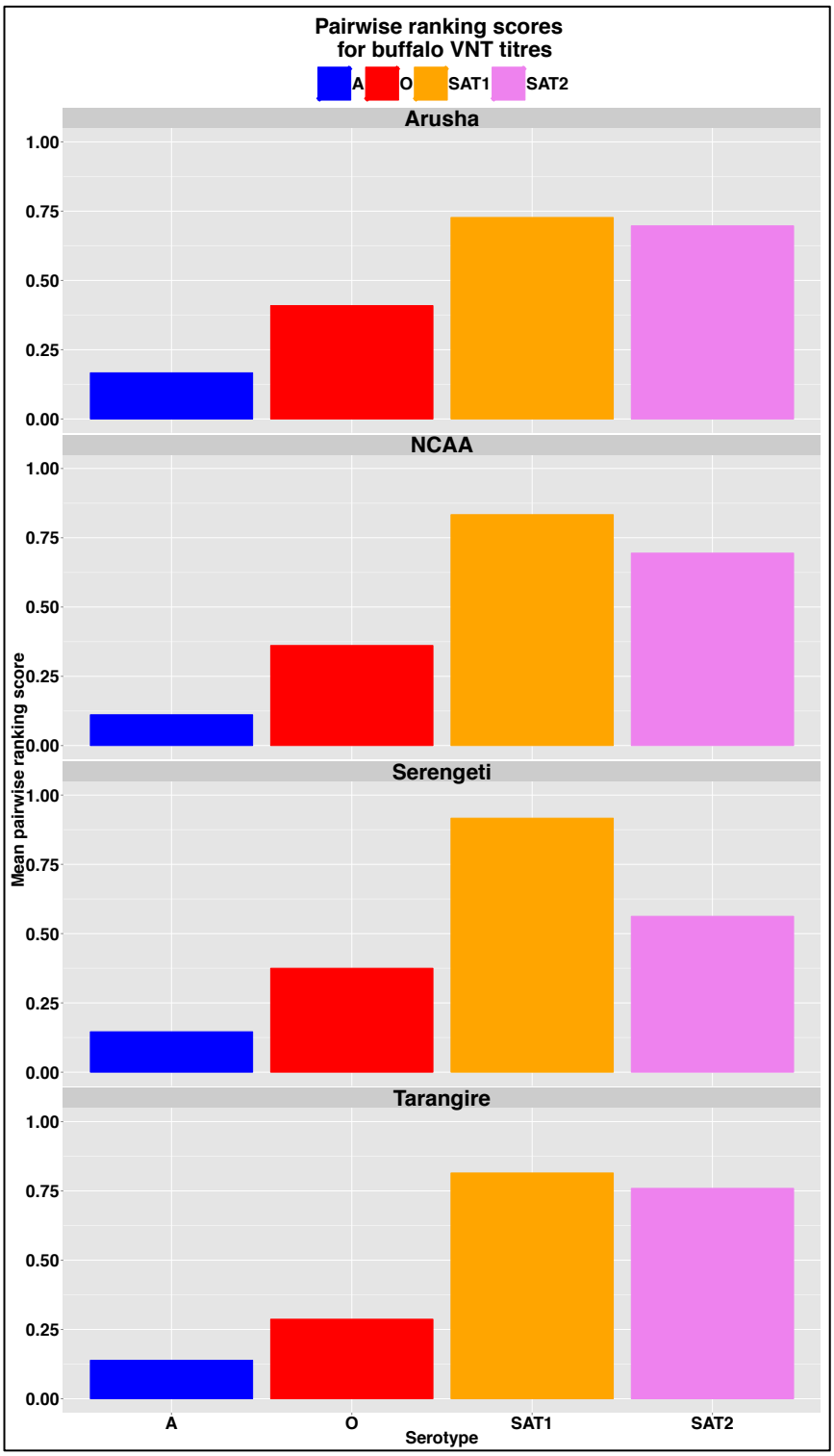


Figure 6.14: Mean pairwise ranking scores for serotypes A, O, SAT1 and SAT2 in buffalo in Arusha National Park, Ngorongoro Conservation Area, Serengeti National Park and Tarangire National Park.

Buffalo infection with specific FMDV serotypes

Serotype A in buffalo

Of the 51 buffalo sera that had conclusive VNT results for serotype A, only one serum from a five-year old female buffalo in Serengeti NP was positive. Serum from this buffalo was also VNT positive for serotypes O, SAT1 and SAT2 and the titres against SAT1 (reciprocal titre = 708) and O (178) were higher than the titre against A (64).

Serotype O in buffalo

Of the 37 conclusive buffalo VNT results for serotype O, there were 7 (18.9%, CI: 8.9-35.2%) positive results, all from female buffalo. Four were from NCA, two were from Tarangire NP and one was from Serengeti NP.

Serotype SAT1 in buffalo

There were 43 conclusive buffalo VNT results for SAT1. Only one serum from a five-year-old male buffalo was negative for neutralising antibodies against SAT1.

Serotype SAT2 in buffalo

There were 47 buffalo sera with conclusive VNT results for SAT2. Of these, 36 (76.6%, CI: 62.0 – 87.7%) were positive.

Comparison of serotype-specific FMDV infection patterns in buffalo and cattle.

Table 6.8 summarises the information available about infection with specific serotypes over time and space in buffalo and cattle in the study area. The information was obtained from both VNT and virus isolation from cattle FMD outbreaks. Serotype-specific information from cattle and buffalo over a similar time window were available in NCA, Tarangire and Serengeti ecosystems. In Serengeti, in July 2011, there was a clear difference in cattle infection patterns (O dominant) and those of buffalo (SAT1 dominant). Comparisons between infection patterns in cattle and buffalo in NCA and Tarangire were less clear-cut due to very few animals fitting the time-window for comparison. In Tarangire in July 2011, SAT1 dominated in buffalo, closely followed by SAT2. Serotype O antibodies were dominant in cattle, but there was also a cattle FMD outbreak caused by

SAT2 in August 2011. Neutralising antibodies against serotype O were dominant in the two NCA buffalo sera from August 2011 that were VN tested.

Table 6.8: Summary of FMDV type specific infection in cattle and buffalo in four ecosystems in Northern Tanzania.
NCA = Ngorongoro Conservation Area, VNT = Virus neutralisation testing, VI = virus isolation and genotyping

z	Date buffalo sampling	N buffalo VN tested	Dominant buffalo VNT serotypes	Cattle serotypes over similar period from VNT/VI	N Cattle with VNT results covering relevant period	N cattle herds with VI results covering relevant period	Comment
Arusha NP / Arusha livestock	Mar-12	11	SAT1 / SAT2	Not available	0	0	Arusha cattle sera were taken in August 2011 and were therefore not comparable to buffalo sera from March 2012
NCA	Aug-11	2	O	SAT1/SAT2	37	2	Loliondo cattle sera were taken between February - July 2011 (SAT 1 dominant). SAT 2 was isolated from cattle in Loliondo and Longido in July and August 2011
NCA	Apr-12	16	SAT1	Not available	0	0	No cattle results from this area and period. Virus isolation from Loliondo: July 2012 - A and O isolated.
Tarangire	Jul-11	6	SAT1 / SAT2	O/SAT2	18	1	Simanjiro cattle sera taken in August 2011 (serotype O dominant). SAT2 isolated from Simanjiro cattle in August 2011
Tarangire	Nov-11	12	SAT1	Not available	0	0	No cattle results from this area and period. Closest are virus isolation results from Simanjiro in spring 2012 - SAT1 and SAT2 isolated. Virus isolation from Loliondo July 2012 - A and O isolated.

6.4. Discussion

The conclusions from this chapter are:

I. There was a pattern of FMDV serotypic dominance over time, with a sequence of serotypes SAT1, O, SAT2 and A between 2010 and 2013 in the northern Tanzania – southern Kenya region.
II. There is no evidence for wildlife contact-related drivers of serotype-specific FMDV infection in our sample of cattle.
III. Buffalo have high levels of FDMV seropositivity, but, in contrast to cattle, their likelihood of seropositivity for exposure to FMDV NSP decreases with age.
IV. Buffalo have different patterns of serotypic dominance of FMDV infection compared to cattle in the same ecosystem.
V. Inference from serology results suggests transmission of FMDV serotypes that are associated with livestock to buffalo in limited instances but virus detection would be necessary to prove this.

The different lines of evidence in this study, drawn from field epidemiological studies, laboratory analyses and statistical modelling, are consistent with the interpretation that the dominant pattern of FMDV circulation in cattle across East Africa is a sequence of serotype-specific outbreaks. Our findings suggest that infection patterns in cattle are not closely linked to those in buffalo. Furthermore, the temporal sequence of serotype-specific outbreaks in cattle may be predictable. In Serengeti district, where most longitudinal FMDV typing data were available, the sequence of serotypes to cause FMD outbreaks after 2011 was inversely related to VNT titres representing antibody levels in the Serengeti cattle population in 2011.

Serological and virus isolation results from the present study are consistent with previous East African studies (Balinda *et al.*, 2010b; Kasanga *et al.*, 2014b, 2012; Namatovu *et al.*, 2015; Wekesa *et al.*, 2013a, b, 2015) in showing that multiple FMDV serotypes are circulating. This study is the first to highlight waves of serotypic dominance sweeping through the northern Tanzanian livestock population over time.

It made sense to extend investigation of these waves of serotypic dominance from northern Tanzania to southern Kenya, due to the close cultural and trading connections between

these two regions which could lead to FMDV trans-boundary movements. Northern Tanzanian cattle are taken to Nairobi and other Kenyan urban areas to generate better prices at market (FAO, 2013a; GFRA, 2013; Di Nardo *et al.*, 2011). Better grazing in northern Tanzania may motivate Kenyan cattle owners to bring their cattle southwards (Prof. Sarah Cleaveland, personal communication). The temporal pattern of antigenic dominance seen amongst the Kenyan isolates fitted with the inferences made from serology data and with the longitudinal virus isolation data from this study in Tanzania. This suggests that FMDV serotypes circulate at a broad regional scale. This predictability of serotypic dominance could allow for the design of control measures, such as targeted vaccination of cattle in advance of serotype-specific outbreaks, to maximise their effectiveness and impact in this resource-limited region.

Turkey is another region that is endemic for multiple serotypes of FMD and that has longitudinal virus typing records. Long term records from Turkey, as well as more detailed studies covering 1990-2002 (Gilbert *et al.*, 2002, 2005) and 1996 – 2004 (Klein *et al.*, 2006; Parlak *et al.*, 2007) have highlighted patterns of FMD circulation in the region. They show that serotype O persists in Turkey and represents an evolutionary continuum. In contrast, different genetic variants of serotype A appear to make incursions from the East. Serotype Asia -1 caused outbreaks in Turkey in in 1973, 1984, 1999 and 2012, but persisted for less than three years on each occasion. FMD outbreaks were associated with host density and short-distance spread, and introduction of a new variants was associated with long distance inward movements into regions where demand for meat out-stripped supply (Gilbert *et al.*, 2005). Turkey appears to have increases in FMD outbreak incidence every few years. For example, peaking in 1996, 1999, 2006 and 2010, it had a series of epidemics caused by Serotype O, serotype Asia1/O/A, serotype A/O and serotype O (respectively) (McLaws *et al.*, 2011). Peaking in 2011, 2012, 2013, 2015 and 2016, a series of epidemics caused by serotypes A, Asia1, O (small), A, and O respectively, were observed (Data from SAP Institute, Turkey and personal communication with Dr. Naci Bulut). Comparison of the drivers of these epidemic waves in Turkey and East Africa would be an interesting area for further research.

The predictability of FMDV antigenic dominance over time in northern Tanzania and southern Kenya could be explained by a combination of competition between FMDV antigenic types and FMDV type-specific immunity in the cattle population. There is likely

to be constant competition between the four serotypes for dominance in the cattle population. A serotype that has been dominant recently has a competitive disadvantage, as there will be high levels of immunity against it in the cattle population. Given the highly contagious nature of clinical FMD, once a serotype obtains a slight advantage and causes clinical infection in a subset of cattle, this will rapidly amplify due to acutely infected cattle shedding large quantities of virus. Once a large enough proportion of cattle have developed post- infection immunity against the dominant antigenic type, it is again the most "disadvantaged" serotype, and another serotype is more likely to become dominant next.

This strong temporal pattern of antigenic dominance in combination with absence of serotype-specific risk factors for serotypes A, O and SAT1 suggests a lack of serotype-specific drivers of FMD infection. The variable that partially explained SAT2 infection was district, and this was related to sera sampled in December 2011 in Monduli district (later than in the other districts), as the wave of SAT2 (evidenced by virus isolation findings from the study area and Kenya) was emerging across the region. This is consistent with waves of serotypic dominance over time rather than serotype-specific risk factors. Similarly to our study, a recent study in Kenya also reported multiple FMDV serotypes circulating in the cattle population with no evidence of any connection to variants circulating in buffalo (Wekesa *et al.*, 2015). In the outbreak tracking study, the same herds, with the same risk factors suffered serial outbreaks caused by different serotypes. This does not support the hypothesis that there are different risk factors for different serotypes. Three strands of evidence consistently support the interpretation that factors other than contact with wildlife drive FMD transmission in the study area: Firstly, the strong temporal pattern of antigenic dominance, secondly the lack of serotype-specific risk factors, and thirdly the same herds suffering outbreaks caused by different serotypes.

Serotype-specific investigations of buffalo sera from this study further support the idea that FMDV circulation in northern Tanzanian cattle is not coupled to that in buffalo. In contrast with the findings in this study suggesting that buffalo are not an important reservoir of FMDV for East African cattle, multiple researchers have associated southern African cattle infection with the SAT serotypes and contact with buffalo (Hargreaves *et al.*, 2004; Miguel *et al.*, 2013; Vosloo *et al.*, 2002a, b). A crucial difference between East and southern Africa may be that cattle in East Africa, where FMD is relatively uncontrolled, may function as an independent maintenance population (Haydon *et al.*, 2002). Greater efforts

are made to control FMDV in southern African cattle, with parts of South Africa being recognised as FMD free areas (OIE, 2015a), which might have altered more natural disease dynamics. There are also major contrasts between the southern African ranch based management system and the vast movements of East African cattle to reach grass and water, to avoid disease, and to fetch the best prices at market.

It has been postulated that buffalo are the ancestral hosts for FMDV (Vosloo *et al.*, 2002b), and it is well accepted that they show few clinical signs of disease compared to cattle and have long-term persistent infections (Condy & Hedger, 1974, 1978; Condy *et al.*, 1985; Gainaru *et al.*, 1986; Hedger, 1972). Early researchers observed that buffalo calves initially become infected as soon as their maternally-derived immunity wanes (Condy & Hedger, 1978). It is believed that the buffalo with most FMDV replication and infectiousness are these acutely affected calves (Thomson, 1995; Vosloo *et al.*, 2009). In contrast to southern Africa where there is a clearly defined buffalo calving season (Thomson *et al.*, 1992), Tanzanian buffalo calve all year round (Prins, 1987), possibly reducing the load of buffalo-related FMDV posing an infection risk to cattle at any one time. This provides a further explanation for the lack of evidence that Northern Tanzanian buffalo and cattle share FMDV antigenic types.

Although cattle-related risk factors are likely to dominate as drivers of infection patterns in northern Tanzania, it is evident that contact and potential transmission between Tanzanian buffalo and cattle can occur. The dominance of antibodies against serotype O in two NCA buffalo at the time when this serotype was most dominant in cattle suggests that cattle-to-buffalo transmission may have occurred. As pastoralist cattle are in frequent contact with wildlife in the NCA, it is plausible that acutely affected cattle shedding large volumes of FMDV type O could infect the buffalo in the area. However, only detection of FMDV serotype O or its genetic material from buffalo could confirm this. Positive buffalo serotype O VNT (Anderson *et al.*, 1979) and ELISA (Di Nardo *et al.*, 2015) results have been previously reported, but outside of experimental infection (Anderson *et al.*, 1979), serotype O, or its genetic material, have never been isolated from am African buffalo.

Another topic for further investigation is the lower seroprevalence of FMD in both cattle and buffalo in the Arusha region. It is unknown whether there is a connection between findings in buffalo and cattle. The smallholders that keep cattle in Arusha have smaller

herds and do not move their livestock as far as the other management systems (Chapter 4), meaning that their animals have less exposure to risk factors for FMD. However, it is more difficult to explain why Arusha NP buffalo have lower FMD seroprevalence compared to the other ecosystems and this requires further research.

As well as suggesting that cattle and buffalo in the study area do not share serotype-specific FMDV infection patterns, this study highlighted that FMDV epidemiology within the buffalo population appears to have different drivers compared to cattle. In this study, the likelihood of FMDV infection (using NSP ELISA seropositivity as an indicator of FMDV replication in the buffalo over the past several years), reduced as the buffalo aged. Evidence that younger buffalo have the highest levels of active FMDV infections is consistent with southern African studies that show that almost 100% of buffalo are infected with all three SAT serotypes by the time that they are two years old (Thomson *et al.*, 1992), and the one to three year old age-group that have the highest levels of persistent infection (Juleff *et al.*, 2012). Southern African buffalo appear to become infected as young as three months old (Condy & Hedger, 1978), but, after a rapid sequence of infection with multiple SAT serotypes, they develop high levels of neutralising antibodies against FMDV (Thomson *et al.*, 1992). Similarly to this and to our study, another study of 483 buffalo spread across East and Central Africa showed early sero-conversion against FMDV NSP with a slight decline in buffalo over 15 years old (Bronsvoort *et al.*, 2008). The reduction in FMD NSP seropositivity in older buffalo in the present study area contrasts with findings in livestock. In Chapter 4, increasing age is a risk factor for seropositivity in cattle and small ruminants. This suggests a difference in FMD epidemiology in cattle compared to buffalo populations.

Although there were no positive SAT3 VNT results amongst the buffalo sera, this does not mean that there are no SAT3 FMDV variants circulating in Tanzanian buffalo. The only SAT3 variant available for VNT was a virus isolated over 30 years ago from Zimbabwe. Buffalo populations in Africa are fragmented (East, 1999) and SAT3 is very rarely isolated from livestock (Dhikusooka *et al.*, 2015; Thomson, 1994). Therefore, it is likely that any SAT3 viruses circulating in Tanzanian buffalo would be divergent from southern African variants. The SAT3 viruses isolated from the closest location to Tanzania (Uganda (Dhikusooka *et al.*, 2015), WRL), were divergent from southern African SAT3 viruses, suggesting that lack of avidity between East African anti-SAT3 serum and southern

African SAT3 test virus may have been a problem. Lack of avidity between test viruses and antibodies in the sera being tested may also explain why a small number of sera were NSP positive but tested negative with the VNT or the SPCE. This lack of avidity could be due to a SAT3 infection or to an infection with a strain of A, O, SAT1 or SAT2 viruss that was dissimilar to those used in the VNT and SPCE. The NSP antigen is far less variable than the capsid antigens that the serotype specific-assays are based on (Grubman & Baxt, 2004), suggesting that the NSP assay may have detected infection where the serotype-specific assays could not. Another possibility is false positives on the NSP assay, although this is more likely to happen in a low prevalence situation (Bronsvoort *et al.*, 2006b).

Despite the lack of power in the GLMMs to investigate serotype-specific risk factors, the consistency of the trends shown from the risk factor analysis with sequence of serotypic dominance over time, the same herds suffering outbreaks caused by different serotypes and the lack of linkage between patterns of infection in cattle and buffalo increase confidence in the conclusions. The potential predictability of outbreaks by given serotypes in northern Tanzanian and southern Kenyan cattle populations heralds exciting possibilities for targeted control options in the future.

Chapter 7: Thesis Discussion

Informed control measures for FMD are highly relevant to global agendas to reduce poverty and improve food security. In this context, the aims of this thesis were to quantify the socio-economic impacts of FMD in East Africa, understand its epidemiology at the wildlife-livestock interface and characterise patterns of infection with specific serotypes. These aims were addressed through collation of field data, laboratory analyses and statistical modelling, focusing on three northern Tanzanian ecosystems where susceptible livestock and buffalo populations live in close proximity.

Key conclusions from the thesis were:

1. FMD is prevalent in the study area and has substantial impacts on rural livelihoods in traditional livestock-keeping systems. FMD control in these systems has the potential to reduce vulnerability to poverty through increased milk and crop production.
2. In contrast to FMD epidemiology in southern Africa, livestock management rather than wildlife related risk factors drive FMD infection in East African livestock. Different patterns of FMD serotype dominance in cattle and buffalo infections suggest that FMDV circulation in these species is not tightly linked, and further support the finding that buffalo are not currently an important source of FMDV for livestock in the study area. Therefore, in the early stages of FMD control efforts in East Africa, addressing livestock management related risk factors for FMD and reducing the FMD burden in livestock through vaccination is more likely to be successful than focusing on strategies to block transmission from buffalo.
3. There was a pattern of FMDV serotypic dominance evident over a four-year period in livestock sampled across northern Tanzania and southern Kenya. A sequence of serotypes (SAT1- O – SAT2 – A) dominated between 2010 and 2014. Inferences of FMD infection histories from serological data through a novel modelling approach were consistent with these findings. Whilst longer term research is necessary to investigate whether this pattern is truly predictable, it raises the possibility that expected serotypes could be targeted by vaccination strategies.

Figure 7.1 links thesis aims, chapters and conclusions.

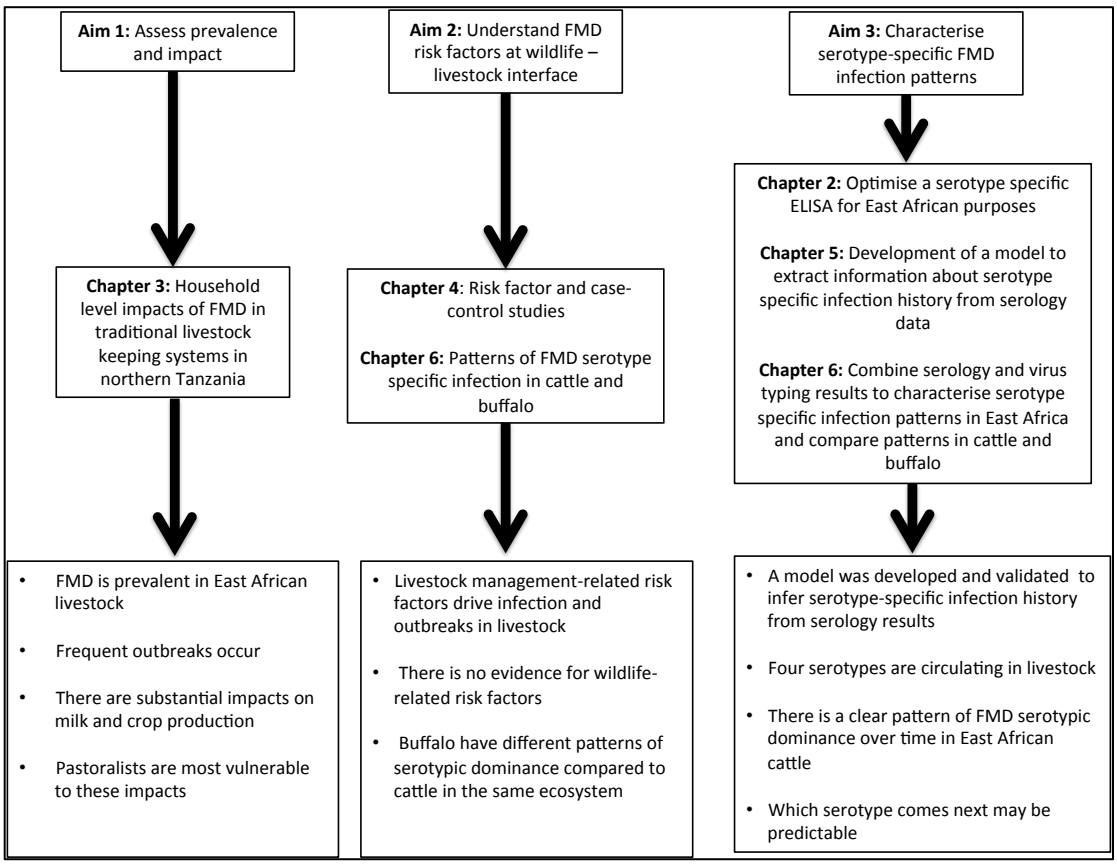


Figure 7.1: Thesis aims, chapters and conclusions.

7.1 Prevalence and impacts of FMD

The high seroprevalence of FMD in livestock in the study area was consistent with other East African reports (Bayissa *et al.*, 2011; Genchwere *et al.*, 2014; Mkama *et al.*, 2014; Namatovu *et al.*, 2013a; Wekesa *et al.*, 2015). Furthermore, substantial impacts on milk and crop production resonated with studies in Ethiopia and Sudan (Barasa *et al.*, 2008; Bayissa *et al.*, 2011).

The present study is the first to confirm the barrage of frequent FMD outbreaks that rural communities suffer, with livestock keepers reporting up to three outbreaks a year. Northern Tanzanian livestock owners were very familiar with FMD, identifying clinical signs consistently with outbreak team investigations and confirmation by virus isolation in the WRL-FMD. In addition, the reported herd outbreak history correlated well with FMD

sero-prevalence. These consistencies increased confidence in household reports where laboratory confirmation was not available, and suggest that livestock owners in the region are highly perceptive of their animals' health status. This attention to livestock and their ailments is not surprising given the integral roles that livestock play in household nutrition and livlihoods. All three livestock management systems reported substantial impacts on milk production and draught capacity due to FMD outbreaks.

Given the soaring human populations in Africa (Worldpop, 2015), things may get even tougher for East African livestock owners. They will need to compete for increasingly scant land reosurces, and more efficient use of land through using fewer, more productive livestock may be necessary. This may be facilitated through control of FMD and other livestock diseases. Heavy livestock disease burdens may generate adversity to trialling more productive breeds of livestock in fear of greater losses (Perry *et al.*, 2002).

In this study, rural smallholders, the management system that had the lowest FMD burden, were also the households with the fewest, but the most productive cattle in terms of milk yield per cow. Another study in the same districts showed that the rural smallholder communities were less vulnerable to childhood health issues and food-insecurity compared to the pastoralists (Lawson *et al.*, 2014). Therefore, it appears that the smallholders in the Arusha area are relatively better off compared to the other management systems in terms of having a lower burden of FMD, more productive livestock, and better human health and nutrition status. In contrast, this study and others (Barasa *et al.*, 2008; Lawson *et al.*, 2014) have highlighted pastoralists as the group that can least afford extra losses from FMD. They are most vulnerable to the impacts of FMD on milk production due to their reliance on milk as a protein source, and very high levels of FMD were evident in their livestock. Further studies are required to understand the potential impacts of FMD on human nutrition and childhood mortality.

Pastoralists were also the only group who reported human illness associated with FMD outbreaks and respondents recognised the potential to contract FMD from drinking milk, which is consistent with what is known wbout human FMD infections (Bauer, 1997). Investigation is required to decipher whether these reports relate to separate concurrent infections during FMD outbreaks, recall bias, or zoonotic FMD infections.

As well as being more vulnerable to impacts due to FMD, pastoralists reported higher morbidity due to FMD in their livestock compared to agropastoralists. A very large range in morbidity (4 -100%) at animal level has been previously reported (Gonzales *et al.*, 2014; Govindaraj *et al.*, 2015; Jemberu *et al.*, 2014; Mersie *et al.*, 1992; Roeder *et al.*, 1994), with 50-100% previously reported in East Africa (Jemberu *et al.*, 2014; Mersie *et al.*, 1992; Roeder *et al.*, 1994). Therefore, it is difficult to compare animal level morbidity to previous reports. However, at herd level, an Ethiopian study found that pastoralists reported signicicantly higher morbidity compared to mixed crop and livestock farmers (Jemberu *et al.*, 2014).

The reasons for this higher reported morbidity in pastoral livestock, as well as the apparent increase in outbreaks amongst pastoral livestock in the wetter season need to be better understood. Given the enormous variation in reported morbidity in this study, modelling efforts could only offer hypotheses to pursue with further studies rather than conclusive answers. The large variation is likely to be genuine, as it is conisitent with other studies in endemic countries (Subramaniam *et al.*, 2013), and livestock owners were accurate where their reports could be compared to laboratory analyses and field observations. It is possible that this highly variable morbidity is partially driven by something that was not measured in this study. For example, herd-immunity subsequent to sequential FMD outbreaks, differences in virulence amongst FMDV strains, aspects of management, interplay with other diseases or climatic conditions could all potentially explain it. The lack of association between herd level seroprevalence and reported morbidity suggests that sub-clinical FMD infections in livestock may be more frequent than currently realised, and separate studies are emerging to substantiate this idea (Dhikusooka *et al.*, 2016; Gonzales *et al.*, 2014; Miguel *et al.*, 2013). Infectiousness at animal level is related to the presence of FMD clinical signs (Charleston *et al.*, 2011). Therefore, as well as helping to understand the impacts of FMD outbreaks, a better understanding of animals with FMD lesions, those with other clinical signs and those subclinically infected would inform epidemiological modelling of outbreaks in endemic areas.

7.2 Patterns and risk factors for FMD at the wildlife-livestock interface

The epidemiological findings from this study raise the potential for improvements in FMD control efforts in East Africa. Cross-sectional, case-control and serotyping studies all suggest that livestock related risk factors are the most important drivers of FMD in livestock in the study area. This provides a rationale for focussing initial FMD control efforts on livestock management and vaccination strategies.

The two criteria for a reservoir of infection were introduced in Chapter 1. These are:

1. FMDV can persist indefinitely in the system without the need for transmission from another system. I.e. the system is a maintenance community for FMDV.
2. FMDV is transmitted from the system to the target population (livestock).

This study suggests that East African livestock are likely to function as a maintenance community for FMDV without the need for contact with wildlife. No wildlife contact related risk factors were identified for FMD infections or outbreaks, whereas there was strong evidence for livestock management related risk factors. Virus typing data, in combination with inferences from serology, showed that livestock underwent sequential sweeps of outbreaks caused by different serotypes, including serotypes that are not associated with buffalo. The same herds, with the same risk factors, had outbreaks caused by serotype A and the SAT serotypes.

As well as being a maintenance community, there are more opportunities for livestock to transmit FMDV to other livestock compared to opportunities for buffalo to livestock transmission. For example, the size and connectivity of the East African livestock community results in many contacts between livestock. FMD was highly prevalent in livestock, and cattle that are acutely infected with FMDV are more infectious than buffalo (Gainaru *et al.*, 1986). Chapter 4 highlights risks for FMD associated with livestock contacting other livestock (larger herds, new animals in the herd), systems with greater connectivity between different herds (pastoral and agropastoral), and animals (cattle) that are naturally more susceptible and that are managed in a manner where they contact more cattle compared to how small ruminants are managed. Livestock movements appear to be key drivers of FMDV transmission in the study area, as has been recognised in many settings (for example Ayebazibwe *et al.*, 2010; Klein *et al.*, 2008, reviewed by Di Nardo *et*

al., 2011). Livestock movements in the East African region have many potential seasonal, economic and cultural drivers. Deeper interrogation of animal movement data from the household questionnaires described in this study, as well as further cross-border studies, may help to improve our understanding of patterns and drivers of livestock movements in the region, and subsequently shed light on critical FMD control points.

Buffalo are also likely to comprise a maintenance population for FMD in East Africa. They had high seroprevalence of FMDV, with highest titres against SAT serotypes. In contrast to livestock, buffalo have fewer opportunities to transmit FMDV to livestock. They are less abundant than livestock, and have few contact opportunities with them. They are also less infectious than cattle (Gainaru *et al.*, 1986). Therefore it makes sense that no buffalo-contact related risk factors for FMD in livestock were identified. Furthermore, measures of contact with potential non-buffalo wildlife intermediaries of infection did not increase the likelihood of FMDV infection in livestock.

Livestock are likely to be the most important source of FMDV for livestock in northern Tanzania, but some transmission from buffalo cannot be ruled out. Any signal from wildlife to livestock transmission is likely to be drowned out by the dominance of livestock related risk factors. The role of wildlife as a potential reservoir of FMDV for livestock in East Africa may only become evident if FMDV is well controlled in livestock. Indeed, intervention studies have been condoned as a useful method for identifying reservoirs of infection (Haydon *et al.*, 2002; Viana *et al.*, 2014). Further approaches to investigate wildlife to livestock transmission could include conducting a more detailed study in the smallholder area, where there are fewer livestock-related risk factors for FMD, or molecular epidemiology studies comparing the genomes of FMDV from wildlife and livestock in the same ecosystem.

This study has integrated all available virus typing data with inferences from serology to strengthen confidence in conclusions, as advocated by Viana *et al.* (2016). Further strands of evidence to develop include investigations into whether livestock and buffalo share similar molecular variants of SAT type FMDV, and, if they do, in which direction is transmission? Virus isolation and genotyping from buffalo could also be used to investigate whether occasional transmission of livestock-associated serotypes to buffalo occurs, as was suggested for serotype O by serology results.

7.3 Diagnostic challenges and strategies to overcome them

This study highlighted that integration of different types of data could greatly enhance their utility for informing conclusions. The novel Bayesian approach described in Chapter 5 increased the information that could be gleaned from serology data. This was combined with virus typing and neutralisation data to inform conclusions about the circulation of specific FMDV serotypes in the study area. However, the project also highlighted how the logistics of producing laboratory results can have a significant impact on power achieved in the study. When there was an accessible and easy-to-use diagnostic assay available, such as the FMDV-NSP commercial ELISA, sufficient data were generated to provide acceptable statistical power in subsequent analyses. Serotype-specific data were more challenging to generate in the laboratory, and this affected the statistical power for the serotype- specific risk factor analyses.

Much effort was put into optimising a serotype-specific ELISA to generate results from a herd with known infection history, and into building a model to interpret data from this ELISA. The rationale for this was to develop an easier tool with which to generate large amounts of serotype-specific data from randomised studies. Unfortunately, due to the temporary closure of facilities in The Pirbright Institute for a building upgrade, the reagents required for the ELISA were not available in the quantities necessary to generate a large serotype-specific dataset from the cross-sectional study.

The difficulties in producing reagents for FMD assays from rabbits and guinea pigs highlight the potential benefits if commercial monoclonal antibody- based kits (Brocchi, 2012b) were widely available. When more of these kits are validated and produced, the combination of these with flexible modelling approaches for interpreting their results would produce a potent and accessible epidemiological aid.

Strategies to mitigate the constraint imposed by the limited supply of reagents included careful selection of sera from the animals most likely to yield information on the recent infection history of each cross-sectional herd. Despite the sample size limitations, integration of results from the serotype-specific risk factor study with longitudinal, outbreak tracking, virus isolation and NSP data provided consistent conclusions regarding the epidemiology of FMD in Tanzania.

Another approach that strengthened the conclusions of this study was the inclusion of southern Kenyan virus isolation data in the analyses. Patterns of circulation were consistent across broader scales. Given the trans-boundary nature of disease circulation, excellent collaborations across the region will be necessary to fully understand the scale of circulation and devise appropriate control strategies. A key challenge however is to develop simplified systems for the systematic generation of surveillance and diagnostic data in areas where infrastructure is limited. The emergence of logistically easier diagnostic approaches for field detection offers promise (Bachanek-Bankowska *et al.*, 2014; Brocchi, 2012; Howson *et al.*, 2015).

7.4 Serotype specific FMDV circulation patterns

The collation of FMDV typing data (Chapter 6) yielded some exciting findings about the potential predictability of the sequence of serotypes to cause outbreaks in livestock in the study area over time. A longer-term study is necessary to confirm this possibility. If it is true, it suggests that herd immunity (driven by the demographics of the livestock population and adaptive immune responses after FMDV infection) may play a role in the relative probability of which serotype will come next. The formidable challenge of reducing the FMD burden in East African livestock through vaccination in the region could be lessened by operating synergistically with post-infection herd immunity. Even partial reduction of outbreaks through this approach could potentially have positive impacts on rural livelihoods.

Field evaluation of FMD vaccination strategies in Turkey indicated that herd immunity from vaccination wanes rapidly due to the births of calves and the short duration of immunity produced by vaccination (Knight-Jones, 2014). However, Chapter 5 highlights that post-infection immunity in cattle serially infected by different serotypes of FMDV might be different. Virus neutralisation testing of a longitudinal dataset indicated that, subsequent to an outbreak caused by a specific serotype, antibodies that neutralised a different serotype of FMDV, possibly a previous serotype that the animal was infected with, were increased. It is many years since the immune responses of livestock serially infected with FMD were previously investigated (Cotral & Gailiunas, 1971; Garland,

1974), and, given its potential relevance to FMD control in East Africa, it is an area that merits further investigation.

7.5 The first steps towards FMD control in East Africa

This study supports focussing initial FMD control efforts on livestock-management and vaccination strategies. The communities of northern Tanzania have a good track record of engaging with research and disease control programmes. Rabies research and control in the Serengeti ecosystem is a good example of this (Cleaveland *et al.*, 2003). This engagement would greatly contribute to the success of any potential FMD control programme, and must therefore be managed carefully. For example, a key element of the eradication of rinderpest in East Africa was the direct involvement of people from the community, who understood local conditions best, as implementers of disease control (Mariner *et al.*, 2012).

East African livestock keepers have shown themselves to be open to embracing disease control options if they are effective. For example, there has been good uptake of East Coast Fever (ECF) vaccine amongst Tanzanian pastoralists, despite its high cost ($6 - $14 per animal), because of its effectiveness in reducing lossess (Di Giulio *et al.*, 2009). Similarly, community workshops in the study area have shown that livestock owners would be willing to use control options for FMD, including vaccination and measures such as not allowing sick and healthy livestock to mix (Lembo *et al.*, 2015).

Given that current FMD vaccines have many potential limitations for use in endemic settings (Chapter 1; Knight-Jones, 2014; Parida, 2009), very good community engagement would be necessary to maintain credibility with livestock owners during any potential intervention studies. Answering questionnaires and allowing their livestock to be sampled for FMD research purposes represents an enormous investment on the part of livestock keepers. This effort is partially motivated by the expectation of better FMD control and improved livelihoods. Therefore, creating the understanding that interventions may not be immediately effective, and FMD control may require long term research and control efforts, would be a vital element in any future FMD related work in the area. Even if experimental herds are purchased for such trials in Tanzania, experience from an MCF vaccine trial has shown that good engagement with communities is critical to maintain local support (Prof Sarah Cleaveland, Personal communication). If such local support was

maintained, and funding was available, potential future work towards FMD control in the region includes:

a) The establishment of a platform for long-term longitudinal virus characterisation and serological studies in the region, which, as well as informing vaccine strain selection, would provide information about the predicteability of antigenic sweeps and post-infection immunity in livestock.
b) The collation of available data as well as further studies to better understand livestock movement patterns in East Africa.
c) The engagement of vaccine manufacturers with the FMD vaccine requirements of East Africa.
d) Vaccine effectiveness trials.
e) Further studies of potential transmission from wildlife to livestock in areas with low FMD prevalence in livestock, or when better FMD control in livestock is achieved.

7.6 Conclusions

This work has highlighted the large burden of FMD in traditional livestock keeping systems in northern Tanzania and the significant impacts this has on the rural poor. There is currently no evidence for wildlife-contact related risk factors for FMD in northern Tanzania, whereas livestock-management related risk factors are important. Therefore initial control efforts should focus on livestock management and vaccination. Incorporating inferences from serology data using a Bayesian model with virus typing data proved a useful approach to understanding serotype specific infection patterns in the study area. There are four FMDV serotypes circulating in northern Tanzanian and southern Kenyan livestock, and further studies are warranted to investigate the potential predictability of the temporal sequence of serotypes causing outbreaks in the region.

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Appendix

Appendix 1: Consent form

Foot and Mouth Disease Project

We are carrying out research on foot and mouth disease in livestock. The aim of this project is to understand more about the disease so that we can find ways to control it successfully. In addition we would like to work out the cost of the disease to farmers.

We would like to collect a sample of blood and a sample from the back of the mouth from some of the cattle, sheep and goats that are owned by your household, and test them for foot and mouth disease. We would also like to ask you some questions about the animals, the way the animals are managed, and also about the costs and losses associated with keeping livestock.

You and your village will be informed of the results of tests carried out on your livestock. We will discuss with you what these results mean and if there are any actions you might want to take. In presenting or publishing this study, your household will be represented by a code number, so that any facts about you or your household are kept private.

You are free to choose whether to be part of this study or not. In the end, this study will lead to better control of foot and mouth disease. However, you will not see this benefit during the study.

If you or someone in the compound regularly handles the animals, we would like to ask their help in catching and holding the animals while we take the samples. Reasonable measures will be taken to ensure that animals are handled properly for their own safety and for the safety of people assisting.

If you have any questions, please contact the study vet, Dr Enos Kamani, on …….If you would like to discuss this study with a vet who is not involved with the study, please contact the District Veterinary Officer, ……. on ……..

The consent form has been explained to me and I agree to my animals taking part in the study.		
Name:……..	**Signature:**……	**Date:**……

Appendix 2: Cross-sectional questionnaire

Questionnaire – Longitudinal first visit

1. General information

1.1 Household identification

Name of interviewer …….

Date ……..……

District ……..

Village ……..……..……...

Subvillage ……..……..……..

Head of household ……

Name of balozi ……..……..……...

Tribe ……..……

Distance from nearest neighbour (estimate in km) …….....

GPS location ……..……...

1.2 Respondent details

Respondent name……..

Gender: ……..……..

Age: ……..……...

Relation to Household: ……..……..

2. Household demographics

For people living in the household at the moment:

Total number of children aged 5 years and under: ….……

Total number of children aged between 6 and 10 yrs: ……..

How many of these go to school? ……..……..……...

Total number of children aged between 11 years and 15 years: ….……

How many of these go to school? ……...

Total no. of people in household aged over 15 years: …….

Fill out the table below for all people in household aged over 15y:

	Sex M/F	Age yrs	Marital status[1]	Role in family[2]	Education[3]	Primary Occupation	Secondary Occupation
Person 1							
Person 2							
Person 3							
Person 4							
Person 5							
Person 6							
Person 7							
Person 8							
Person 9							
Person 10							
Person 11							
Person 12							
Person 13							
Person 14							
Person 15							

[1] **M** Married, **S** single, **W** widowed, **D** divorced
[2] Household head, Relative to household head: spouse, son, daughter, brother, sister, father, mother, nephew, niece etc
[3] Highest level of education reached
[4] **F** employed on farm, **S** self-employed off farm, **E** employed off farm – agriculture, salaried, other

Does the head of the household have other households? Yes No

If yes, how many? .....

How long has the household lived in this place?

.....

If moved in last 10 years, where did you move from?

.....

Why did you move?

.....

Assets and income (not livestock related)

3.1 Assets

Asset	Number of Units	Purchase Price TSh (If purchased)	Age	Working Y/N
Ox plough				
Ox cart				
Bicycle				
Motorbike				
Vehicle				
Tractor				
Mobile phone				
Radio				

3.2 House details

How many houses are there? .....

How many other buildings are there? .....

Do any living quarters have a metal roof? Yes No

Are any living quarters built of concrete block or brick? Yes No

What is the number of rooms in all living quarters combined? .....

Latrine? indoor outdoor none

Electricity? none grid off grid

if off grid, specify ……

What is your primary water source? private well community well river pond

other: ……

How long does it take you to travel to obtain drinking water (one way)?

……

How many times per week do go you to obtain drinking water?

.....

Energy sources used for cooking: electricity gas kerosene cow dung

firewood charcoal other

How long does it take you to travel to collect firewood (one way)?

……

How many times per week do you go to collect firewood?

.....

3.3 Income sources

What are your income sources (tick all which apply):

Livestock sales ( ) Milk sales ( ) Other livestock income ( )

Crops related ( ) Honey related ( ) Wildlife related ( )

Food relief ( ) Off-farm employment ( )

3.4 Land use

Is the land you use for grazing: owned by you rented from others common land other

Is the land you plant for crops: owned by you rented from others common land other

Is the land where the house is built: owned by you rented from others common land other

3.5 Crops

Have you harvested any crops in the past four months? Yes No

If yes, fill in the table below for crops harvested in the past four months:

	Crops harvested		Crops sold			Crops given away to others	
	No. (units)	Month	No. (units)	Price per unit	Month sold	No. (units)	Month
Rice							
Millet							
Maize							
Sesame							
Cassava							
Sweet potato							
Bean							
Cabbage							
Lettuce							
Vegetable							
Tomato							
Banana							
Cotton							
Other							

3.6 Off-Farm employment

If the household reported any off farm employment, please fill in the table below:

	Net Income/week (TSh)	Time spent per week working for cash income outside the home
Member 1		
Member 2		
Member 3		
Member 4		
Member 5		
Total from children <15y		
Household Total		

Does the head of the household have a savings account? Yes No

What is the current balance of this savings account?

.....

How many other people in the household have savings accounts?

3. Livestock summary

Number of animals currently at household and owned by the household (Ad > 1yr; Juv 0-1yr):

	Cattle	Goat	Sheep	Donkeys	Chickens	Ducks	Dogs	Cats	Other …….
Adult	M: F:	M: F:	M: F:	M: F:	Total:	Total:	Total:	Total:	
Juvenile	Total:	Total:	Total:	Total:					

Number of animals currently owned by the household (Ad > 1yr; Juv 0-1yr) but kept elsewhere:

	Cattle	Goat	Sheep	Donkeys	Chickens	Ducks	Dogs	Cats	Other …….
Adult	M: F:	M: F:	M: F:	M: F:	Total:	Total:	Total:	Total:	
Juvenile	Total:	Total:	Total:	Total:					

Number of animals currently at household, but not owned by the household (Ad > 1yr; Juv 0-1yr):

I

	Cattle	Goat	Sheep	Donkeys	Chickens	Ducks	Dogs	Cats	Other …….
Adult	M: F:	M: F:	M: F:	M: F:	Total:	Total:	Total:	Total:	
Juvenile	Total:	Total:	Total:	Total:					

If the household has animals that belong to other people, fill in the table below:
Please use a new line for each group (eg adult male cattle, juvenile goats)

Number of animals	Species	Age (ad, juv)	Sex	Who do they belong to?	Where do they come from?	What month did they come into the herd

4. Livestock movements, demography and disease

These questions are only about cattle, sheep and goats which are owned by the household and kept at this boma.

Please fill in the table below for the number of animals appearing or disappearing from herd in the last four months (Ad > 1yr; Juv 0-1yr):

				Animals leaving herd				Animals joining herd			
		# Born	# Died	# Slaughtered	# sold	# given away	# lost or stolen	# bought	# received (gift)	# Moved into herd[1]	# Moved out of herd
Cattle	Ad M										
	Ad F										
	Juv M										
	Juv F										
Goat	Ad M										
	Ad F										
	Juv										
Sheep	Ad M										
	Ad F										
	Juv										

[1] E.g. elsewhere for grazing; gone temporarily to a relative, etc.

New livestock

If you acquired any new animals in the last 4 months please fill in the table below

Species	Sex	Age (juv, adult)	Date acquired	Where from	Reason: bought, gift, bride price	Price paid if bought (TSh)

If any animals have moved into the herd from elsewhere (eg for grazing or from relatives) in the last four months, please fill in the table below (one line for each animal):

Species	Age (juv, adult)	Sex	Where did it come from?	Why?	When did it return?

Have any of your animals gone to market but come back to the household again in the last four months? Yes/No
If yes, please fill in the table below (one line for each animal):

Species	Age (juv, adult)	Sex	Where did it go?	When did it go?	When did it return?

5. Farm income related

These questions relate to all species (ie including chickens, ducks etc). Poultry refers to chickens and ducks together.

What do you use your livestock for?

Cattle: Milk ( ) Meat ( ) Draught ( ) Sale ( )
Other ( ) specify……

Goats: Milk ( ) Meat ( ) Sale ( )
Other ( ) specify……

Sheep: Milk ( ) Meat ( ) Sale ( )
Other ( ) specify……

Poultry: Eggs ( ) Meat ( ) Sale ( )
Other ( ) specify……...

Sold livestock

If you sold any livestock (all species) in the last 4 months, please fill in the table below:

		Number	Average price of livestock sold (or range) (Tsh/head)	Where sold?	Reason for selling?
Cattle	calf (<12 months)				
	Adult male (>12 months)				
	Adult female (>12 months)				
Goats	Total				
Sheep	Total				
Poultry	Total				
Other	Total				

Consumed livestock

If you slaughtered any livestock in the last 4 months, fill in the table below:

Species	Sex	Age (juv/adult)	Date	Consumed at home? Y/N	If no, where/what used for?

Livestock products produced and sold

If you sold any livestock products in the last four months please fill in the table below:

	Product	Amount produced per day	Amount sold per day	Price sold at (TSh/litre)	Average number of animals producing each day
Cattle	Milk (liters/day)				
Goats	Milk (liters/day)				
Sheep	Milk (liters/day)				
Poultry	Eggs/day				
Other					

Did you purchase any of these things over the last 4 months?

Veterinary services/products	Feed	Supplements	Labour	Other
Yes No	Yes No	Yes No	Yes No	Yes No

For cattle, what was the total cost of these purchases in the last four months?
(TSh)……

For other livestock, what was the total cost of these purchases in the last four months?
(TSh)……

Did you have to purchase anything relating to crop production over the last four months?
Yes No

If yes, how much did you spend in total on crop production expenses in the last four months? ……

Household food consumption

Please fill in the table for the amount of food purchased per week

	Amount purchased per week	Cost per unit	Of food consumed in the household		
			% purchased	% produced at home	%other sources
Cow milk (litres/week)					
Goat milk (litres/week)					
Sheep milk (litres/week)					
Poultry eggs					
Beef (kg)					
Other Meat (kg)					
Other food expenditures					
Cooking fuel purchase					

How many times per week do you eat meat in your meals?
.....

Other household expenditure:

- Clothes over the last four months:
  TSH…….
- Human health care over the last four months:
  TSH…….
- Education over the last four months:
  TSH…….

6. Morbidity/mortality

If cattle, sheep or goats died in the last four months, indicate the cause:

Species	Sex	Age (juv, adult)	Date	Cause (*Disease/Predation/ Drought/Snake bite/Accident/ Others*)

Were any cattle, sheep or goats sick or died of disease in the last four months? If yes, fill out the table below:

Species	Sex	Age (juv, adult)	Sampled Y/N	Sample ID	Date sick	Signs	Diagnosis if known	Died Y/N

7. Herd management practices

Where do you take your cattle for grazing – wet season?
.....
How long do you walk to reach it (one way)?
.....

Where do you take your cattle for water – wet season?
.....

How long do you walk to reach it (one way)?
.....

Where do you take your cattle for grazing – dry season?
.....
How long do you walk to reach it (one way)?
.....

Where do you take your cattle for water – dry season?
.....
How long do you walk to reach it (one way)?
.....

If you have them, do cattle, sheep and goats graze together? Yes No
If no, then where do sheep and goats go for grazing and watering?
.....

Do you graze your animals with other people's animals? Yes No
If yes, with which species?
.....
Why do you graze your animals with other people's animals?
.....

Do you confine your animals at night? Yes No
Where do you confine them? Boma at household Elsewhere
.....

Do you confine cattle, sheep and goats together? Yes No

Do you slaughter your own animals? Yes No
If no, do you take them to a slaughter slab? Yes No
If yes, where?
.....

Do you borrow bulls from other herds or sent cows to other herds for servicing? Yes No

Do you use artificial insemination in your herd? Yes No

8. Contact with wildlife

Do you see wild animals in your village? Yes/No
If yes, fill out the table below:

Species	Y/N	Frequency (tick)		
		Every day	Once/twice per week	Less often
Wild carnivores				
Buffalo				
Wildebeest				
Zebra				
Topi				
Kongoni				
Gazelle				
Impala				
Warthog				
Eland				
Elephant				
Others				

Do you see wild animals near your house? Yes/No
If yes, fill out the table below:

Species	Y/N	Frequency (tick)		
		Every day	Once/twice per week	Less often
Wild carnivores				
Buffalo				
Wildebeest				
Zebra				
Topi				
Kongoni				
Gazelle				
Impala				
Warthog				
Eland				
Elephant				
Others				

Do you see wild animals near your livestock when you take them for watering/grazing? Yes/No
If yes, fill out the table below:

Species	Y/N	Frequency (tick)		
		Every day	Once/twice per week	Less often
Wild carnivores				
Buffalo				
Wildebeest				
Zebra				
Topi				
Kongoni				
Gazelle				
Impala				
Warthog				
Eland				
Elephant				
Others				

9. Foot-and-mouth disease knowledge

Do you know foot-and-mouth disease? Yes No

What are the signs of foot-and-mouth disease in animals?

.....

Do people get sick with foot-and-mouth disease? Yes No

Do you know how people get foot-and-mouth disease?

.....

What are the signs of foot-and-mouth disease in people?

.....

Do you know these diseases?		Rank them in order of importance (1=very important, 7= not very important
Foot and mouth disease	Yes No	
Trypanosomiasis	Yes No	
ECF	Yes No	
Black quarter/anthrax	Yes No	
Tick borne diseases – heartwater, babesia	Yes No	
Malignant catarrhal fever	Yes No	
Brucellosis	Yes No	

Why have you ranked them in this order?

.....

10. Disease preventive measures

Do you know how animals get foot-and-mouth disease?

.....

Do you know how to prevent foot-and-mouth disease in animals? Yes No

If yes, explain the measures you take to prevent foot-and-mouth disease in your animals?

.....

.....

.....

11. Vaccination

Have you ever vaccinated for FMD? Y N

What year did you first vaccinate for FMD? : .....

Have you vaccinated annually for FMD? : .....

Who vaccinates the cattle? (organization/veterinarian): .....

If you travel to obtain vaccination treatments, what is the distance traveled (one way)?

.....

Are any government subsidies provided for the vaccination? : ……...

Was access to the vaccine restricted so that you could not treat as many livestock as desired? Yes or No

How did the household pay for the vaccination treatments? [Cash, loan, sell cattle, other asset, in-kind trade] : .....

12. History of foot-and-mouth in livestock in the village/herd

Have you ever had any cases of foot-and-mouth in your animals? Yes/No

Have you had an outbreak of foot-and-mouth disease in your animals in the past year? Yes/No

Fill out the table below and tick which species affected for outbreaks in the last year:

Outbreak #	Date	Cattle	Sheep	Goats	Other species
1					
2					
3					
4					

Have you heard of any cases of foot-and-mouth disease in animals in this area during the past 12 months? Yes No
If yes, please fill in the table below:

Where	Date	Species affected				Description (plus head of household if known)
		Cattle	Goats	Sheep	Other	

Collect the following information for the latest outbreak only:

Species		Number of animals affected	Signs (tick all that apply)												Did any animals have abortions? (number)	Did any animals die? (number)
			Mouth lesions	Salivation	Foot lesions	Lameness	Anorexia	Depression	Fever	Loss of milk	Weight loss	Diarrhoea	Staring coat	.....		
Cattle	juvenile (<12 months)															
	Adult male (>12 months)															
	Adult female (>12 months)															
Goats	Juvenile															
	Adult															
Sheep	Juvenile															
	Adult															
Other																

Did you treat FMD infected cattle during the outbreak? Yes No
What was the treatment? .....

What was the cost per animal for the treatment (TSh): .....

Do you know where the disease came from (how it got into the herd)? .....
Did you do anything to try and stop the disease spreading? Yes No
What did you do? .....

Do animals which have FMD show any signs after they have recovered from the disease?
Yes No If yes, describe: .....

Have you heard of animals developing a very thick haircoat or heat intolerance after FMD?
Yes No

Did cow milk production decrease during the FMD outbreak?
Yes No No milking during outbreak
If yes, how many litres produced per day during the outbreak? .....

Did goat milk production decrease during the FMD outbreak? Yes No
If yes, how many litres produced per day during the outbreak? .....

If milk production decreased, did you stop selling milk during the outbreak? Yes No
If milk production decreased, did you stop consuming milk during the outbreak? Yes No

If you own working draft animals, did you perceive that FMD affected their productivity for traction? Yes No
Because of FMD did you alter the amount or type of crops you produce?
.....

Did the FMD outbreak cause you to alter time spent on off farm work? Increase, decrease, or not change? By how much? .....

Did you slaughter and consume any animals with FMD?
.....
If animals died, were they consumed?
.....
If not, did you dispose of animals in other ways (bury, burn) that died because of FMD?
.....
If so, how much time did this take?
.....

Did FMD affect whether you sold livestock or not? Yes No
If yes, why?
.....

Did you sell animals during the FMD outbreak? Yes No
Did you sell animals exhibiting FMD characteristics? Yes No
Did you have to sell any animals because of the outbreak? Yes No
How many? .....
Why? .....

Did you alter your grazing or watering practices because of the FMD outbreak?
If yes, why? .....

13. History of FMD in people in the village/household

Were any people in your household sick at the same time as the FMD outbreak? Yes No
Do you think people were sick because of FMD? Yes No
What signs did they show?

.....
.....
.....

Have you heard of any cases of foot-and-mouth disease in other people in this area during the past 12 months? Yes/No
If yes, please fill in the table below:

Name	Sex	Age	Date	Where	Description, including signs/symptoms	Recovered Y/N

Appendix 3: Case-control study questionnaire

RISK FACTOR FORM

Head of household: …… Village: ……..
Tel.No.: …….. Tribe: ……
GPS location: …….. Today's date: ……

No. of adults (≥ 15 yrs) in household: …… No. of children (< 15 years): …….

For herds affected by FMD:
When did you observe the first case of FMD in this outbreak? …… ……..

Type of animal	Total number in herd/flock	Number affected by FMD in this outbreak (still alive)	Number that died during this outbreak	Comments
Lactating cows				
Other female cattle				
Adult male cattle				
Juvenile cattle (< 1 yr)				
Adult goats				
Juvenile goats (< 1 yr)				
Adult sheep				
Juvenile sheep (< 1 yr)				

Please provide information for one month prior to first case observed in the village. For the 'control' herds in the village, collect information for the same time period.

Acquisitions
Did any new animals join the household or herd YES/NO
If YES, please complete the MOVEMENTS FORM.

Did you bring any animal products into the household? YES/NO
If YES, Meat / hides / skins / milk /manure/ other

Did you bring in any feedstuff for your livestock? YES/NO
If YES, describe……

Grazing/water
Did you take your cattle more than 1 hr walking from the village for grazing? YES/NO

If NO, is zero grazing practiced? YES/NO

While grazing, were animals from other herds present? YES/NO
If YES, from which village(s) ……

Did you take your sheep/goats more than 1 hr walking from the village for grazing? YES/NO

While grazing were animals from other herds present? YES/NO
If YES, from which village(s) ……..

Did you take your cattle more than 1 hr walking from the village for water? YES/NO

At the water point, were animals from other herds present? YES/NO
If YES, from which village(s) ……..

Did you take your sheep/goats more than 1 hr walking from the village for water? YES/NO

Were sheep/goats from other herds present at the same time? YES/NO
If YES, from which village(s) ……..

Are these locations any different from usual for this time of year? YES/NO
If YES, explain ……

Have your cattle been in contact with any of the following wild animals at the household or while grazing/watering? (circle if YES) Wildebeest /Impala / Gazelles / Buffalo / Elephant / Warthogs

Interventions
Have you taken your cattle for any vaccinations? YES/NO
If YES, give date: ……..

Where were cattle vaccinated? ……..
What vaccination was given? ……

Were cattle from other herds present at the same time? YES/NO
If YES, from which village(s) ……

Have you taken your cattle for dipping? YES/NO
If YES, give date: ……..

Which dip tank? ……

Were cattle from other herds present at the same time? YES/NO
If Yes, from which village(s) ……

Have you taken your sheep/goats for any vaccinations? YES/NO
If YES, give date: ……...

Where were sheep/goats vaccinated? ……
What vaccination was given? ……

Were sheep or goats from other herds present at the same time? YES/NO
If YES, from which village(s) ……

Have you taken your sheep or goats for dipping? YES/NO
If YES, give date: ……

Which dip tank? ……...

Were sheep/goats from other herds present at the same time? YES/NO
If Yes, from which village(s) ……...

People/Vehicles
Have any vets or livestock officers visited your herd/flock? YES/NO

If YES, name of person: ……
Contact number: ……....
Date of visit: ……..……
Reason for visit: ……

Has anyone from outside the village collected milk from your herd/flock? YES/NO

If YES, name of person……
Contact number: ……..
Date of visit: ……..……..

Have any livestock trucks visited your premises to transport animals to another location? YES/NO

If YES, date of visit: ……
Name of haulier: ……..
Contact number: ……...

Has anyone visited herd/flock for artificial insemination? YES/NO

If YES, name of person……
Contact number: ……..
Date of visit: ……..……..

Has anyone come into the household to look after the cattle, sheep or goats? YES/NO

If YES, name of person……
Date of arrival:……..……
Where did he/she come from? ……

History of outbreaks
Has your herd or flock suffered any FMD outbreaks in the past 2 years? YES/NO

Have you heard of any villages that had FMD outbreaks before the outbreak in this village? YES/NO

Name of village: ……..
Date of the most recent outbreak:……

Name of village: ……..
Date of the most recent outbreak:……

Name of village: ……..
Date of the most recent outbreak:……

Name of village: ……..
Date of the most recent outbreak:……

Appendix 4: Extra data for Chapter 5

Appendix 4 Table 1: Numbers (N) of samples from Herd 1 (training data) from each sampling date that were ELISA tested. NSP = FMDV non structural protein ELISA.

Sampling point	Date	N cattle in herd	N cattle NSP tested	N cattle A ELISA tested	N cattle O ELISA tested	N cattle SAT1 ELISA tested	N cattle SAT2 ELISA tested
1	13/01/2011	100	99	87	85	94	75
2	15/03/2011	100	74	73	82	10	9
3	11/04/2011	100	0	0	9	9	8
4	26/04/2011	100	98	97	92	98	95
5	10/05/2011	96	86	0	9	90	7
6	24/05/2011	96	0	76	84	9	79
7	21/06/2011	96	74	75	84	30	84
8	18/07/2011	96	96	89	74	85	83
9	20/08/2011	96	77	76	0	74	76
10	20/09/2011	96	79	84	93	92	91
11	28/03/2012	96	66	76	71	71	70
12	22/05/2012	96	28	27	23	27	0
13	12/07/2012	96	42	36	7	41	41
14	31/08/2012	96	86	87	82	63	85
15	09/01/2013	52	49	49	45	41	47
16	18/04/2013	51	44	46	50	49	49
17	28/06/2013	51	41	48	48	46	44
18	02/08/2013	51	39	39	41	38	39
19	28/11/2013	51	47	43	48	47	48

Appendix 4 Table 2: A summary of the five FMD outbreaks as by the herd managers between January 2011 and November 2013 as well as virus isolation data for these outbreaks.

Date of reported outbreak	Spring 2011	August 2011	July 2012	September 2012	June 2013
Herd managers' perception of outbreak severity	Mild	Severe	Less severe	Mild	Unknown
Number of animals in herd at time	100	96	96	96	51
Number of animals with photographic evidence of lesions or lesion material submitted to WRL	0	18	10	0	7
Number of lesions from which FMDV was genotyped at world reference laboratory (serotype detected)	0	2 (SAT2)	2 (SAT1)*	0	2 (A)
Predicted infection status model 2B	Uninfected = 35 Infected = 65	Uninfected =6 Infected = 90	Uninfected = 23 Infected =73	NA	Uninfected = 16 Infected = 35

Appendix 4 Table 3: Numbers of samples from Herd 2 (test data) from each sampling date that were ELISA tested

Sampling point	Date	N cattle in herd	N cattle NSP tested	N cattle A ELISA tested	N cattle O ELISA tested	N cattle SAT1 ELISA tested	N cattle SAT2 ELISA tested
1	13/12/2011	100	84	54	84	87	55
2	14/02/2012	100	2	2	2	99	NA
3	28/03/2012	99	93	86	92	92	90
4	22/05/2012	98	15	NA	31	80	NA
5	12/07/2012	96	10	10	9	10	8
6	31/08/2012	96	89	89	86	86	85
7	09/01/2013	96	89	95	93	96	96
8	15/03/2013	96	92	89	90	89	88

Appendix 5: Method for selection of sera from cross-sectional study for serotype specific assays

The study design for the cross-sectional study is described in Chapter x. A subset of 128 sera of the 2694 available cross-sectional samples was selected for virus neutralisation testing (VNT).

For each village in the cross-sectional study, cattle were ordered according to youngest age and then by the highest FMDV NSP ELISA PI (reflecting high anti-FMDV antibodies). An algorithm was developed in the R statistical environment (R development core team, 2008) to list the "top three" chosen cattle in each village according to youngest age then highest NSP PI. However, if the next youngest animal outside of the "top-three", had a higher NSP PI compared the animal with the lowest NSP PI amongst the chosen cattle, this animal was added to the list, and so on, until the minimum NSP PI on the list was as high as any other NSP PI amongst the cattle in the village.

Serum selections for VNT were made from the algorithm generated list for each village balancing the aims of including sera from the youngest cattle, sera with the highest NSP PI and sera with sufficient volume available for VNT testing.

After two sera from each village were prioritized for VNT testing, the algorithm and selection process was repeated at herd level with the aim of including at least one serum from each herd.

Appendix 6: Relating the shape and rate parameters of a gamma distribution to mode and standard deviation.

c = shape parameter of gamma distribution

q = rate parameter of gamma distribution

σ^2 = variance parameter of gamma distribution

σ = standard deviation parameter of gamma distribution

μ = mode parameter of gamma distribution

From Evans *et al*., (2001):

$$\sigma^2 = \frac{1}{q^2} \times c$$

$$\mu = \frac{1}{q} \times (c - 1)$$

$$c = \mu \times q + 1$$

Algebra to relate shape (c) and rate (q) to mode (μ) and standard deviation (σ):

$$\sigma^2 = \frac{1}{q^2} \times c$$

$$\sigma^2 = \frac{1}{q^2} \times (\mu \times q + 1)$$

$$\sigma^2 \times q^2 = \mu \times q + 1$$

$$\sigma^2 \times q^2 - (\mu \times q) - 1 = 0 \quad \text{(Quadratic equation)}$$

Relationship between rate, mode and standard deviation: As the rate parameter must have a value greater than zero (Evans *et al*., 2001), and given the prior in the model for σ (2 to 20 days), the positive solution to the quadratic equation was selected.

$$q = \frac{-\mu \pm \sqrt{\mu^2 - (4 \times \sigma^2 \times 1)}}{(2 \times \sigma^2)}$$

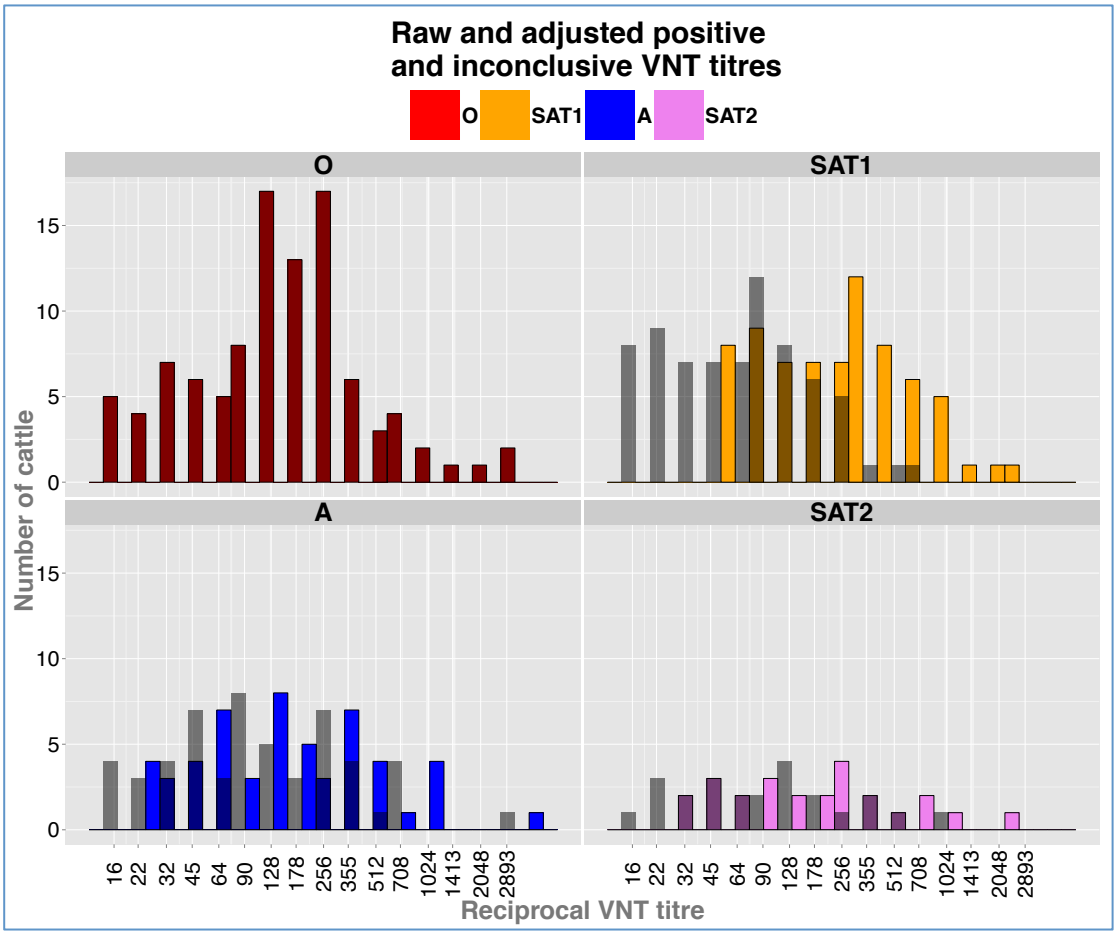
Once rate is calculated, the shape parameter can be worked out from rate and mode:

$$c = \mu \times q + 1$$

Reference for Gamma distribution parameters: Evans, M., Hastings, N. and Peacock, B. (2001). Chapter 22: Gamma Distribution. In: Statistical Distributions, Third Edition. Wiley, Hoboken, New Jersey.

Appendix 7: Method for cattle virus neutralisation titre adjustment to take into account variation in avidity between the sera and test viruses

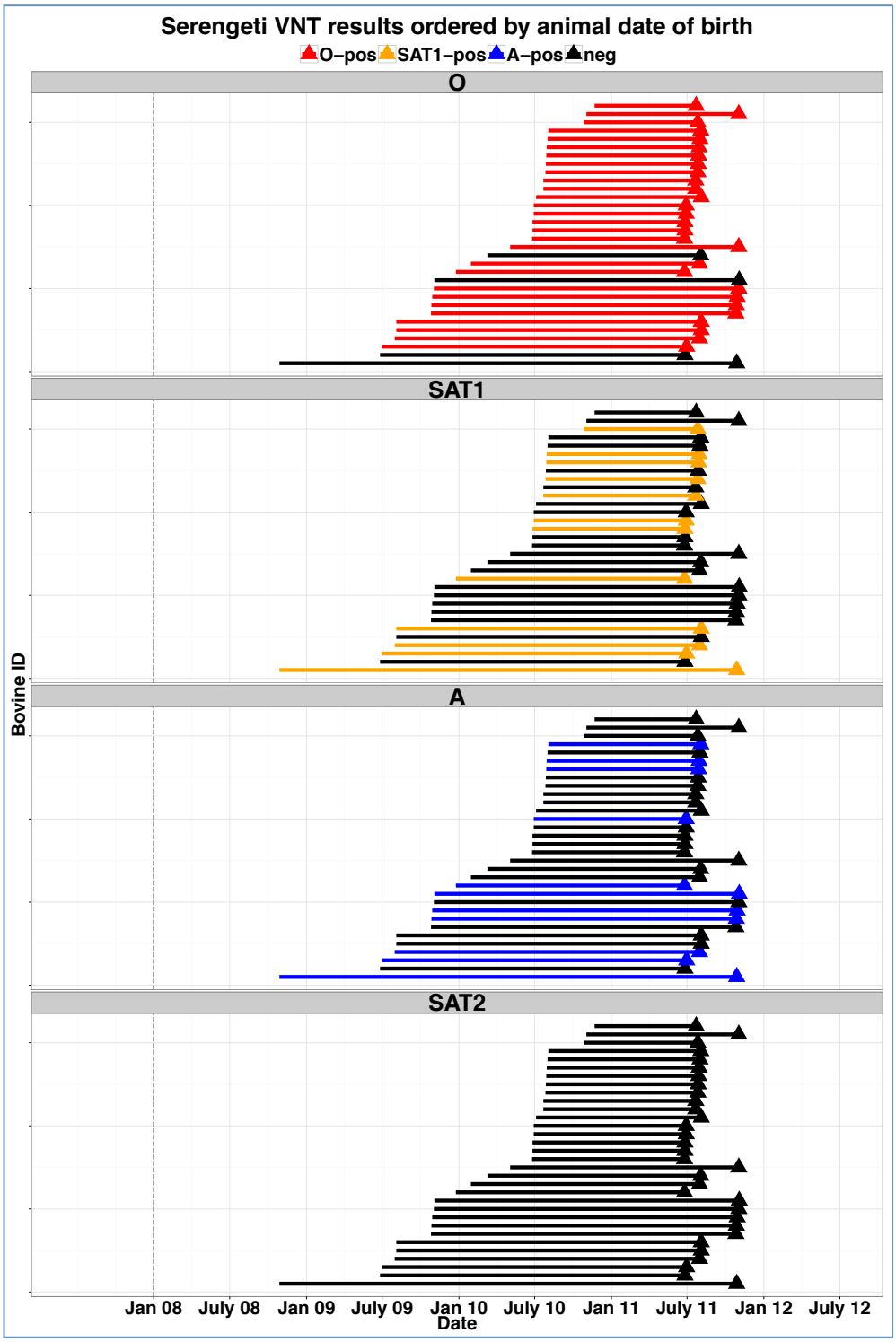
1. For each VNT serotype, the five highest cattle serum titres were selected and averaged.
2. The offset for each VNT serotype was calculated by subtracting the biggest "top five mean titre" out of the four serotypes from the "top five" mean titre for each serotype.
3. Negative VNT results were not altered.
4. The titres for each serotype were adjusted by subtracting the offset for that serotype from each positive and inconclusive result (Appendix 7 Figure 1).



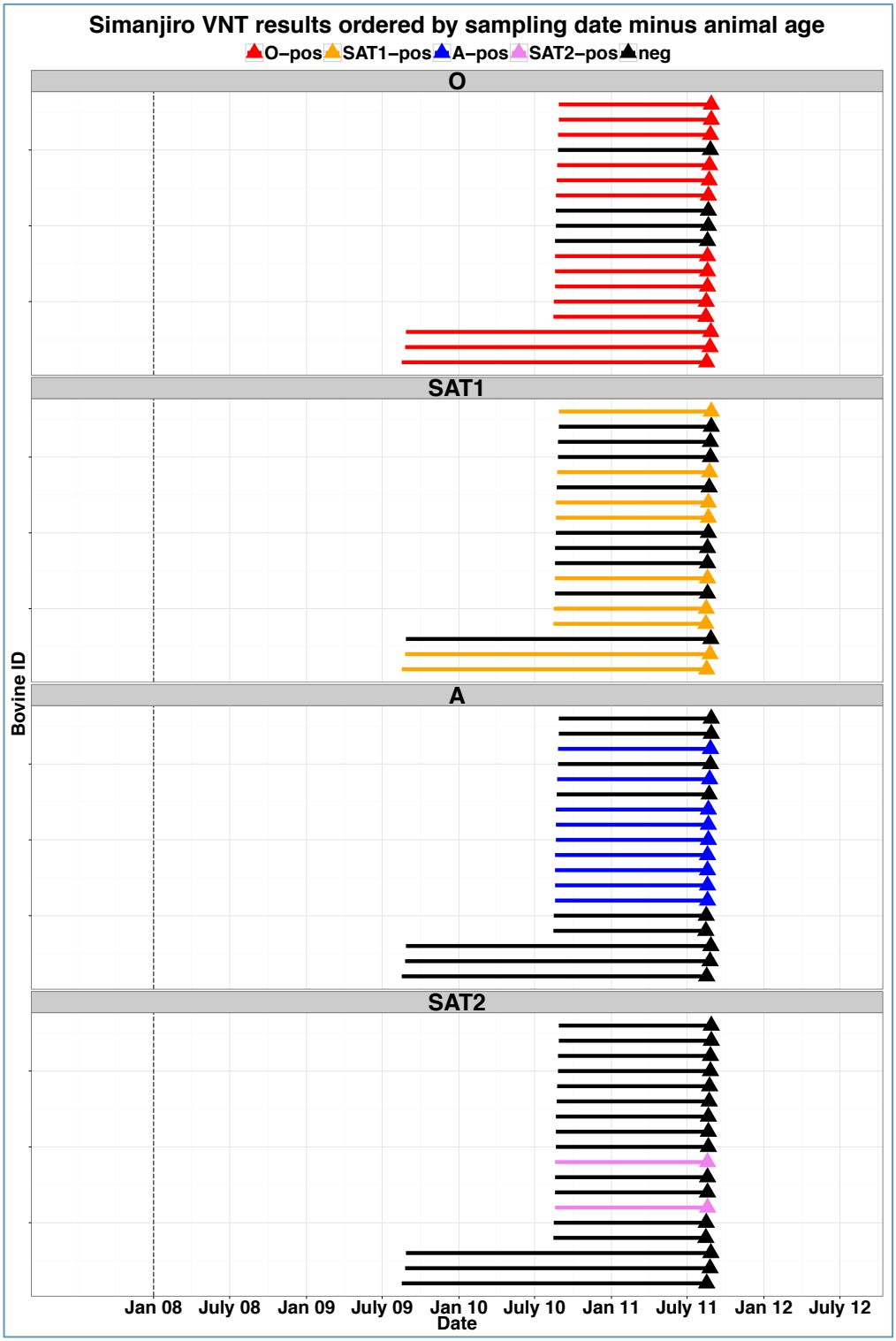
Appendix 7 figure 1: A summary of raw and adjusted VNT titres against the four serotypes. The grey histograms represent the original titres and the coloured histograms represent the adjusted titres.
The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.

Appendix 8: Results for serotype specific seroprevalence from virus neutralisation testing of cattle sera in relation to sampled cattle ages in each district

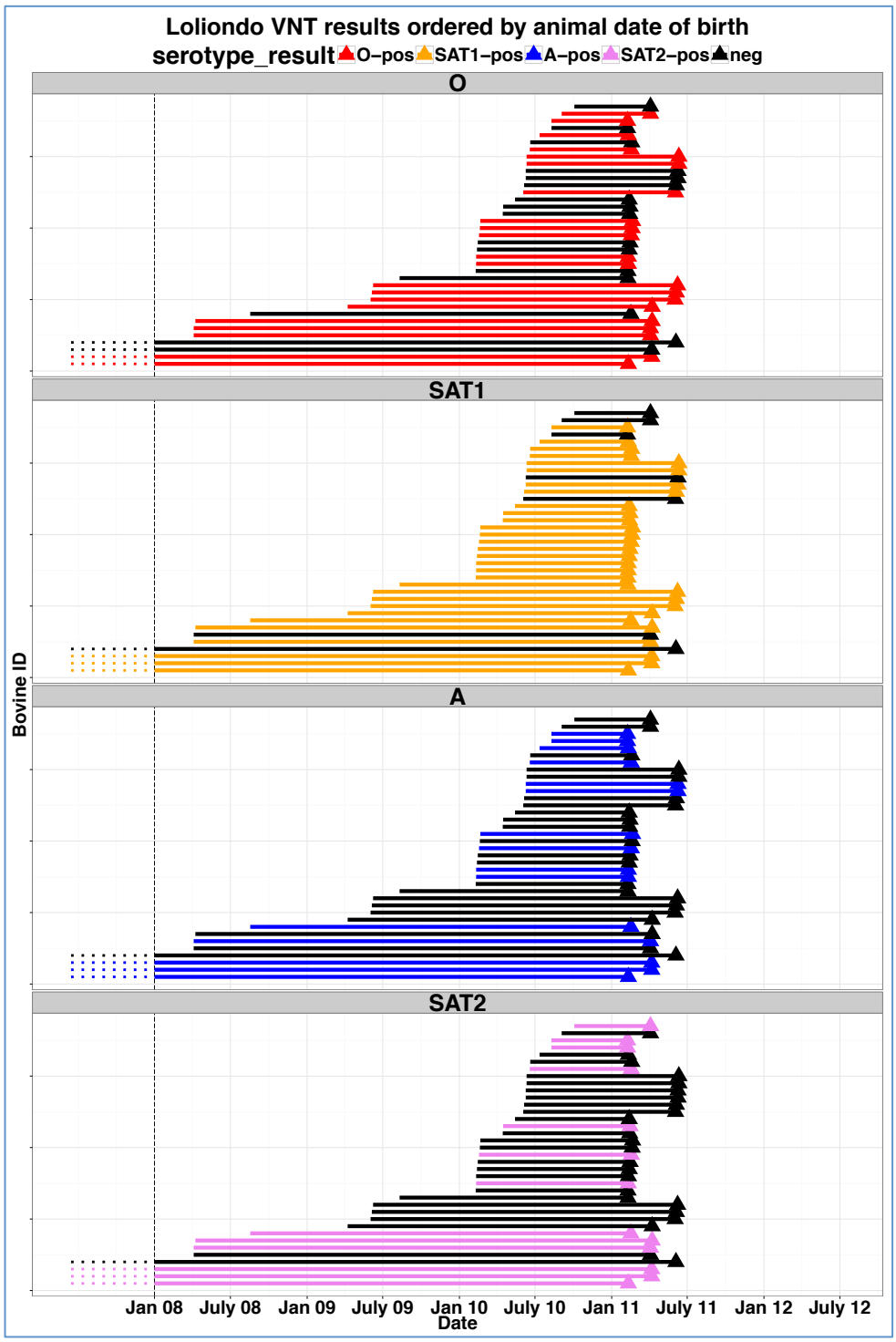
Appendix 8 figures 1 – 5 show the seroprevalence of each serotype in cattle in Serengeti district, Simanjiro district, Loliondo area, Monduli district and Meru (Arusha) area with the ages of the cattle sampled.



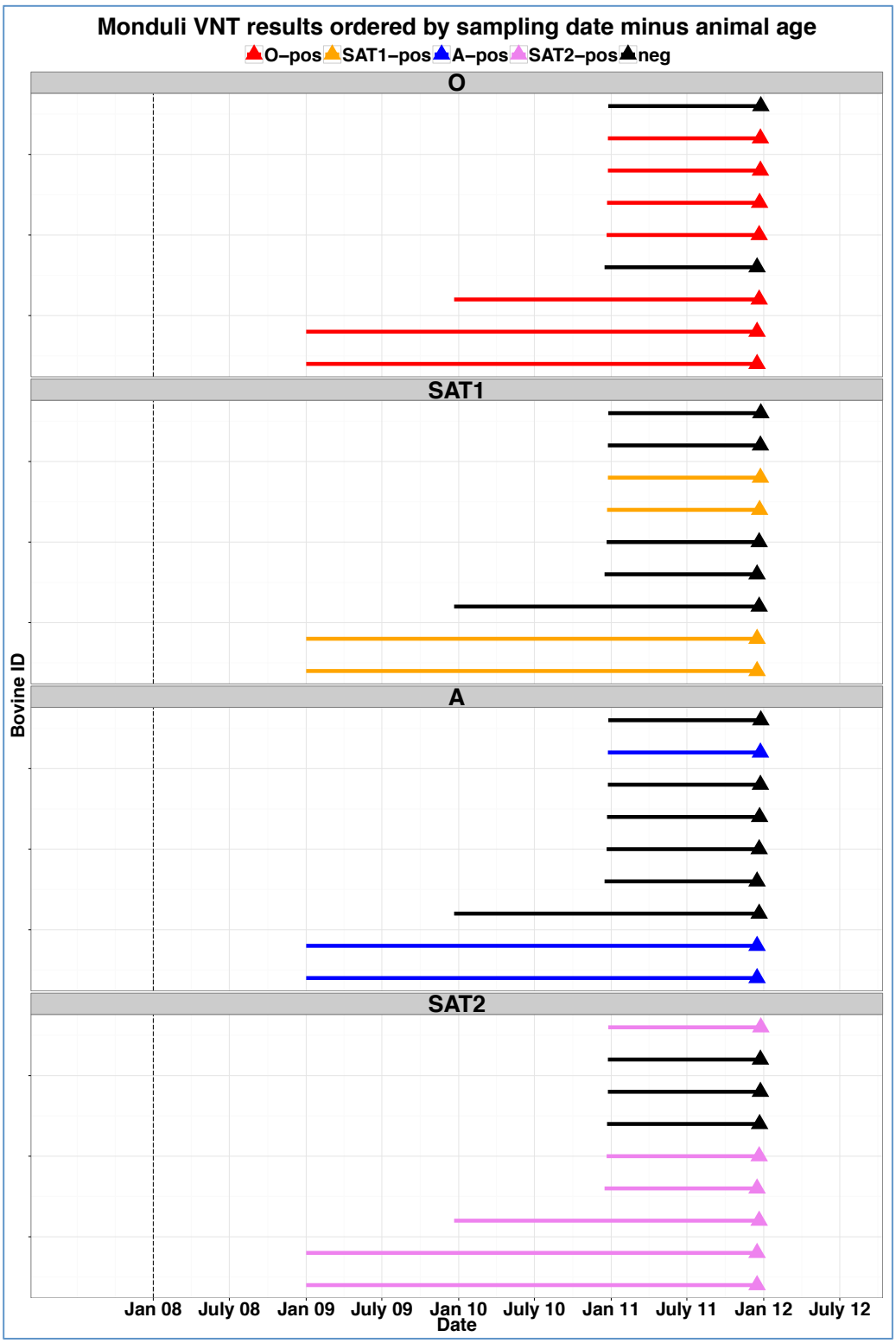
Appendix 8 figure 1: A plot showing virus neutralisation testing results in sera from cattle sampled between in Serengeti district. The triangles represent the date of serum sampling. Each horizontal line on the plot begins at the animal's date of birth (to a minimum cut-off of January 2008) and ends at the sampling date. The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.



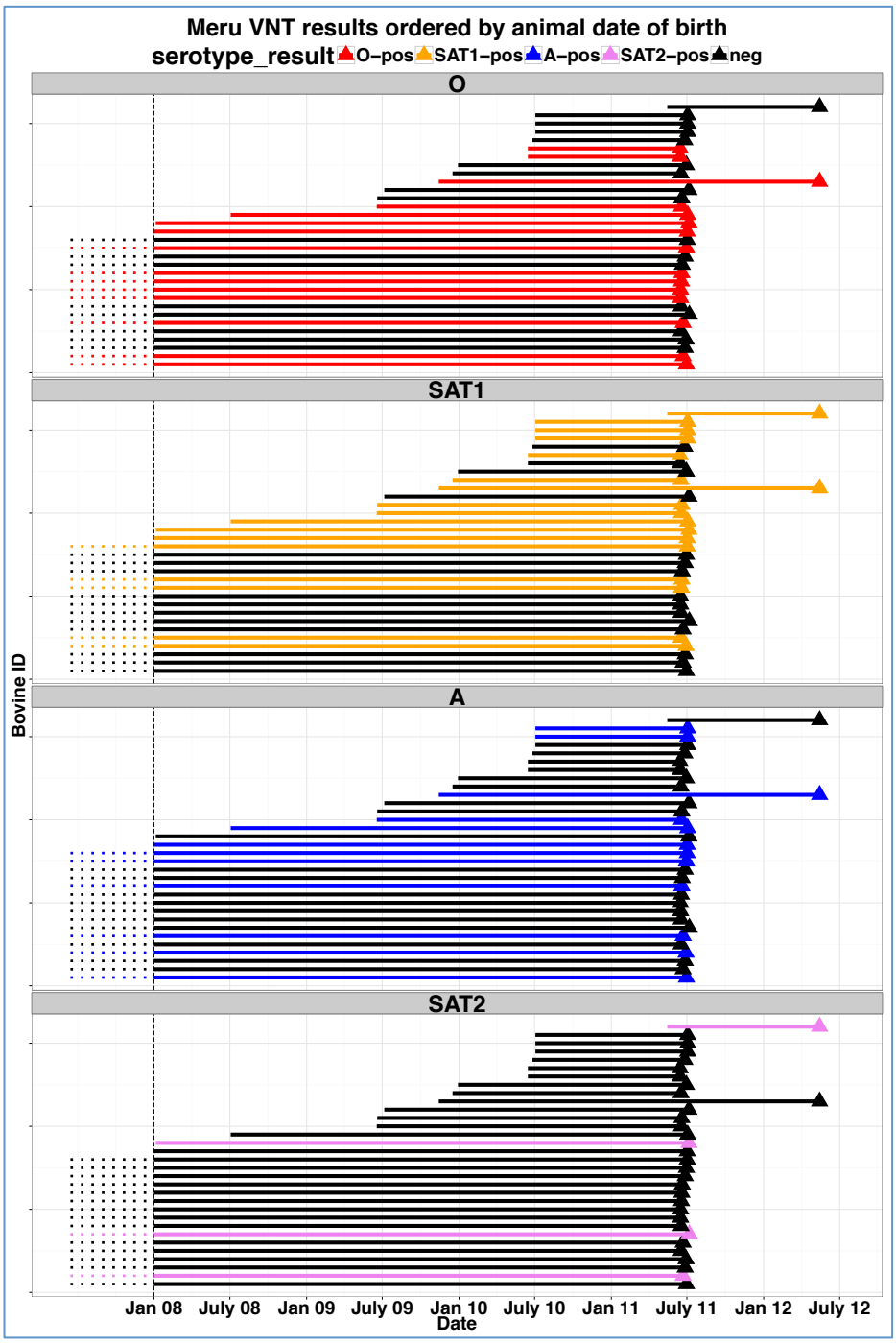
Appendix 8 figure 2: A plot showing virus neutralisation testing results in sera from cattle sampled between in Simanjiro district. The triangles represent the date of serum sampling. Each horizontal line on the plot begins at the animal's date of birth (to a minimum cut-off of January 2008) and ends at the sampling date. The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.



Appendix 8 figure 3: A plot showing virus neutralisation testing results in sera from cattle sampled between in Loliondo area. The triangles represent the date of serum sampling. Each horizontal line on the plot begins at the animal's date of birth (to a minimum cut-off of January 2008) and ends at the sampling date. The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.



Appendix 8 figure 4: A plot showing virus neutralisation testing results in sera from cattle sampled between in Monduli district. The triangles represent the date of serum sampling. Each horizontal line on the plot begins at the animal's date of birth and ends at the sampling date. The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.



Appendix 8 figure 5: A plot showing virus neutralisation testing results in sera from cattle sampled between in Arusha area (also termed Meru). The triangles represent the date of serum sampling. Each horizontal line on the plot begins at the animal's date of birth (to a minimum cut-off of January 2008) and ends at the sampling date. The colour red represents serotype O, yellow represents serotype SAT1, blue represents serotype A and violet represents serotype SAT2.

Appendix 9: Results from GLMMs in Chapter 6

Appendix 9 Tables 1-4 show the resulst from generalised linear mixed models testing whether management or wildlife contact risk factors explained exposure to serotypes A, O, SAT1 and SAT2 in cattle.

Appendix 9 Table 1: Generalised linear mixed model results for serotype A testing whether management or wildlife contact risk factors explained exposure to serotype A in cattle

SEROTYPE A (NO ROBUST EXPLANATORY VARIABLES)					
		LRT	**p**	**Estimate (95% CI)**	**Odds ratio (95% CI)**
Total cattle		1.77	0.18	0.002 (-0.002-0.006)	1.002 (0.998-1.006)
Buffalo sighting weekly or more often		1.25	0.26	0.575 (-0.438-1.589)	1.778 (0.645-4.898)
Other wildlife sighting		0	0.99	-0.008 (-0.917-0.9)	0.992 (0.4-2.46)
Distance to buffalo area		0.09	0.76	0.005 (-0.029-0.04)	1.005 (0.971-1.04)
Total animals acquired in the herd over past 4 months		1	0.32	-0.011 (-0.033-0.011)	0.989 (0.967-1.011)
Maximum minutes walked to reach grazing and water		0.33	0.57	0.001 (-0.002-0.003)	1.001 (0.998-1.003)
Total small ruminants		2.99	0.08	0.001 (0-0.003)	1.001 (1-1.003)
District (compared to Arusha)		1.73	0.78		
	Monduli			-0.405 (-2.445-1.635)	0.667 (0.087-5.127)
	Ngorongoro			-0.56 (-1.869-0.75)	0.571 (0.154-2.117)
	Serengeti			0.201 (-1.117-1.518)	1.222 (0.327-4.565)
	Simanjiro			0 (-1.435-1.435)	1 (0.238-4.198)
Livestock practice (compared to Agropastoral)		1.08	0.58		
	Pastoral			-0.546 (-1.624-0.533)	0.58 (0.197-1.703)
	Rural Smallholder			-0.201 (-1.518-1.117)	0.818 (0.219-3.056)

Appendix 9 Table 2: Generalised linear mixed model results for serotype O testing whether management or wildlife contact risk factors explained exposure to serotype O in cattle

SEROTYPE O (NO ROBUST EXPLANATORY VARIABLES)					
		LRT	**p**	**Estimate (95% CI)**	**Odds ratio (95% CI)**
Total cattle		4.09	0.04	0.007 (-0.002-0.016)	1.007 (0.998-1.016)
Buffalo sighting weekly or more often		0.01	0.91	0.065 (-1.108-1.237)	1.067 (0.33-3.445)
Other wildlife sighting		2.51	0.11	-0.891 (-2.036-0.253)	0.41 (0.131-1.287)
Distance to buffalo area		0	0.98	0 (-0.04-0.039)	1 (0.961-1.04)
Total animals acquired in the herd over past 4 months		3.65	0.06	0.031 (-0.006-0.069)	1.032 (0.994-1.071)
Maximum minutes walked to reach grazing and water		0	0.96	0 (-0.003-0.003)	1 (0.997-1.003)
Total small ruminants		0.1	0.75	0 (-0.001-0.002)	1 (0.999-1.002)
District (compared to Arusha)		6.54	0.16		
	Monduli			0.875 (-1.538-3.289)	2.4 (0.215-26.823)
	Ngorongoro			0.049 (-1.285-1.383)	1.05 (0.277-3.985)
	Serengeti			1.686 (-0.091-3.464)	5.4 (0.913-31.934)
	Simanjiro			1.281 (-0.526-3.088)	3.6 (0.591-21.932)
Livestock practice (compared to Agropastoral)		4.19	0.12		
	Pastoral			-1.194 (-2.81-0.422)	0.303 (0.06-1.525)
	Rural Smallholder			-1.686 (-3.464-0.091)	0.185 (0.031-1.095)

Appendix 9 Table 3: Generalised linear mixed model results for serotype SAT1 testing whether management or wildlife contact risk factors explained exposure to serotype SAT1 in cattle

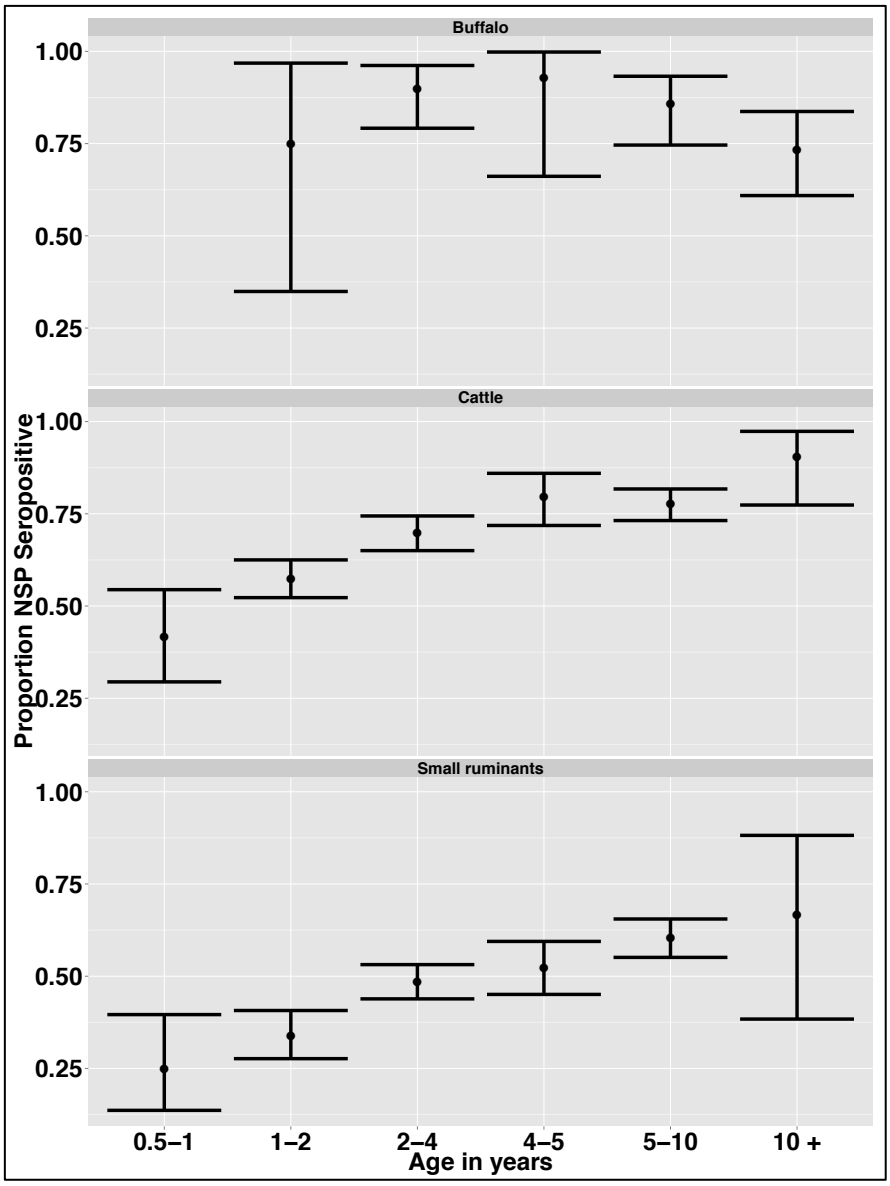
SEROTYPE SAT1 (NO ROBUST EXPLANATORY VARIABLES)					
		LRT	**p**	**Estimate (95% CI)**	**Odds ratio (95% CI)**
Total cattle		1.46	0.23	-0.002 (-0.006-0.002)	0.998 (0.994-1.002)
Buffalo sighting weekly or more often		0.02	0.88	-0.1 (-1.345-1.146)	0.905 (0.26-3.145)
Other wildlife sighting		0.21	0.65	-0.26 (-1.388-0.867)	0.771 (0.25-2.38)
Distance to buffalo area		0.01	0.94	-0.001 (-0.041-0.038)	0.999 (0.96-1.038)
Total animals acquired in the herd over past 4 months		0.17	0.68	-0.005 (-0.031-0.02)	0.995 (0.97-1.02)
Maximum minutes walked to reach grazing and water		0.14	0.71	-0.001 (-0.003-0.002)	0.999 (0.997-1.002)
Total small ruminants		1.91	0.17	-0.001 (-0.003-0.001)	0.999 (0.997-1.001)
District (compared to Arusha)		6.38	0.17		
	Monduli			0.154 (-1.89-2.198)	1.167 (0.151-9.006)
	Ngorongoro			1.595 (0.027-3.162)	4.926 (1.027-23.628)
	Serengeti			-0.051 (-1.374-1.273)	0.951 (0.253-3.571)
	Simanjiro			0.036 (-1.411-1.484)	1.037 (0.244-4.411)
Livestock practice (compared to Agropastoral)		2.32	0.31		
	Pastoral			0.841 (-0.409-2.091)	2.318 (0.664-8.094)
	Rural Smallholder			-0.012 (-1.499-1.475)	0.988 (0.223-4.372)

Appendix 9 Table 4: Generalised linear mixed model results for serotype SAT2 testing whether management or wildlife contact risk factors explained exposure to serotype SAT2 in cattle. * The confidence interval includes infinity as there were no SAT2 VNT positives from Serengeti District.

SEROTYPE SAT2					
		LRT	**p**	**Estimate (95% CI)**	**Odds ratio (95% CI)**
Total cattle		4.4674	0.0345	0.004 (-0.001-0.009)	1.004 (0.999-1.009)
Buffalo sighting weekly or more often		2.9993	0.0833	1.041 (-0.075-2.156)	2.831 (0.928-8.633)
Other wildlife sighting		3.55	0.0595	1.151 (-0.072-2.374)	3.161 (0.93-10.741)
Distance to buffalo area		0.413	0.5205	-0.014 (-0.059-0.03)	0.986 (0.943-1.03)
Total animals acquired in the herd over past 4 months		0.2994	0.5843	0.007 (-0.017-0.031)	1.007 (0.983-1.032)
Maximum minutes walked to reach grazing and water		4.8961	0.0269	0.003 (0-0.006)	1.003 (1-1.006)
Total small ruminants		15.2067	0.0001	0.005 (0.001-0.008)	1.005 (1.001-1.008)
Livestock practice (compared to Agropastoral)		12.839	0.0016		
	Pastoral			18.919 (-7748.091-7785.93)	164659663.887 (0-Inf)
	Rural Smallholder			18.46 (-7748.55-7785.471)	104054578.639 (0-Inf)
District (compared to Arusha)		19.87	0.0005		
	Monduli			1.01 (-1.214-3.233)	2.745 (0.297-25.367)
	Ngorongoro			0.295 (-1.225-1.814)	1.343 (0.294-6.137)
	Serengeti			-36.372 (-28065834.993-28065762.248)	0 (0-Inf)*
	Simanjiro			-2.375 (-5.187-0.437)	0.093 (0.006-1.548)

Appendix 10: Comparison of age-stratified buffalo, cattle and small ruminant seroprevalence measured by the FMD-NSP ELISA

Appendix 10 figure 1 compares FMD NSP ELISA seropositivity levels in different age groups of buffalo, cattle and small ruminants.



Appendix 10 figure 1: The proportion of FMD NSP seropositive buffalo, cattle and small ruminants from the cross-sectional study that tested positive on the Prionics foot and mouth disease non structural protein ELISA. The bars represent binomial 95% confidence intervals for proportions. The points represent the proportions.

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Chapter 2

Patterns of Foot-and-Mouth Disease Virus Distribution in Africa

The Role of Livestock and Wildlife in Virus Emergence

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THE DISEASE

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hooved animals caused by FMD virus (FMDV), a positive-sense, single-stranded RNA virus of the family *Picornaviridae* (genus *Aphthovirus*) that exists as seven serotypes (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3). Disease in susceptible animals is characterized by a high fever and development of blisters in the mouth and on hooves. Weight loss and reduction in milk production are commonly observed. While the disease has been reported since the 16th century (Francastorius, 1546), FMD poses an increasing challenge to the international community with circulation of highly divergent virus serotypes and strains that have great potential for transboundary spread. FMDV has many of the characteristics of a successful emergent pathogen: it has high genetic and antigenic variability (Carrillo, 2012; Vosloo et al., 2010); it has a spectrum of variants suited to very different epidemiological conditions; it can infect over 70 different species (Hedger 1981; Shimshony, 1988; Bengis & Erasmus, 1988; Pinto, 2004; Arzt et al., 2011a; Karesh, 2012) and it is highly contagious in the acute stages of disease, but can also survive subclinically

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for years in persistently infected animals, so-called "carriers" (Burrows, 1966; Bengis et al., 1986; Alexandersen et al., 2003).

Carriers are defined by the World Organisation for Animal Health (Office International des Épizooties: OIE) as persistently infected animals which are recovered, vaccinated or exposed and from which FMDV can be isolated from the oropharynx for more than 28 days after acute stages of disease (OIE, 2009). About 50% of ruminants are thought to become persistent carriers (Arzt et al., 2011b). Cattle are capable of maintaining the virus for up to 3.5 years, sheep for at least 9 months, goats for 4 months and African buffalo (*Syncerus caffer*) for 5 years (Condy et al., 1985; Alexandersen et al., 2002; Arzt et al., 2011b). However, there is much uncertainty about whether these animals transmit FMDV to other animals, and, if they do, which particular factors cause a persistently infected animal to recommence virus shedding to the extent that it can infect another animal (Thomson, 1996).

GLOBAL DISTRIBUTION AND POTENTIAL FOR EMERGENCE

While FMD was eradicated in most of Western Europe by the late 1980s, five out of the seven known FMDV serotypes (O, A, SAT 1, SAT 2 and SAT 3) are present in Africa, whereas A, O and Asia 1 serotypes are found in Asia. Serotypes A and O have the widest global distribution. Conversely, serotype C has been very rarely reported over the past 15 years, the last confirmed outbreaks occurring in Brazil and Kenya in 2004 (Rweyemamu et al., 2008). In Asia, South America and Africa, FMDV can be further divided into seven major pools of infection (Paton et al., 2009). It is generally considered that FMDV originated in Africa due to the long-term subclinical infection of African buffalo (involving co-evolution with that species) and the greater genetic diversity of the SAT serotypes compared to the Eurasian types (Vosloo et al., 2002); however, the earliest reliable descriptions of FMD come from Europe, leading others to conclude that its origin lies on that continent (Tully & Fares, 2008). Additionally, it has been suggested that FMD was present in the 11th century in India since Lokopakara (1025 AD) compiled by Chavundaraya (Ayangarya, 2006) described "boils of gum and hoof" as a distinct disease in cattle (Nene, 2007).

The genome of FMDV is highly plastic and evolves rapidly as a consequence of errors that are introduced and inherited during replication. These characteristics allow nucleotide sequence data to be used to reliably reconstruct the relationship between viruses recovered from different locations, or at different times. At the broadest scale, analyses of sequences encoding a capsid protein (VP1/1D) are widely used to categorize field strains into discrete variants (or topotypes) that frequently show geographical clustering based on the historical distribution of FMDV. The pattern of serotypes and variants around the world is not static and sequencing of these viruses allows us to precisely characterize new isolates of FMDV and trace their origin and

movements across international boundaries (Samuel & Knowles, 2001; Knowles & Samuel, 2003).

The escape of FMDV strains from their endemic pools into other regions is a matter of great concern due to the potential for disease emergence in new areas previously naïve to those strains. These introductions can have considerable consequences in terms of disease spread and severity even if resident FMDV strains are already present, because of poor cross-protection against exotic strains (Vosloo et al., 2010). The recent outbreaks of SAT 2 in the Middle East and North Africa or the PanAsia strain of serotype O in the UK in 2001 are examples of this (Knowles et al., 2001; Di Nardo et al., 2011; Valdazo-González et al., 2012). Host vulnerability to new strains, for instance, was evident in a recent incursion of SAT 2 into Egypt, where mortality rates as high as 20% were reported in livestock (Ahmed et al., 2012).

HISTORICAL EMERGENCE OF FMD IN AFRICA

Human activity has had major impacts on the epidemiology of FMD. This is particularly evident in sub-Saharan Africa largely as a consequence of movements of animals and infectious diseases following European colonization. The rinderpest (cattle plague) pandemic, which swept across Africa in the late 19th century following the importation of livestock from India into Ethiopia, decimated more than 90% of cattle, buffalo and other susceptible species in eastern and southern Africa. The pandemic has played a central role in the social and political history of Africa, in the epidemiology of many livestock and wildlife diseases present on the continent today (including FMD), and in shaping African ecosystems (Sinclair, 1979; Reid et al., 2005; Sinclair et al., 2007). Its repercussions are still observable today (African Union, 2010).

Reports of animals with FMD in southern Africa are as old as 1795 (reviewed by Knowles, 1990). However, the rinderpest pandemic largely removed populations susceptible to FMD and, as a result, FMD occurrence declined around the turn of the century, with cases in southern Africa only being reported again in 1931 (Thomson, 1995). It is likely that currently circulating lineages of SAT serotypes re-emerged from small numbers of buffalo that survived the rinderpest pandemic once buffalo and livestock numbers had recovered.

Anthropogenic factors are also likely to have been critical in the introduction and spread of other serotypes in Africa, and phylogenetic analyses are consistent with the interpretation that Eurasian FMDV serotypes (O, A and C) were re-introduced through trade and restocking of livestock from Asia or Europe following the ravages of rinderpest. For instance, there is evidence for a relatively recent (within the past 100 years) common ancestral history between FMDV O topotypes that are currently present in Africa, Asia and South America (Figure 2.1), consistent with emergence of O strains into susceptible animal populations of Africa as a result of introduction with cattle. Furthermore, a more diverse serotype O sequence obtained from a Sudanese FMD virus in the 1960s

may be a sole representative sequence of FMDVs present in Africa prior to the rinderpest pandemic (J.M. Stirling and N.J. Knowles, unpublished data).

Over the past century, and continuing to this day, the unfenced rangelands of eastern Africa have supported abundant wildlife populations, with frequent opportunities for close contact between wildlife and livestock. The control of rinderpest through cattle vaccination in the 1950s and 1960s may have played a major role in livestock–wildlife interactions in the region. In the Serengeti, for example, rinderpest vaccination was associated with dramatic increases in wildebeest and buffalo numbers (Sinclair, 1979), with the potential for increased interactions with neighboring pastoral livestock populations. There are arguably now more susceptible hosts, more contact between them, and more intra- and

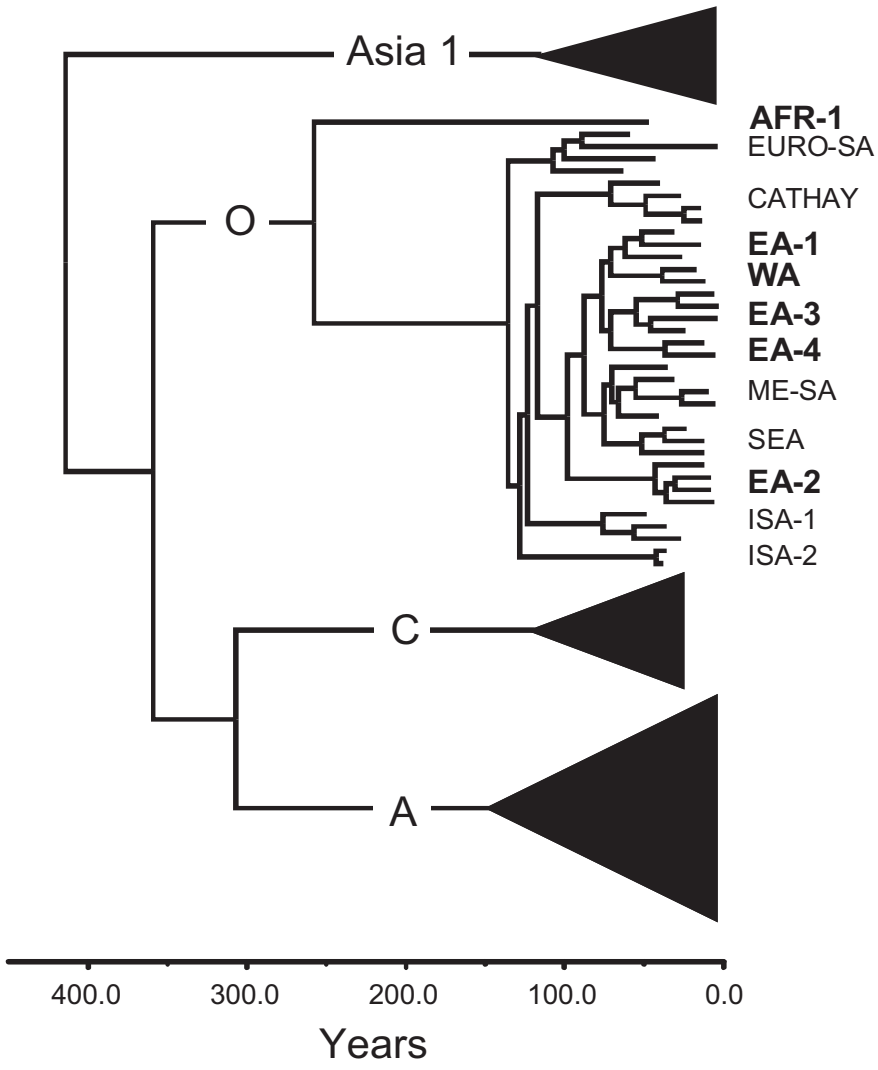

FIGURE 2.1 **Impact of the rinderpest pandemic upon current FMDV distribution in Africa. Bayesian phylogenetic tree for representative VP1 (1D) sequences for serotype O FMD viruses. These results indicate that current FMDV topotypes present in Africa (highlighted in bold) have diverged from other global FMDV topotypes within the last 100 years. A single isolate representative of the putative FMDV strains that were present in sub-Saharan Africa prior to the rinderpest pandemic in 1889–1997 is also shown (AFR-1).**

interregional livestock movements than at any other time in recent history. This creates an ideal environment for the emergence of novel FMDV strains and may explain the greater diversity of FMDV serotypes and topotypes than in any other regions. Genomic analyses may provide a useful approach to explore this hypothesis, and to gauge whether viral populations are diversifying more rapidly in areas with high levels of wildlife–livestock interactions, and/or high mobility of livestock. However, a true picture of diversity is difficult to obtain retrospectively, as there is much bias from the patchy sampling coverage and disease reports from many areas in the last century.

MAINTENANCE OF FMD IN DIFFERENT RESERVOIR POPULATIONS IN SUB-SAHARAN AFRICA

Although buffalo are considered the ancestral host of SAT serotypes and important maintenance host populations in southern Africa (Thomson et al., 1992; Vosloo et al., 2001, 2002, 2010), many features of the epidemiology of FMD in Africa remain unclear, particularly in relation to the role of livestock and wildlife in maintaining different FMDV serotypes in other parts of Africa (Figure 2.2). While SAT 1 and SAT 2 are known to be maintained in buffalo, these serotypes have also been able to "escape" from sub-Saharan Africa to cause extended livestock outbreaks in North Africa, the Middle East and Europe without involvement of buffalo or any other wildlife species (Ahmed et al., 2012; Bastos, 2003; Dimitriadis & Delimpaltas, 1992; Rweyemamu et al., 2008). This suggests that SAT 1 and SAT 2 can be maintained independently in both livestock and buffalo populations (Figure 2.2A). However, in the wildlife-rich rangelands of East Africa, the degree to which SAT 2 outbreaks are sustained by re-introduction from buffalo is still unclear. In contrast to SAT 1 and SAT 2, serotype SAT 3 appears to be mainly confined to buffalo with only a small number of outbreaks reported in domesticated species (Figure 2.2B) (Thomson, 1995; Bastos et al., 2003; Thomson et al., 2003). Conversely, although maintenance hosts for SAT serotypes, buffalo are not believed to be reservoirs of Eurasian FMDV serotypes (Anderson, 1979; Ayebazibwe et al., 2010) (Figure 2.2C).

The role of other wildlife hosts in the epidemiology of the disease is even less clear. In contrast to buffalo populations that show consistently high levels of exposure (Thomson et al., 1992, 2003; Bronsvoort et al., 2008; Ayebazibwe et al., 2010), seroprevalence in other wild ungulates, for example impala (*Aepyceros melampus*), giraffe (*Giraffa camelopardalis*), eland (*Taurotragus oryx*), tsessebe (*Damaliscus lunatus*), kudu (*Tragelaphus strepsiceros*), waterbuck (*Kobus ellipsiprymnus*), sable antelope (*Hippotragus niger*), bushbuck (*Tregelaphus sylvaticus*), nyala (*Nyala angasii*), warthog (*Phacochoerus africanus*), bushpig (*Potamochoerus larvatus*), redbuck (*Redunca spp.*) and wildebeest (*Connochaetes gnou*), is very low, suggesting that they are spill-over hosts rather than maintenance populations (Anderson et al., 1993) (Figure 2.2D). However, in some parts of southern Africa it is suggested that spill-over from

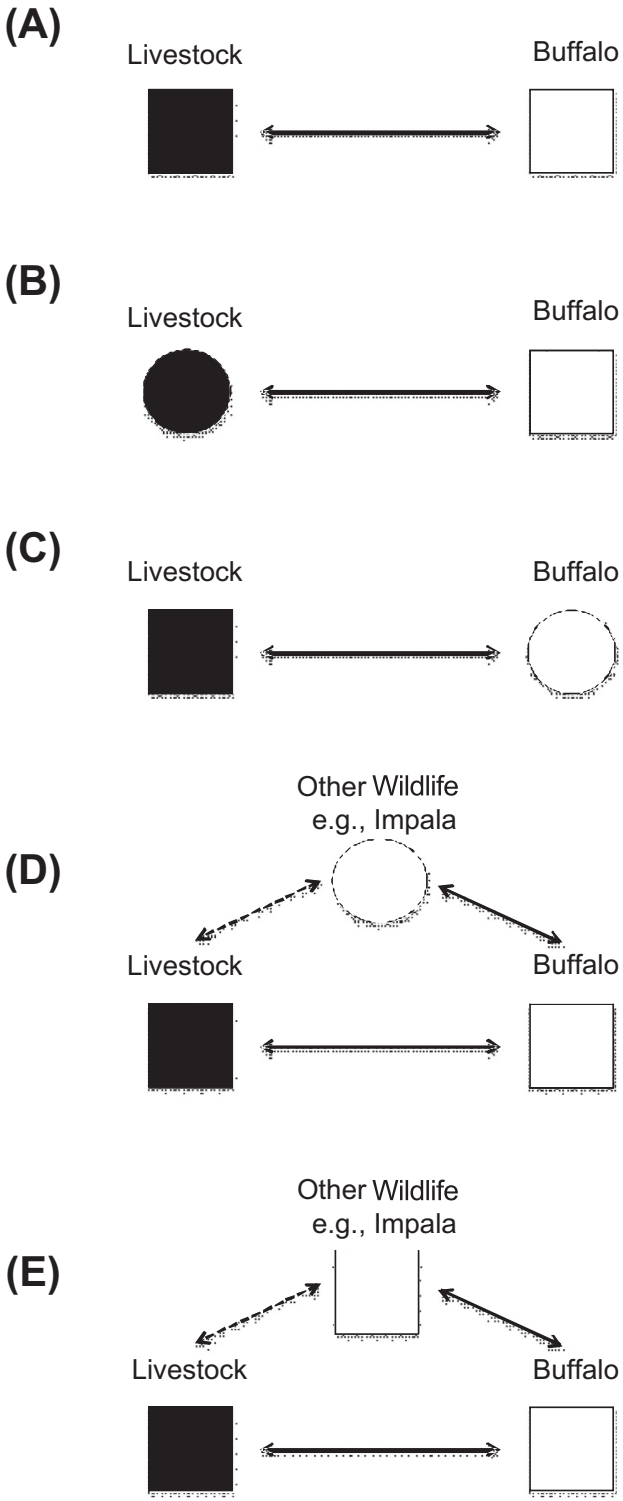

FIGURE 2.2 **Simple models that outline possible FMDV reservoir systems in sub-Saharan Africa. Squares represent maintenance populations and circles show non-maintenance populations. Schematics show different scenarios where: (A) Livestock and buffalo can both maintain FMDV independently of one another, as is thought to be the case for SAT 2 in different parts of Africa; (B) Buffalo, but not livestock can maintain FMDV independently, for example in the case of SAT serotypes in South Africa where livestock control measures are in place; (C) Livestock, but not buffalo, can maintain FMDV independently, as is thought to be the case for serotypes A and O; (D) Livestock and buffalo can both maintain FMDV independently of one another. FMDV may spill over to other susceptible animals such as impala but cannot be maintained independently in this other wildlife population, as is the case in most non-buffalo wildlife in Africa; and (E) Livestock, buffalo and other wildlife can all maintain FMDV independently of one another but can also transmit it between each other, as is proposed for some high density impala populations in South Africa.**

buffalo to impala may occur frequently and that denser impala populations may be capable of self-sustained circulation (Vosloo et al., 2009) (Figure 2.2E). Impala have also been implicated as intermediate hosts between buffalo and cattle (Bastos et al., 2000; Hargreaves et al., 2004; Vosloo et al., 2006).

OPPORTUNITIES FOR BUFFALO-TO-LIVESTOCK TRANSMISSION

African buffalo are of particular concern where they act as potential reservoirs of FMDV for livestock, and as a maintenance source of persistently infected animals (carriers) where antigenic diversity may be generated (Vosloo et al., 1996). Across southern Africa, where the disease is well controlled in livestock, buffalo are implicated as the likely source of many new livestock outbreaks (Bastos et al., 2000; Hargreaves et al., 2004; Thomson et al., 2003; Vosloo et al., 2001). However, much less is known about the role of buffalo elsewhere in Africa, and the importance of buffalo-to-livestock transmission in triggering new outbreaks and sustaining endemic cycles of infection.

Acutely infected buffalo develop FMD lesions that shed virus, albeit in quantities lower than cattle (Gainaru et al., 1986). Buffalo calves become infected with FMD between 3 and 6 months (Condy and Hedger, 1978), with the proportion of persistently infected animals peaking in the 1–3 year age group (Juleff et al., 2012a). It is speculated that acutely infected buffalo calves may be a source of virus for other animals (Thomson et al., 2003). However, clear experimental evidence for FMDV transmission from artificially infected buffalo to livestock has been elusive. In the two experiments where transmission was achieved, cattle only became infected 5 and 10 months after the acute stage of the disease in the buffalo (Dawe et al., 1994; Vosloo et al., 1996). A further four studies reported absence of infection in cattle despite protracted contact with persistently infected buffalo (Bengis et al., 1986; Gainaru et al., 1986; Condy & Hedger, 1974; Anderson et al., 1979). In the studies where transmission occurred, male buffalo were mixed with female cattle, and cattle became infected only after the buffalo reached sexual maturity. This led to the hypothesis that FMD can be transmitted by the sexual route. However, FMD virus was retrieved from semen and sheath wash from only one out of twenty FMDV seropositive male buffalo (Bastos et al., 1999), and therefore the importance of possible sexual transmission of FMD from buffalo to cattle remains inconclusive. Although there are few experimental reports of buffalo-to-cattle transmission, epidemiological field data and phylogenetic evidence in southern Africa demonstrates that transmission from buffalo to FMD-free cattle does occur (Bastos et al., 2000; Hargreaves et al., 2004; Thomson et al., 2003; Vosloo et al., 2009).

Tanzania, Zimbabwe, Zambia, Democratic Republic of Congo and South Africa represent the five countries with the highest estimated buffalo numbers, with Tanzania having at least six times more buffalo than any other country (Table 2.1, Figure 2.3). In most of these countries buffalo populations

TABLE 2.1 Estimated African Buffalo Population Sizes and Population Trends in the Ten African Countries with the Highest Buffalo Populations (East, 1999)

Country	Estimated total number of buffalo (in 1998)	Population trend
Tanzania	>342,450	Stable/decreasing
Zimbabwe	>50,330	Stable/decreasing
Zambia	>40,090	Stable/decreasing
Democratic Republic of Congo	>39,180	Decreasing
South Africa	>30,970	Increasing
Botswana	>26,890	Stable/decreasing
Uganda	>20,220	Stable/increasing
Kenya	>19,560	Decreasing
Gabon	>20,000	Stable/decreasing
Central African Republic	>19,000	Decreasing

are stable or decreasing (East, 1999). Livestock densities in these areas are also considerable. Ethiopia, Sudan and South Sudan and Tanzania, for example, have the highest populations of cattle in Africa and Nigeria, Sudan and South Sudan, and Ethiopia have the highest combined sheep and goat populations (FAO, 2013; Chilonda, 2005). Hence, together with maximal FMDV diversity (Rweyemamu et al., 2008), East Africa also contains the largest pool of susceptible hosts.

Achieving a better understanding of the relative importance of buffalo in the epidemiology of FMDV in East Africa is of particular relevance for disease control in livestock-keeping communities living at the wildlife–livestock interface, particularly given the ecological and economic importance of buffalo in these areas. Buffalo are bulk grazers, and open up habitats preferred by short grass grazers. They are one of the "big five," that are sought by tourists, both for game viewing and sport hunting. In 7 out of the 14 Southern African Development Community countries, the revenue from the game hunting industry is estimated to be worth $192 million, with wildlife-watching tourism revenue worth $3.2 billion for 10 of these countries where data are available (Booth, 2010). To develop effective FMDV control strategies that support both livestock-based livelihoods and wildlife conservation, much information is still needed on how wildlife species interact with livestock, how and where cross-species transmission occurs, and the possible role of wildlife, other than buffalo, as intermediaries in transmission.

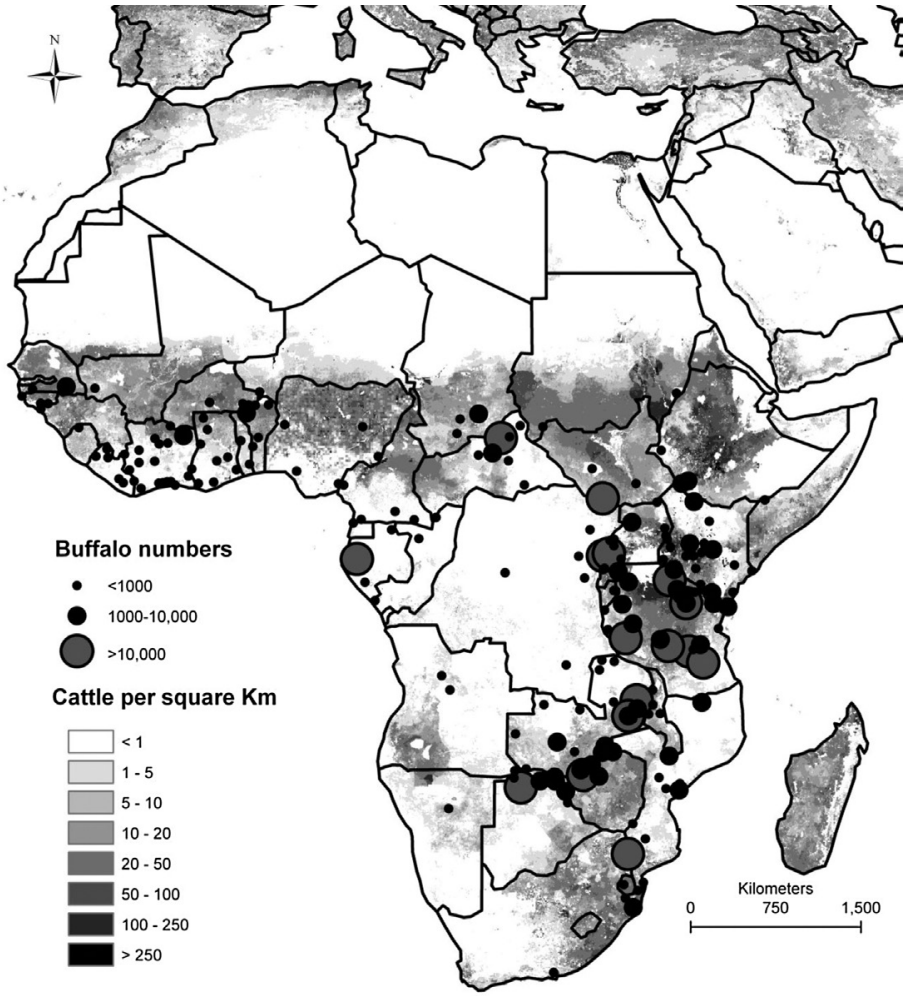

FIGURE 2.3 Estimated geographical distribution of buffalo and cattle in Africa. *Data from East, 1999; Robinson et al., 2007.*

IMPORTANCE OF FMD IN DEVELOPING COMMUNITIES IN AFRICA

Although the devastating consequences of FMD outbreaks in industrialized countries are well recognized, there is relatively little data available to quantify FMD impacts in endemic settings which are often developing countries. The clinical disease has often been regarded as of little significance to livestock health in traditional livestock-keeping systems in Africa, but it is clear that even in extensive, low-production systems, FMD has important consequences on livelihoods and food security, as a result of both direct and indirect effects of the disease. The impact on human poverty has been assessed on the basis of treatment costs, reduced productivity of animals, loss of draft power for tillage and transport, disruption of access to markets, the cost of risk management, limitation of land usage in areas with high disease risk, and risk adversity to embracing advances in animal management (Perry et al., 2002). Based on weighted analysis of socioeconomic criteria and national impacts that also affect the poor,

FMD was ranked third (after gastrointestinal helminths and neonatal mortality syndrome) among animal diseases having greatest impact on overall poverty (Perry et al., 2002).

Livestock owners in East Africa consistently rank FMD among the top five most important livestock diseases (Jost et al., 2010; Ohaga et al., 2007; Bedelian et al., 2007; Cleaveland et al., 2001) with anecdotal evidence for an increasing frequency of outbreaks in pastoral herds and flocks. Studies in Ethiopia, Cameroon, Sudan and Tanzania showed that endemic FMD is associated with calf deaths, reduced milk supply, poor reproductive performance and heat intolerance syndrome (Cleaveland et al., 2001; Catley et al., 2004; Barasa et al., 2008; Rufael et al., 2008). Milk yield may be reduced for the animal's entire lactation period after FMD infection, and lack of milk is likely to be a contributory factor to calf death in traditional livestock keeping regions (Barasa et al., 2008). FMD udder damage may also increase susceptibility to mastitis (Saini et al., 1992). Many people in Africa rely on unpasteurized milk from their animals as an important source of nutrition. Although this is the most common means whereby humans contract FMD (Bauer, 1997), and pastoral communities frequently report a self-limiting febrile disease in people at the time of FMD outbreaks in cattle (Shirma, 2005), no studies have investigated the prevalence of zoonotic FMD infections in African countries.

PROSPECTS FOR FMD CONTROL IN AFRICA

FMDV control presents multiple challenges across the African continent. In East Africa, the broad spectrum of FMDV diversity, large-scale animal movements, which are often unregulated, and an abundance of potential FMDV wildlife hosts makes the region a theater for FMDV emergence and one of the most challenging areas in the world to control the disease. However, new disease control issues are also emerging in southern Africa, with the development of trans-frontier conservation areas (TFCAs) that promote conservation and sustainable management of ecosystems with cross-border tourism. While TFCAs have a clear conservation and political rationale, they are at odds with conventional FMD control methods in southern Africa, such as veterinary fencing and movement controls. Physical segregation of buffalo and livestock, which has traditionally been used in southern Africa to prevent transmission, is not only incompatible with the TFCA vision, but would also be infeasible in many other parts of Africa (e.g., East Africa) due to concerns about negative consequences for wildlife migration and dispersal, which are central to the ecological integrity of many of these unfenced ecosystems (Ferguson et al., 2013).

The African countries that have established zonal FMD freedom have utilized a combination of animal movement control, separation of livestock and wildlife and vaccination of livestock (Brückner et al., 2004).

This geographically based approach may need to be adapted to balance the needs of people, livestock and wildlife, but could, for example, be introduced in areas far from wildlife-protected areas and exploit geographical features that could act as natural barriers to movement of animals and people. Sustainability of control measures also remains uncertain and challenging, particularly in the face of volatile dynamics in livestock markets and in areas with political instability (Thomson, 1995; Vosloo et al., 2002; Batho, 2003).

Another catalyst to FMD control in the East African region would be for more achievable targets to be allowed for entry into lucrative markets for livestock products. At present, African producers are locked out of many markets due to stringent, geographically based rules on FMD status and importation (OIE, 2011). Africa accounts for 7% of global beef consumption, but less than 2% of global trade (Morgan & Tallard, 2007), leaving much potential for growth of commodity-based trade in livestock products both within and outside of Africa. Incentives for positive steps in FMD control, such as commodity-based trade (Thomson et al., 2004; 2009), may provide a welcome injection of funds to further FMD control measures in a positive feedback loop.

Despite the potential for improving FMD control in Africa, the lack of effective FMDV vaccines remains a critical constraint. Current FMDV vaccines produce immunity lasting a maximum of 6 months, need a continuous cold-chain until inoculation, and give very little cross-protection between strains (Vosloo et al., 2002; Paton et al., 2009; Domenech et al., 2010), which limits their usefulness against the high diversity of circulating strains in East Africa. A key step towards effective control and potential elimination of FMD in Africa must be the development of stable vaccines that produce long-lived immunity to a broad spectrum of strains

CONCLUSIONS

East Africa, with its large populations of susceptible ungulates, vast movements of livestock and diversity of FMDV strains, presents the ideal cauldron from which novel strains of FMDV can emerge. Implementation of measures that are sympathetic to wildlife conservation and pastoralism presents a formidable challenge for the control of FMD and the emergence of new epidemic cycles. However, the incentives for control are great; it would indisputably contribute to an improved quality of life for humans and animals and support economic development, acting as an important tool to break cycles of poverty. Where FMD is well controlled in livestock, African buffalo appear to be a critical source of infection. However, in East African countries where FMDV is endemic, livestock and human-related factors are likely to contribute as additional important drivers of FMDV emergence.

Glossary of terms used in the text

Term	Definition	Reference	Possible example in context of FMD	Reference for FMD example
Carrier (epidemiology)	An animal that harbors a specific infectious agent without discernible clinical disease and serves as a potential source of infection	Martin et al., 1987	Examples of asymptomatic animals transmitting FMD are rare	Vosloo et al., 1996; Dawe et al., 1994
OIE FMDV carrier definition	Animals which are recovered, vaccinated or exposed and in which FMDV can be recovered from the oropharynx more than 28 days after acute stages of disease	OIE, 2009	Approximately 50% of domestic ruminants. High proportions of African buffalo (peaking at 1–3 years old)	Alexandersen et al., 2002; Condy et al., 1985; Arzt et al., 2011b; Juleff et al., 2012b
Critical community size	The minimum size of a closed population within which a pathogen can persist indefinitely	Bartlett, 1960		
Maintenance host	A population larger than the critical community size: disease will be maintained within the population even if transmission into the population from the outside is prevented. A combination of nonmaintenance communities can still combine to make a maintenance community	Haydon et al., 2002		

Reservoir	One or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined population of interested (target population)	Haydon et al., 2002	Livestock and buffalo in FMD endemic countries. Buffalo in South Africa	Thomson et al., 2003
Spill-over transmission	Interspecies transmission from a maintenance host to a nonmaintenance host	Daszak, 2000; Power & Mitchell, 2004	Transmission from livestock and buffalo to impala and other susceptible wildlife. Amplification of FMDV may occur in spill-over hosts with secondary transmission to the target population. Independent maintenance proposed in high densities of impala in Kruger National Park	Anderson et al., 1993; Bastos et al., 2000; Vosloo et al., 2009
Commodity-based trade	A focus on the attributes of the product (quality, food safety) rather than the disease status of the place of origin. Transmission of diseases such as FMD	Thomson et al., 2004	Deboned beef poses little threat of FMD transmission. Proponents of commodity-based trade argue that countries that are taking all possible measures to reduce the risk of FMDV transmission from their products should be allowed to trade these products	Rich & Perry, 2011; Thomson et al., 2009

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