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**Foot-and-Mouth Disease in Kenya: Epidemiology, disease impact
and vaccine effectiveness on large-scale dairy farms**

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Declaration of own work

I, Nicholas Anthony Lyons, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Foot-and-mouth disease (FMD) is endemic in Kenya where serotypes A, O, SAT1 and SAT2 are frequently encountered. Despite the importance of the dairy industry and the frequent reporting of disease, the epidemiology of FMD and field-based vaccine effectiveness has been poorly described in these endemic settings. Additionally, the disease impact has been inadequately characterised, despite the importance of such information when allocating scarce resources for animal health in national disease control strategies. The objectives of this doctoral thesis were to gain field experience of FMD in endemic settings and to use appropriate outbreaks to assess the vaccine effectiveness, gather evidence to optimise the use of vaccines and inform national policy, and to estimate disease impact.

Outbreaks on two large-scale dairy farms located within Nakuru County, Kenya, were investigated and detailed descriptions of the outbreaks are presented. Both farms regularly used locally produced, aqueous adjuvanted, non-NSP purified quadrivalent (A, O, SAT1, SAT2) vaccine every 4-6 months. The first attended outbreak was caused by serotype SAT2 and evidence was found of limited or no vaccine effectiveness. At the second outbreak, due to serotype O, there was evidence of increasing protection with increasing number of doses. The reasons behind the vaccine poor effectiveness are discussed and are likely to include poor match with the field strain and inappropriate schedules in youngstock. Virus neutralisation test data were made available from the vaccine manufacturer who sample animals on farms using routine prophylactic vaccination. The influence of maternally derived antibody on the response to vaccination was investigated with these data and recommendations on vaccine schedules and future research priorities are made based on the evidence presented.

On the farm that had SAT2, analysis of the disease impact was performed using individual animal data. Longitudinal analysis of individual milk yields utilising generalised estimating equations and an autoregressive variance structure to account for the correlation of yields for individual animals was performed. Predictions of 305-day milk yields were made based on previous lactations in the same herd. Despite a clear herd level impact, no difference was found between recorded clinical FMD cases and non-cases. More detailed analysis revealed significant reductions among older animals in earlier stages of lactation but younger cows were able to recover sufficiently so that no overall impact was seen. The impact of clinical disease on the rate of clinical mastitis and culling was analysed utilising a historical cohort approach with survival analysis over a 12-month period after the commencement of the

outbreak. Hazard ratios (HR) were generated using Cox regression accounting for non-proportional hazards by inclusion of time-varying effects. There was good evidence of an increased rate of mastitis in the first month after the onset of the outbreak (HR=2.9, 95%CI 0.97-8.9, P=0.057) although the effect on culling was less clear. The implications of these findings for policy and further research are discussed.

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List of abbreviations and acronyms

AHA	Animal Health Assistant
BVS	Bovine Vaccinal Serum
CFT	Complement Fixation Test
DPC	Days Post Challenge
DPV	Days Post Vaccination
DVO	District (Sub-County) Veterinary Officer
DVS	Department of Veterinary Services
EP	European Pharmacopoeia
EPP	Expected Percentage of Protection
EuFMD	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
GEE	Generalised Estimating Equations
GLM	Generalised Linear Model
HF	Holstein-Friesian
IDL	Intradermolingual
KEVEVAPI	Kenya Veterinary Vaccines Production Institute
KVA	Kenya Veterinary Association
LPBE	Liquid Phase Blocking ELISA
MDA	Maternal Derived Antibody
NSP	Non-Structural Protein
OIE	Office International des Epizooties (World Organisation for Animal Health)
PCP	Progressive Control Pathway
PD ₅₀	50% Protective Dose
PPG	Protection from Podal Generalisation
SAT	Southern African Territories
SP	Structural Proteins
SPBE	Solid Phase Blocking ELISA
SPCE	Solid Phase Competition ELISA
TAD	Transboundary Animal Disease
VM	Vaccine Matching
VNT	Virus Neutralisation Test

Chapter 1. Introduction

1.1 Foot-and-mouth disease – the virus, the disease and its impact

Foot-and-mouth-disease (FMD) is caused by a non-enveloped single stranded RNA picornavirus within the *Aphthovirus* genus. It has the ability to infect all species of the order Artiodactyla (i.e. animals with cloven/even-toed hooves) including cattle, pigs, sheep and goats. Camelids are not considered to have an important role in transmission although they may be infected in certain circumstances (Wernery and Kaaden, 2004). Several species of wildlife are known to be susceptible to FMD virus (FMDV) infection and the African buffalo (*Syncerus caffer*) has been implicated as a maintenance host (Thomson et al., 1992).

Disease is most apparent in cattle and pigs, varying from subclinical to malaise, pyrexia and vesicular lesions on the tongue, dental pad, interdigital space and teats. A drop in milk yield is often apparent in lactating cattle (Kitching, 2002). The characteristic appearance of the lesions over time means their age may be estimated which is helpful in epidemiological investigations (DEFRA, 2005). Mortality rates are generally reported as less than 5%, with deaths mostly occurring in youngstock subsequent to acute myocarditis (Alexandersen et al., 2003). In severe cases, adults may die secondary to starvation. Reports of a “heat intolerance and hair overgrowth syndrome” after FMD has been reported in East Africa, that may be associated with viral induced changes to the pituitary gland (Catley et al., 2004; Suttmoller et al., 2003). Small ruminants (i.e. sheep and goats) tend to have milder clinical signs that often go unnoticed.

FMDV is considered to be highly transmissible and spreads predominantly through aerosol or ingestion (Alexandersen et al., 2003). Virus is shed in all excretions and secretions of an infected animal and transmission through contact with contaminated fomites is also possible. FMDV is one of very few infections that have been reported to be spread over long distances by wind. This has been considered as the most likely route of infection to the Isle of Wight from Brittany in 1981 (Garland and Donaldson, 1990) and distances up to 16 km were speculated in the UK 2001 epidemic based on field investigations and meteorological analysis (Gloster et al., 2005). This method of transmission is particularly favoured in cold and humid conditions (Donaldson, 1972). Ruminants tend to be more susceptible to FMDV through aerosol spread than pigs although the latter tend to produce more aerosolised virus (Kitching et al., 2005). Transmission may also occur through animal products including milk, meat or

offal. Pigs are considered particularly prone to infection by ingestion of virus. The feeding of illegally imported non-heat-treated swill to pigs is considered the likely route of introduction to the UK in 2001 (Paton et al., 2009). Typically the post-mortem decrease in carcass pH will inactivate the virus although this does not extend to lymph nodes, bone marrow and some offal, so deboned meat may be traded internationally in some circumstances (Alexandersen et al., 2003).

The published incubation period range is 2-14 days. Animals are usually infectious before the onset of clinical signs with milk and semen having been reported to have virus up to four days before. Acute clinical signs typically resolve within two weeks although secondary infections can lead to protracted disease and milk yields may not recover in the current lactation (Kitching, 2002). Up to 50% of animals may harbour virus in the pharynx more than 28 days after clinical recovery, the period that defines an animal as a virus "carrier" (Salt et al., 1996). This may occur in vaccinated animals without the presence of clinical signs (Cox et al., 2005). Carrier status has not been demonstrated in pigs. The role of carriers in subsequent transmission is unclear and controversial. Whereas there is some evidence from the field that transmission may occur between carrier African buffalo and cattle, in an experimental setting Dawe et al., (1994a, 1994b) were unable to demonstrate this. Moreover, when data in the literature from experimental transmission studies in cattle were used to parameterise a mathematical model, an estimated average of 0.026 (95%CI 0.008-0.059) animals were infected by a FMDV carrier for each month it was present (Tenzin et al., 2008). Field evidence for the transmission of FMDV from carrier to susceptible cattle is old and anecdotal (Sutmoller et al., 2003). Despite this, the possibility of carriers has large implications for control policy and trade in countries free of disease.

FMD is endemic in many regions of the world including parts of South America and across most of Africa, the Middle East and Asia (Figure 1.1). The whole of sub-Saharan Africa is endemic except for parts of South Africa near the Kruger National Park where infection appears to be maintained in the African buffalo population.

There are seven serotypes of FMDV, denoted A, C, O, South African Territories (SAT) 1, 2, 3 and Asia-1. The serotypes are grouped into pools depending on their geographical distribution with serotype O being the most widespread (Figure 1.1). Serotypes are further subdivided into topotypes based on a 15-20% difference in the genetic sequence of the virus protein 1 (VP1) region that follows broad geographical regions (Samuel and Knowles, 2001). The antigenic

diversity varies between serotypes, with A and SAT2 being particularly diverse (Bastos et al., 2003; Upadhyaya et al., 2014).

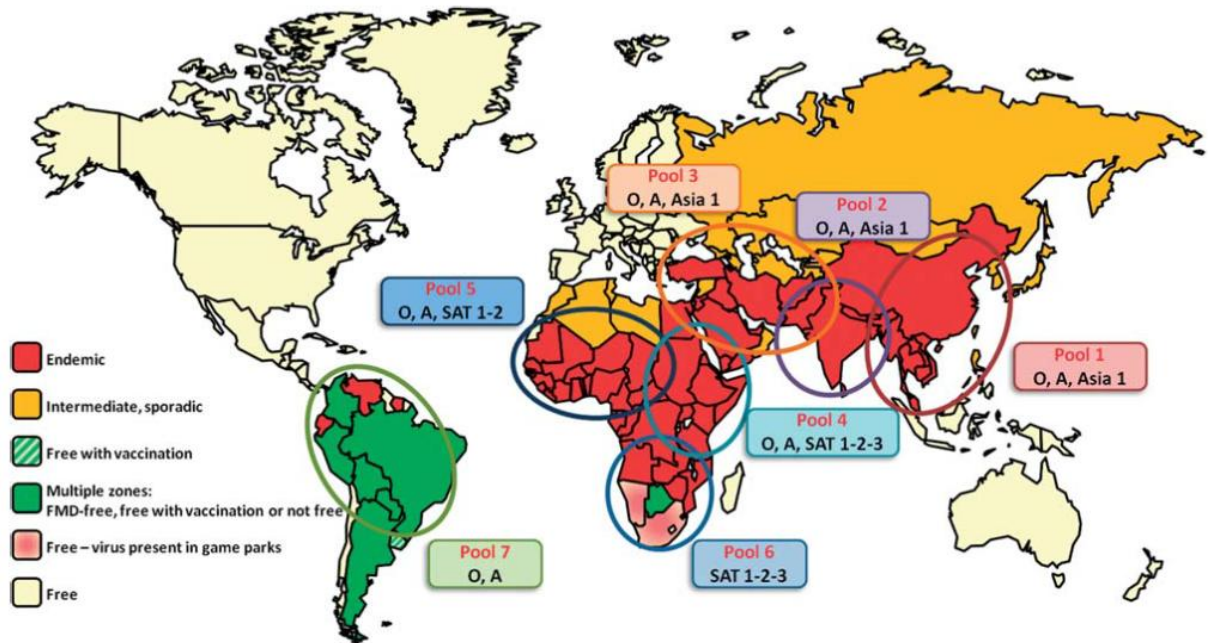


Figure 1.1. Geographical distribution of FMD and location of serotype pools. Source: Di Nardo et al. (2011)

Immunity is considered to be serotype specific, though there is evidence for cross-protection under certain circumstances. For example infection with multiple serotypes may decrease the severity of signs when exposed to other serotypes (Doel, 1996). As is common with RNA viruses, the genome replication is error-prone resulting in a highly mutable virus such that animals which have recovered from one infection may be susceptible to infection with a different strain of the same serotype, hindering control efforts (Domingo et al., 2002; Grubman and Baxt, 2004).

The annual global economic impact of FMD has been estimated as US\$11 billion (90% range 6.5-20) in endemic regions alone although this is a likely underestimate because it only considers the costs from decreased production and vaccination (Knight-Jones and Rushton, 2013). Field data on economic impact are limited particularly in endemic settings and estimates are often based on expert opinion and assumptions (James and Rushton, 2002). Viral incursions into countries free of infection can have devastating economic consequences. The

UK 2001 outbreak is estimated to have cost between 12.3 and 13.9 billion USD through control measures, subsidising farmers, prohibited international trade and effects on tourism (Thompson et al., 2002).

The distribution of countries with endemic infection tends to parallel regions of low household income. Within these countries, there is limited opportunity for international trade since trade is only allowed with other FMD endemic countries. For the individual farmer, the disease has direct economic impact through decreased milk production, reduced growth rates, loss in draught power, increased perinatal mortality, increased associated disease (e.g. mastitis), poorer fertility (including abortion) and increased culling from prolonged lameness (Doel, 2003). A recent estimation of the cost of disease among smallholder cattle farmers in Cambodia revealed the average cost to be between 216 and 371 USD per affected animal (Young et al., 2012). Although FMD is often considered a disease of particular importance to wealthier countries that wish to remain free of disease, its control is also likely to have a role in poverty reduction in poorer countries with endemic disease (Perry and Rich, 2007).

1.2 FMD control policies

FMD control policies vary depending on the epidemiology and available resources. “Stamping-out” policies whereby susceptible animals on infected farms are culled along with “dangerous contacts” (animals on farms at high risk of infection) implicated through outbreak investigations has formed the basis of control measures in many FMD-free countries. This approach is typically combined with movement controls, surveillance strategies, appropriate carcass disposal and cleaning and disinfection of affected farms. In the UK 2001 outbreak, the highly controversial “contiguous cull” strategy involved the slaughtering of neighbouring farms irrespective of clinical signs or other risk factors for the introduction of infection, as this was suggested by mathematical models to be the most effective control method (Ferguson et al., 2001). This policy, and the models it was based on, were later subjected to extensive criticism and described as “seriously flawed” and with inherent biases (Mansley et al., 2011).

Vaccination has been used in FMD-free settings in response to introduced infection, combined with other measures outlined above. Recent examples include outbreaks in the Netherlands (2001), Argentina (2001), South Korea (2010) and Uruguay (2001). In the Netherlands, vaccination was limited to a particular zone whereas in the other examples nationwide vaccination was performed. Vaccine may also be applied as a permanent buffer zone as is the

case in western Turkey to reduce the chances of spread into the EU, and in Botswana to prevent spread to a FMD-free zone. Vaccine policy has been heavily influenced by trade implications (Sutmoller et al., 2003). This comes from the theoretical possibility that vaccinated animals may become virus carriers and the difficulties in distinguishing between infected and vaccinated animals. Vaccination in *free* settings may be either “protective” or “suppressive”. Both are aimed at decreasing the likelihood of transmission but “suppressive” vaccination is combined with the culling of infected animals to hasten the re-establishment of a FMD-free status. This was the strategy used by the Netherlands in 2001 (Bouma et al., 2003). According to the World Organisation for Animal Health (Office International des Epizooties, OIE), whole countries or zones within may be classified as “free with vaccination” or “free without vaccination” with this status affecting the ability to trade with other countries. The countries free without vaccination are represented in Figure 1.1. Currently, Uruguay and the Republic of Korea are recognised by the OIE as FMD-free with vaccination. Countries that have FMD free zones are shown in Table 1.1.

Table 1.1. Countries with zones classified as FMD free either with or without vaccination according to the OIE (source: <http://www.oie.int/animal-health-in-the-world/official-disease-status/fmd/list-of-fmd-free-members/>, accessed 1/10/2014)

FMD free zone (no vaccination)	FMD free zone (with vaccination)
Argentina	Argentina
Bolivia	Bolivia
Botswana	Brazil
Brazil	Colombia
Colombia	Paraguay
Malaysia	Peru
Moldova	Turkey
Namibia	
Peru	
Philippines	
South Africa	

In endemic areas, culling is not usually considered a viable control option due to the associated costs and stakeholder resistance. Therefore, FMD is generally controlled through a combination of movement controls and vaccination. Countries with sufficient resources may opt for mass vaccination as in parts of South America, India and Israel. This strategy was successful in eliminating disease from Europe, which ceased vaccinating in 1991. In some circumstances, as in Europe before 1991 and Uruguay, only cattle were vaccinated as impact is usually greatest in this species. This strategy appeared to be effective in eliminating infection from other susceptible species in these countries. Where resources are limited, which tends to be the case in endemic areas (Rweyemamu et al., 2008), vaccination may be strategically applied for example using “ring vaccination” in a pre-defined area surrounding an outbreak as an attempt to limit spread. Vaccine may be whole or part subsidised by the government or alternatively farmers may have to pay full vaccine costs themselves.

FMD is classified as a transboundary animal disease (TAD) with control efforts seen as an international public good. Sustainable control in endemic areas has been advocated as a global necessity not only to reduce the impact in these settings but also to reduce the source of infection to free countries (Rweyemamu et al., 2008). In 2012, a joint FAO/OIE Global Conference on FMD control in Bangkok formally embraced the “Progressive Control Pathway” (PCP) for FMD control (FAO, 2011). This strategy, initially developed by the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) within the FAO, was designed to assist endemic areas to progressively reduce disease impact and virus circulation so that countries could eventually apply for official OIE endorsement of their control plan and then achieve freedom from disease either with or without vaccination. There are five stages (Figure 1.2): understanding the epidemiology and developing a risk based control plan (Stage 1); implementing control measures to reduce disease impact (Stage 2); reducing virus circulation (Stage 3); achieving OIE recognised stages as free-with-vaccination (Stage 4) and ultimately to free-without-vaccination (Stage 5). Countries need to provide evidence for their progression and are aided by FMD experts. This process may also apply to zones within a country. Vaccination is an integral part of the PCP process in reducing disease impact and virus circulation but is just one component of many that should be utilised for effective FMD control (Rweyemamu et al., 2008). The PCP process is described as “outcome based” acknowledging that control strategies are likely to vary between different regions such that a prescriptive strategy is not appropriate. This is also likely to be the case for vaccination strategies.

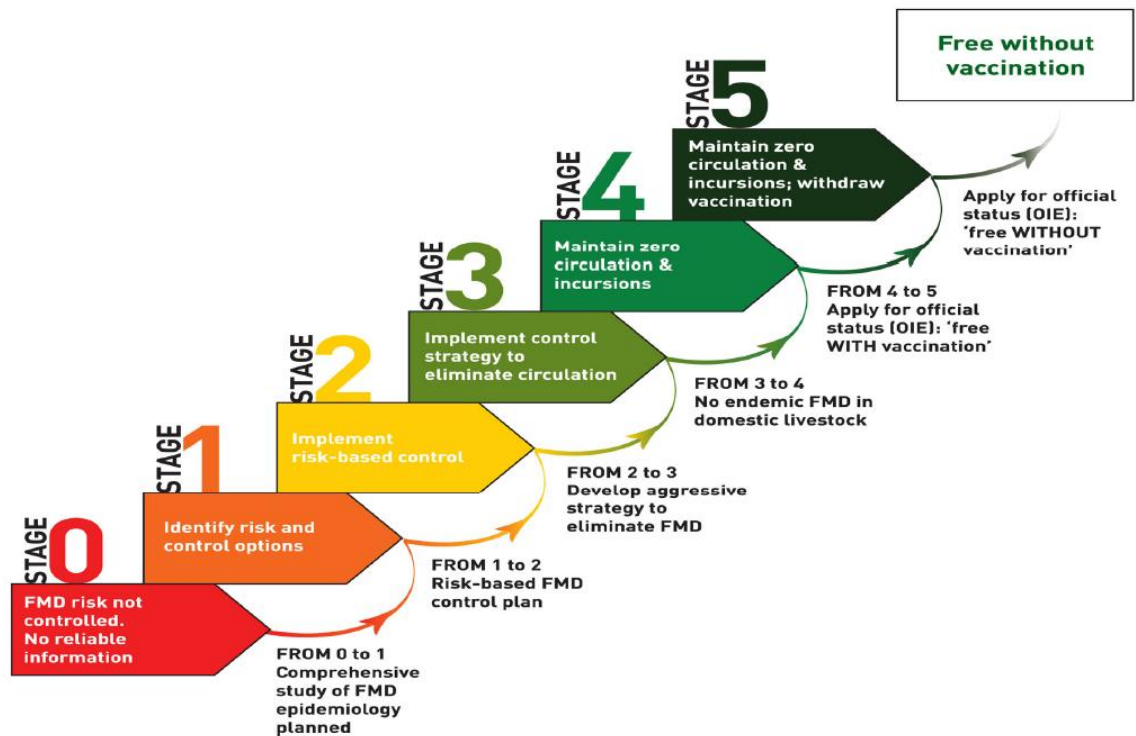


Figure 1.2. Stage progression in the Progressive Control Pathway (PCP). Source: FAO (2011)

1.3 FMD Vaccines

FMD vaccines have been produced on a large-scale since the 1940s (Barteling, 2002). The pioneering “Frenkel” method involved culturing virus in bovine tongue epithelium cells which was then inactivated with formaldehyde and adjuvanted with aluminium hydroxide. This was later replaced by the more efficient baby hamster kidney (BHK) cell line and inactivation with binary ethylenimine (BEI) due to reports of incomplete inactivation seen with formaldehyde leading to vaccine associated outbreaks. Saponin was added to aluminium hydroxide as an additional adjuvant, and oil-based vaccines became available with claims that they were more effective in pigs, induced more persistent titres and were better able to circumvent interference from maternally derived antibody (Cloete et al., 2008; Gomes, 1984; Gomes et al., 1980).

FMDV vaccines are now produced by at least 56 commercial and governmental institutions around the world (Mezzer, O., Vallée SA, Personal communication, 2014). All contain killed/inactivated virus or virus antigen with either aluminium hydroxide/saponin (known as “aqueous”) or oil adjuvant. The latter have been extensively used in South America. Most

companies use BEI inactivation although a minority continue to use formaldehyde. Various types of oil adjuvants are used including a double oil emulsion (otherwise known as a water-in-oil-in-water emulsion) (Barnett and Carabin, 2002). When used prophylactically in endemic settings, vaccines are often given twice in a primary course 4-6 weeks apart followed by boosters every 4-12 months depending on the species and epidemiological situation (Parida, 2009).

Vaccines often contain strains from more than one serotype or multiple strains within a serotype. As well as the epidemiological situation, the selection of a strain will also depend on its ability to grow in cultures, stability and antigenicity (Barteling, 2002). The 146S particle of the VP1 protein has been found to be the most important for antigenicity. Payloads of between 1 and 10µg per strain are usually used in each dose, although there is some variation between serotypes (Doel, 1996, 2003).

All FMD vaccines are purified to some extent to remove cellular debris that may cause adverse reactions and also to concentrate the vaccine. The latter is part of the process in producing “high potency vaccines” since potency is generally related to the antigen concentration. The OIE and European Pharmacopeia prescribe official vaccine potency tests (described and discussed in detail in Chapter 2) that are based on virus challenge into the tongue of experimentally vaccinated and non-vaccinated cattle. Serological correlates (also described in Chapter 2) are also extensively used particularly for licensing vaccines in South America in circumstances in which a correlation with protection has been demonstrated. A protocol has been adopted for pigs but no equivalent tests have been described for small ruminants or buffalo (Parida, 2009). The potency of a vaccine may be influenced by several factors including the antigen quantity, antigen quality, the particular strain used, adjuvant and vaccine schedule (Paton et al., 2005). High potency vaccines have been associated with a more rapid onset of protective immunity and a greater capacity for cross-topotypic protection compared to low potency vaccines (Brehm et al., 2008; Doel et al., 1994).

Vaccines may be further purified by removing non-structural proteins (NSP). Animals given a NSP-purified vaccine will usually not have antibodies present to these proteins unless they have been exposed to infection. This allows the use of NSP serology to distinguish vaccinated and exposed animals. Unlike the structural protein (SP) equivalents, for which serological tests are also available, NSP antigens are conserved across viral serotypes so NSP antibodies do not distinguish between serotypes. However, animals receiving many doses of a NSP-purified vaccine may still seroconvert to NSP. Therefore NSP serology is particularly useful in FMD-free

settings where typically only one or two doses of a NSP-purified vaccine are used. This may also be helpful for the detection of carriers since vaccinated animals may be infected and become a carrier without showing clinical signs although vaccines can decrease the risk of becoming a carrier particular in high antigen doses (Cox et al., 2005; Parida, 2009; Paton et al., 2006). However, where a non-NSP purified vaccine is used, multiple doses may be required in order to induce an increase in NSP titres (Sutmoller et al., 2003). NSP antibodies are measured through a blocking ELISA, whilst serotype specific SP antibodies are generally measured through virus neutralisation tests (VNT), liquid-phase blocking ELISA (LPBE), solid-phase competition ELISA (SPCE), or solid-phase blocking ELISA (SPBE).

Several problems with current FMD vaccines limit their effective use and warrant the evaluation of their effectiveness in the field. Close match of vaccine virus to that in the field is important to ensure effectiveness. Serological **vaccine matching** studies may be performed which can indicate likely effectiveness of a vaccine against a particular strain, but there are several limitations to this approach including uncertainty over the identity of the precise vaccine strain and standardisation of vaccine derived sera (Paton et al., 2005). Recently the sensitivity of conventional vaccine matching tests has also been questioned (Brito et al., 2014, discussed in Chapter 2). Effectiveness is also limited by the short **duration of protection**, estimated as only six months after an initial two dose regimen (Doel, 2003) although duration may be longer with a high potency oil adjuvanted vaccine (Cox et al., 2010). Given the high transmissibility of the virus, high levels of **coverage** are required for herd immunity (Paton et al., 2009). Passive **maternally-derived antibody** (MDA), passed to the neonatal calf through immunoglobulin-rich colostrum, is known to interfere with the vaccine response (Kitching and Salt, 1995). The half-life for FMD antibodies in the calf is estimated at around 22 days with “significant titres” persisting for up to six months in endemic settings (Nicholls et al., 1984). A “window of susceptibility” (otherwise known as an “immunity gap”) therefore exists between waning passive immunity and the ability to respond to the vaccine (Doel, 2003). Vaccine antigens degrade at temperatures above 8°C and the **cold chain** is often difficult to maintain in resource poor countries where FMD is endemic (Garland, 1999). There is also evidence that the antibody response post vaccination varies with **breed** with one recent study showing lower responses in Jersey sires’ offspring compared to Holstein (Di Giacomo et al., 2013).

Despite these problems, vaccines are used extensively to control FMD. It is estimated that over two billion doses of vaccine are used globally each year mainly in China, South America, India and the Middle East (Paton et al., 2009). This includes government subsidised mass vaccination

as part of national control strategies, “ring” (or “reactive”) vaccination in response to outbreaks to reduce impact and spread, and use by individual farmers as prophylaxis in case of exposure. With the considerable use of a problematic vaccine, rigorous evaluation is needed.

1.4 FMD in Kenya

1.4.1 Agricultural background

Like most of East Africa, Kenya’s economy is dominated by the agricultural sector. According to the World Bank in 2012 the value added % of GDP for agriculture was 30% (Source: <http://data.worldbank.org/>, accessed 2nd October 2014). Milk is the most important livestock product, representing around 70% of the total gross value of livestock’s contribution to the agriculture sector and four times more important than meat on a GDP basis. National milk output from cattle has been estimated to be 5.8 billion litres per year (Behnke and Muthami, 2011). Most of the dairy cattle population is in the western part of Kenya, in the southern part of the Rift Valley (Figure 1.3). Smallholder farmers are estimated to produce over 70% of the national milk supply. These farmers are generally based on 1.2-2.0 hectare plots of land, own 2-5 cattle and yield around 5kg milk per cow per day (Muriuki, 2011). The remaining is supplied by large-scale farms that - although they contribute relatively less milk nationally - are less prone to seasonal fluctuations in production mainly due to the greater capacity for storage of fodder. It is estimated that 70% of national milk output is produced from “grade” cattle (i.e. pure-breed European dairy cattle or their crosses with indigenous breeds) making up almost all marketed milk (Muriuki, 2011).

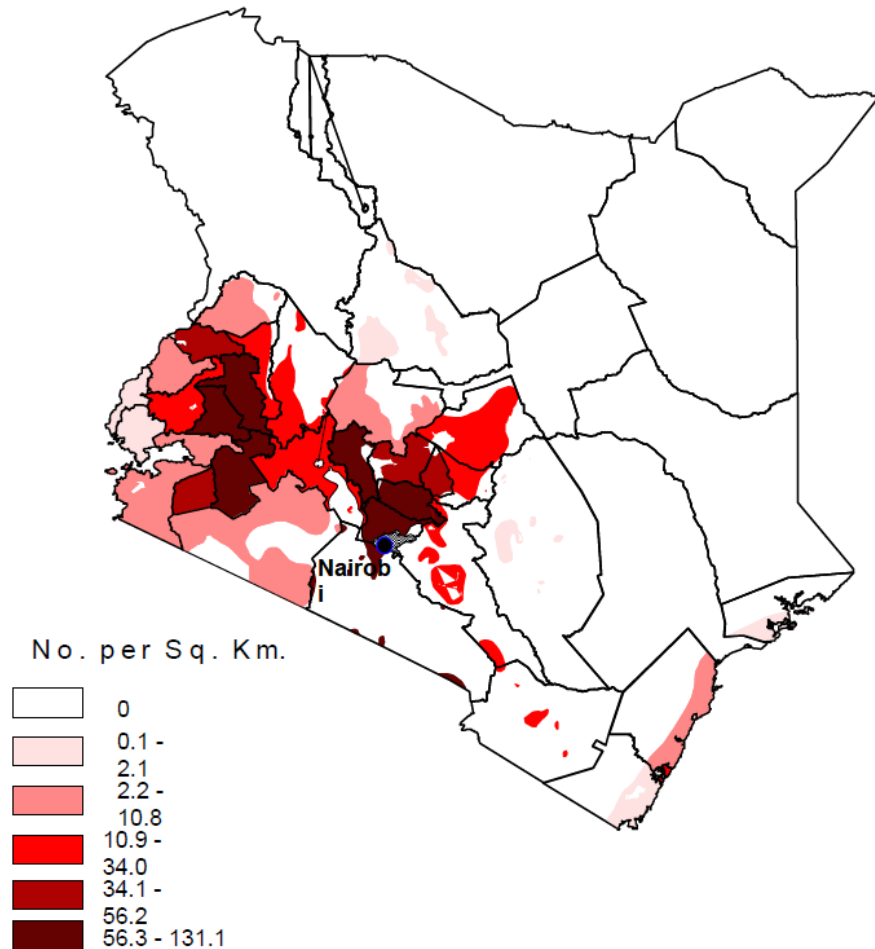


Figure 1.3. Dairy cow density in Kenya. Source: Omore et al., (1999) <http://ilri.org/infoserv/Webpub/fulldocs/InvestingInDairy/DOX/>, accessed 2nd October 2014.

1.4.2 FMD occurrence and control

FMD is endemic in Kenya with O, SAT1 and SAT2 serotypes in apparent constant circulation with occasional outbreaks of serotype A. Serotype C was last isolated in 2004 (Sangula et al., 2011). The FMD susceptible population is mainly small ruminants although a large number of cattle are also present (Figure 1.4). Surveillance is mainly passive with reports made from farmers to local animal health assistants (private or government-employed), private veterinary practitioners, or local government veterinary officers. The farm is then visited by a government veterinary officer to confirm that the suspicion is consistent with FMD and a sample is taken and submitted to the National FMD Laboratory in Embakasi, for diagnosis and serotype testing by antigen (Ag)-ELISA. A recent review of laboratory capacities for FMD in East Africa shows

Kenya as being the best equipped in the region having the ability to do Ag-ELISA, Complement Fixation Tests (CFT), PCR and virus isolation and to serve as a laboratory to several other East African countries. Available serological assays include VNT, LPBE and NSP-ELISA (Namatovu et al., 2013a).

Figure 1.5 shows the number of confirmed FMD outbreaks and the serotype detected by the national FMD laboratory over a ten year period. This does not include the number of *unconfirmed* outbreaks and as surveillance is mainly passive, there is likely to be underreporting. However, the relative numbers of different serotypes is likely to reflect actual patterns. FMD is considered to be associated with the dry seasons (normally January-March and July-September) coinciding with increased movements of pastoralist cattle searching for grazing. Information gleaned from veterinarians working in Kenya suggests FMD is present at all times of the year with a higher incidence during the dry seasons.

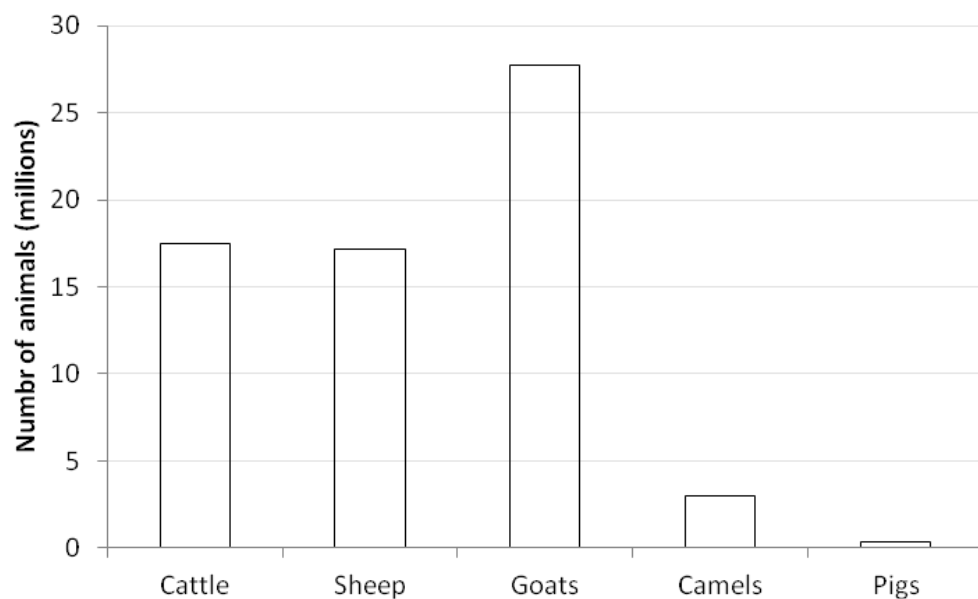


Figure 1.4. Population of different FMD susceptible species in Kenya created from the 2009 agricultural census. Source: <https://www.opendata.go.ke/>. Accessed 2nd October 2014.

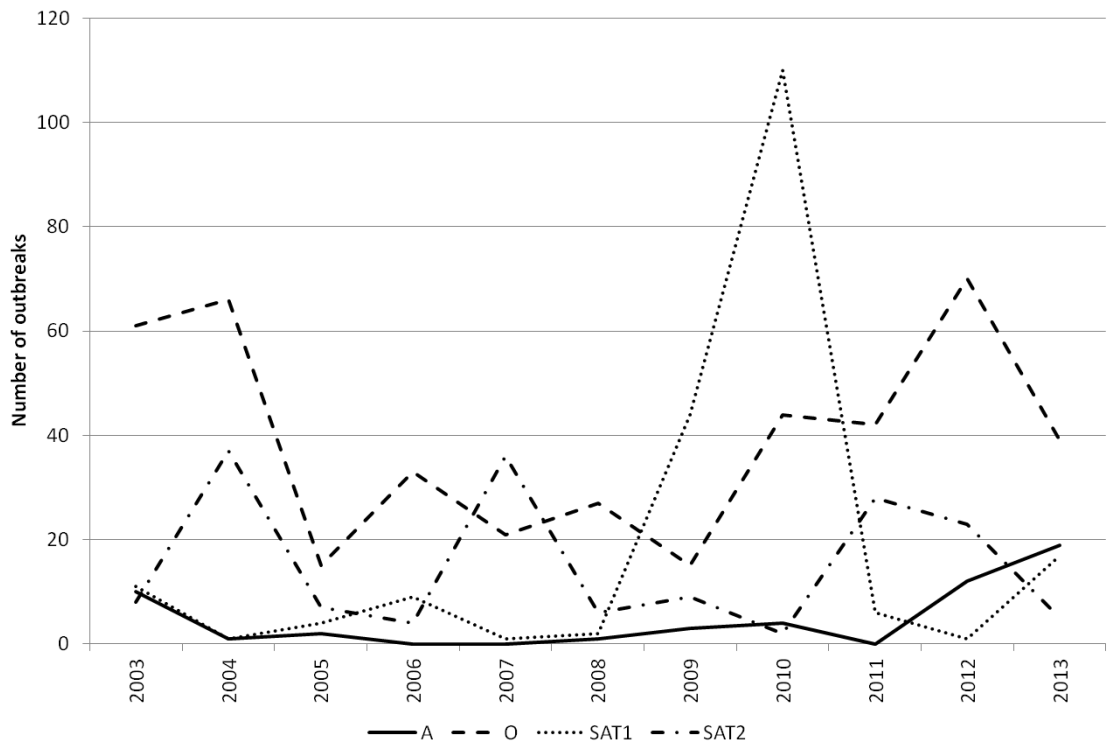


Figure 1.5. Trends in FMD serotypes detected in Kenyan outbreaks from 2003 to 2013 inclusive. Serotype C not included (one report only from 2004). Source: Kenyan FMD laboratory, Department of Veterinary Services.

FMD is a notifiable disease in Kenya and so control is driven by the government through the Department of Veterinary Services (DVS). In 1967/1968, a plan for disease-free zones in Nakuru and Laikipia was created and successfully implemented. Through the regular use of mass vaccination and strict movement controls, meat exports to Europe were possible in the 1970s. A lack of resources and higher demand for meat within Kenya led to this export status being eventually lost and it has not since been regained (Rweyemamu, 1984a). Current methods employed in Kenya include movement restrictions, market closures and ring vaccination (Namatovu et al., 2013a). The only licensed FMD vaccines in Kenya are produced by the government owned Kenya Veterinary Vaccines Production Institute (KEVEVAPI) and include quadrivalent (O, A, SAT1, SAT2), trivalent (O, SAT1, SAT2 or O, A, SAT2) and monovalent vaccines. The current vaccine is aqueous adjuvanted (aluminium hydroxide and saponin) and not NSP-purified. According to government sources, KEVEVAPI produces around 15 million monoequivalent doses of vaccine annually, with around 7 million used in Kenya.

Evaluation is through the PD₅₀ assay as recommended by the European Pharmacopeia and OIE (OIE, 2009).

Among *smallholders*, vaccination is typically limited to government-subsidised ring vaccination in response to a confirmed outbreak. This involves offering farmers a single dose of subsidised vaccine per animal to areas surrounding a confirmed outbreak after a period of publicising the dates and locations. Vaccination usually takes place at a communal handling facility such as a cattle dip or on a farm that has a working crush. Alternatively vaccination teams will move from farm to farm offering vaccine. Usually only cattle are included in these campaigns. Data on vaccination use is relayed back to the central DVS where estimates on coverage are made based on the doses used and the 2009 animal census figures for the area. This vaccination policy is dependent on vaccine and finance availability and consequently vaccine coverage is typically low estimated by veterinary officers at around 10%. Larger *commercial farms* often employ prophylactic vaccination, which may be supplied through the government at a subsidised rate, but often is sourced directly from the manufacturer at an unsubsidised cost. This is in part because of a lack of certainty over vaccine availability through the local government and distrust of government cold chain practices.

In some parts of Kenya, in particular the Central province, the Kenyan Veterinary Association (KVA) has organised vaccination campaigns passing discounts associated with bulk vaccine purchase on to the farmer. However, in these campaigns FMD is considered alongside other diseases including Lumpy Skin Disease (LSD) and Clostridial disease, meaning FMD vaccine is not included in all areas. Records of vaccinations are not routinely kept at the individual animal level.

As part of a government campaign, “Kenya Vision 2030”, there are now plans to set up five disease free zones to enable export of animal products to disease free countries (Source: <http://www.vision2030.go.ke>). FMD is one of the target diseases and the campaign specifies improvements in vaccination as an important strategy. FMD vaccine evaluation is therefore timely for Kenya.

1.4.3 FMD research in Kenya

1.4.3.1 Phylogenetics

Several research studies have been carried out and published related to FMD in Kenya. The majority of studies focus on the phylogenetic analysis and characterisation of isolated virus

strains with possible implications for vaccination. Two studies looking at serotype O isolates from 1964 to 2011 revealed four topotypes to have been present in Kenya during this time (East Africa [EA]-1, EA-2, EA-3 and EA-4). The vaccine strain is based on an EA-1 topotype strain. Of the 66 viruses sequenced in these two studies, a EA-1 topotype has only been detected on one occasion (in 2007) since the year 2000 raising concerns over the potential for the vaccine to be effective in the field (Sheila N. Balinda et al., 2010; Wekesa et al., 2013). A recent analysis of 38 serotype A strains collected between 1964 and 2013 showed five lineages within the African topotype (two of which now appear extinct) all having a countrywide distribution (Wekesa et al., 2014b). This study also raised concerns over the similarity of more recent isolates with the vaccine strain currently in use having an average difference in VP1 sequence of 13% for viruses isolated between 2003 and 2013. One study of serotype C suggested that given the limited nucleotide diversity of samples taken over 40 years it is possible that re-introductions of improperly inactivated vaccine strains may have been responsible for the outbreaks (Sangula et al., 2011).

A study of SAT1 isolates from East Africa suggested that of two independent groups of strains introduced from South Africa those seen in Kenya and Tanzania were similar enough to suggest transboundary exchanges of virus from movements of livestock or wildlife (Sangula et al., 2010a). Several studies have analysed sequences of SAT2 throughout sub-Saharan Africa. One of these focussed on isolates from Kenya and found two distinct lineages to be present between 1957 and 2007 (Sangula et al., 2010b). Again there have been concerns over the vaccine since only one SAT2 strain from one of these lineages is present in the vaccine with unknown cross protection capacity.

Di Nardo et al. (2011) related the virus topotype distributions to livestock movements and concluded that the diversity in ecosystems and the adaptation of pastoralists to seasonal trends in climate and grazing are important determinants in the distribution of the viruses and may bring cattle into contact with susceptible wildlife. Cross-border movements of animals are also important for FMD circulation with large numbers of animals moving from neighbouring Tanzania and Ethiopia to Nairobi, where higher prices can be obtained.

1.4.3.2 Serosurveys

Several serosurveys have been published on different species in Africa. A report by Paling et al. (1979) described clinical signs and positive neutralising titres in domesticated eland (*Taurotragus oryx*) on a large ranch in south east Kenya, likely due to SAT2 as this was isolated

from clinically affected cattle on the same ranch at the same time. Virus could still be isolated from the cattle 10 months after the outbreak but not from buffalo (*Syncerus caffer*) and eland, although for the latter two species low numbers of samples were taken.

A later study by Bronsvort et al. (2008) determined the seroprevalence of NSP and SP antibodies in wildlife that had contact with livestock populations in parts of Eastern and Central Africa (including Kenya). Sera were collected from 27 different species between 1994 and 2002 as part of Rinderpest surveillance. Of the 731 samples taken, 483 were from African buffalo (*Syncerus caffer*). VNT for SP antibodies were measured in these sera for all three SAT serotypes. 327/483 (67.7%) of the buffalo were positive to NSP antibodies compared to 11/248 (4.4%) in the non-buffalo species demonstrating the relative importance of buffalo. Age stratification of the results from the buffalo indicated over 60% seroconversion by the age of nine months indicating early lifetime exposure. The age distribution was closely matched by the VNT result for SAT2.

A serosurvey in the “Somali Eco-System” was performed utilising 499 samples collected from cattle over a two year period beginning in January 2007 (Chepkwony et al., 2012). These had been collected as part of the final evaluation of the Rinderpest eradication programme. VNT for SP antibodies were performed to five serotypes (O, A, C, SAT1, SAT2). 226/499 (45.3%, 95%CI 41.0, 49.7) samples were seropositive to one or more serotypes with serotype O being the most common (in 118/499 [23.6%] of samples). Similar prevalences were found for serotypes A, SAT1 and SAT2 at around 15% each although it was significantly lower for C at 1.6%. Seropositivity was significantly different for age categories and appeared to show a linear increase (Table 1.2). There was no significant difference in seropositivity between sexes. No vaccination campaigns had been recorded as having taken place in this region.

A nationwide cross sectional study of cattle was performed in 2010 by Kibore et al. (2013). They tested 3,709 samples from 39/47 counties taken over a two month period for NSP antibodies. Overall 1947/3709 (52.5%) were positive similar to that reported by Chepkwony et al (2012) although NSP seroprevalence by age was not presented (Table 1.2). A sub-selection of NSP-positive samples (n=738) was analysed by LPBE on which a risk factor analysis was based (Table 1.2). In this latter analysis, animals were defined as positive if seropositive to one or more serotypes by LPBE. Limited inference can be made from this analysis because only NSP positive samples were chosen and no trend was analysed between the number of serotypes to which each age category was positive (only zero or ≥ 1). Risk factor analysis of NSP seropositivity would have been more appropriate but was not possible from the data

presented. Additionally, insufficient data and details on study design were presented to understand the geographical distribution of seropositivity and the implications of using a non-purified vaccine were not discussed. An opportunistic NSP serosurvey of pigs was also conducted alongside this cattle serosurvey (pigs only being sampled if found at the randomly selected cattle sites) which revealed 101/191 (53%) to be positive despite no history of clinical disease (Wekesa et al., 2014a). Those positive for NSP antibodies were also tested for serotype specific antibodies using SPBE and VNT which revealed SAT1 to be the causative serotype that was also prevalent during cattle at the time. Some of the pigs had been vaccinated but a history of vaccination was not associated with seropositivity consistent with the consensus that aqueous based vaccines are ineffective in this species.

Table 1.2. Summary of recent serosurveys in Kenya analysing seroprevalence by age category in cattle.

Study	Test	Age category	Seropositive	Total	Percentage (95% CI)	P-value	
Chepkwony et al, 2012	VNT	1-2 years	111	290	38.3 (32.7-43.9)	<0.001	
		2-3 years	107	199	53.8 (46.8-60.7)		
		3-4 years	8	10	80.0 (51.2-98.5)		
		Total	226	499	45.3 (41.0-49.7)		
Kibore et al, 2013	NSP	All animals	1947	3705	52.5 (50.0-55.0)		
		LPBE ^a	<1 year	181	227		79.7
			1-2 years	157	203		77.3
			>2 years	253	308		82.1
			Total	591	738		80.1

Note: For VNT and LPBE, animals were defined as seropositive if positive to one or more serotypes. The survey by Chepkwony et al (2012) was performed in the Somali Eco-System whereas the study by Kibore et al (2013) was nationwide. ^a LPBE were performed on a subset of NSP positive samples.

1.4.3.3 Economics

Two papers have presented case reports on the economic impact of FMD on large-scale dairy herds in Kenya (Kimani et al., 2005; Mulei et al., 2001). Using participatory methodology among pastoralists in Narok, Onono et al. (2013) scored East-Coast Fever (ECF) and FMD to be the highest ranking diseases in terms of impacts on production. FMD was reported to be the disease most frequently encountered in cattle of those discussed. These studies are considered in more detail in Chapter 5 and Chapter 6.

1.4.3.4 FMD vaccination in Kenya

Immune response to the locally available quadrivalent vaccine was assessed by Berger et al. (1975). In two different field trials they investigated whether there was a difference in the response to SAT serotypes if included in polyvalent vaccines. In the first trial, 46 Friesian or Ayrshire cattle aged between 20 and 36 months were vaccinated at zero, three and 27 weeks. In the second, six groups of 20 Boran steers were given a single dose of vaccine containing different serotype combinations. In both trials, animals were periodically blood sampled and tested by VNT to the vaccine strains and mean titres over time were presented. They concluded that polyvalent vaccines with SAT serotypes produced satisfactory levels of immunity, although three doses were needed for the SAT1 vaccine and there was some evidence that a better response is seen to SAT2 when used in monovalent form. The SAT1 strain is the only one that is still included in the current vaccine.

Evidence for field effectiveness of FMD vaccines in Kenya is limited. In the aforementioned study by Paling et al. (1979) (section 1.4.3.2), an outbreak of SAT2 occurred among cattle despite biannual vaccination although no details were given to sufficiently appraise the vaccine effectiveness. Ndiritu et al. (1983) later published the results of vaccine matching tests which compared nine SAT2 field isolates to four vaccine viruses to identify the vaccine strain with the broadest serological spectrum for inclusion in the vaccine.

Anderson et al (1974) compared the prevalence of carrier cattle in vaccinated and non vaccinated areas with no recent outbreak history. The former were sampled at slaughter in the abattoir whilst for the latter probangs were performed on live animals. The prevalence of 3.3% in the non vaccinated area (n=2,219) and 0.49% in vaccinated areas (n=1,231) led the authors to conclude that carrier cattle were not a significant risk to virus elimination in routinely vaccinated areas.

Although primarily focussed on the treatment of affected animals, one study did report an outbreak on a cattle dairy farm that affected 95/166 (57.2%) of animals over a 21 day period despite using prophylactic vaccination three times a year (Gakuya et al., 2011). The most recent vaccination had occurred four days before the outbreak began and was the locally produced quadrivalent vaccine (A, O, SAT1, SAT2). Ag-ELISA revealed the serotype to be SAT1 and VP1 sequencing at the World Reference Laboratory (WRL), Pirbright, showed it was 10.56% different to the vaccine strain. There were no results of any vaccine matching tests. Data on the number of animals affected per day and the age category they belonged to (>2 years, 1-2 years and <1 year) were presented, but no denominators were presented; thus it is not possible to calculate incidence by age or number of vaccine doses received. However, most of the cases were in animals over two years of age (66/95, 69.5%). An epidemic curve created from these data is shown in Figure 1.6. Given the dissimilar VP1 sequences, it is unlikely that the vaccine was the source of the virus but this study does present evidence of a lack of vaccine effectiveness for the SAT1 serotype.

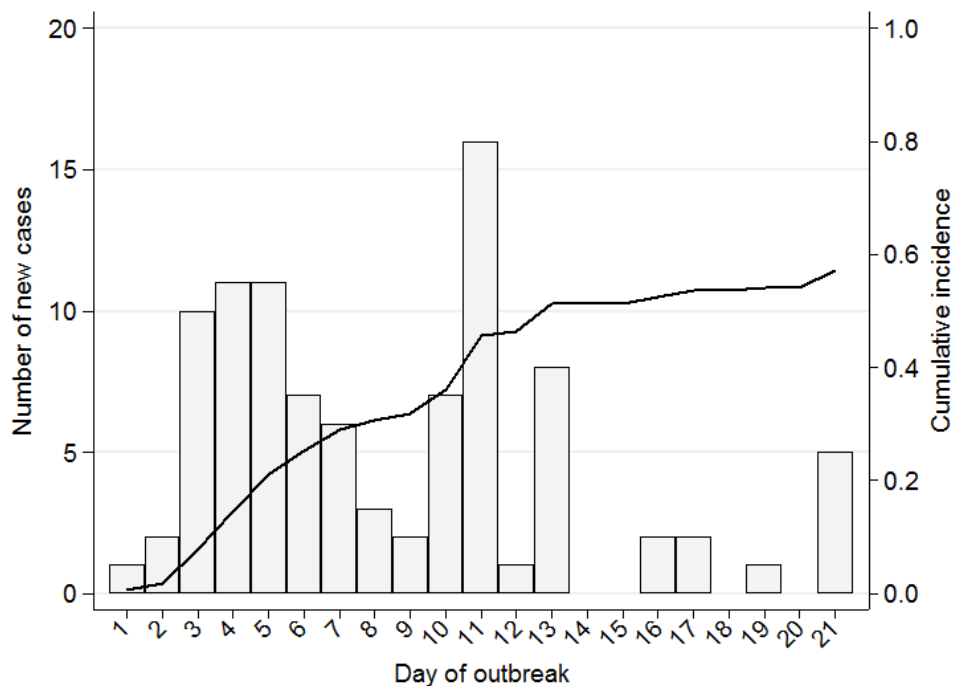


Figure 1.6. Epidemic curve and cumulative incidence over time, taken from the data presented by Gakuya et al. (2011) on a farm that had an outbreak of FMD due to SAT1 four days post vaccination.

Similarly a study by Mulei et al. (2001), that focussed on the economics of a FMD outbreak on a commercial dairy farm in Kenya, reported an outbreak due to serotype O affecting 123/207 (59.4%) of cattle despite vaccinating 104 days earlier. Vaccine schedules and animal ages were not given, but from the outbreak description, the incidence tended to be higher in adults affecting 20.8% of lactating cows compared to less than 5% in weaned and suckling calves.

1.5 Vaccine effectiveness

Vaccines may have direct and indirect protective effects. Direct protection refers to effects in vaccinated animals themselves. Indirect protection is a population level effect whereby vaccination reduces transmission and risk of exposure (and therefore infection or disease) irrespective of whether an individual has been vaccinated or not. Vaccines may also protect against infection, infectiousness or disease. Different study designs can be utilised to compare different populations or subpopulations depending on the specific aspects of protection that are being analysed (Halloran et al., 1999, 1997).

Vaccine **efficacy** is defined as “ $1-RR$ ” where RR is the risk or rate ratio which is the incidence in the vaccinated divided by the incidence in the (similarly exposed) unvaccinated populations. This is measured under controlled trial conditions to ensure equal exposure of the vaccinated and unvaccinated groups. Other assumptions include random administration of the vaccine and a homogenous population with all individuals equally susceptible to infection. These studies are typically done through randomised controlled trials (Smith et al., 1984).

Vaccine **effectiveness** has an identical calculation but is measured in field conditions and is therefore more reflective of “real-life” conditions. Vaccine efficacy studies are usually carefully performed and can be considered performance under “ideal” conditions that are unlikely to be possible in routine programme conditions. Alternatively, the vaccine effectiveness estimate reflects the effectiveness of a vaccine *programme* at a farm, regional and/or national level. Vaccine effectiveness therefore has important implications for policy. The study population is heterogeneous and therefore calculations usually need to be adjusted for potential confounders such as age, sex and previous exposure. These studies are typically observational using a cohort or case-control approach (Smith et al., 1984), although cluster randomised trials may also be used whereby the herd immunity effects of vaccination can be assessed (Clemens et al., 2011). Effectiveness studies are an essential component of post-licensure monitoring in human vaccines.

In human vaccinology, these evaluation issues are widely accepted but among veterinary vaccines these terms have not been standardised (Knight-Jones et al., 2014b). Vaccines in veterinary species are extensively used, including for FMD, and in many different species. Despite this, vaccine effectiveness studies are rarely performed for veterinary vaccines, and vaccine evaluation tends to rely on experimental challenge or seroconversion. These assessments do not account for the heterogeneity of field conditions and therefore extrapolating their outcomes to a wider population is uncertain. Additionally, there is a tendency for veterinary vaccine manufacturers to not publish fully the results of their trials which for ethical reasons are made available to national control agencies such as the National Institute for Biological Standards and Control (NIBSC) in the UK or Food and Drug Administration (FDA) in the USA for human vaccines. As a result, there is a great need to carry out rigorous field based evaluations of veterinary vaccines, especially when public money subsidises their use and where diseases have implications for human health, household income and animal welfare. This is particularly pertinent in developing countries where agriculture is so important.

Despite the importance of FMD to Kenya and the East African region and the not-insignificant investment in vaccination, no studies evaluating the field performance of the vaccines have ever been published.

1.6 Overall aim of thesis

The overall aim of this thesis is to gain a greater understanding of FMD field epidemiology, evaluate the performance of FMD vaccination in Kenya, and to measure the impact of disease among Kenyan dairy cattle.

Objectives:-

1. To conduct a review of the literature on current FMD evaluation methods
2. To gain experience of FMD in the field and gather data on its epidemiology
3. To find outbreaks of disease that have occurred on farms that have used recent vaccination and perform vaccine effectiveness studies
4. To use farm records on production parameters to estimate the impact of disease
5. To use recent post-vaccination serological data from the vaccine manufacturer to complement the epidemiological studies

1.7 Structure of the thesis

This thesis is a research paper style thesis that has three complete papers (Chapter 4, Chapter 5, Chapter 6), and two additional non-published results chapters (Chapter 7 and Chapter 10). For consistency in style, the latter two have also been written in the style of research papers. Chapter 2 is a literature review on FMD vaccine evaluation which leads onto Chapter 3 which gives details of the research questions and specific objectives of the thesis including the field work activity.

There are two parts to the thesis. Part A is on epidemiology, and Part B is on serology.

Part A presents the analyses of two outbreaks of FMD on large-scale dairy farms that used routine prophylactic vaccination. The first outbreak is described in Chapter 4 which includes an evaluation of the vaccine and has been accepted for publication in *Acta Tropica*. Chapter 5 and Chapter 6 use this same outbreak to describe the impact on clinical mastitis, culling and milk yield and include separate literature reviews on these subjects. Both of these chapters have been submitted for publication. The second outbreak is similarly described in Chapter 7, and a separate discussion comparing the two outbreaks is presented in Chapter 8.

Part B of the thesis evaluates serological data made available from the vaccine manufacturer in the light of the epidemiological findings presented in Part A. A review on the impact of maternally derived antibody on the response to vaccination is presented in Chapter 9 followed by the analysis in Chapter 10.

An overall discussion of both parts of this thesis is presented in Chapter 11.

Chapter 2. Literature review 1 – FMD vaccine evaluation

The objective of this review is to provide a comprehensive background on FMD vaccine evaluation methods. Only commercially available vaccines and their application in cattle were considered. A search of the literature in the English language was performed in Pubmed/Medline and Web of Science using the search terms: (fmd OR foot-and-mouth) AND (bovine OR cattle OR cow) AND (vaccine OR vaccination). Of the 241 articles initially found, a screen based on the titles and abstract refined these to 62 articles that were found relating to the evaluation of vaccines in individual or populations of cattle. The OIE Terrestrial Manual was also included. On the basis of these results, the following areas were reviewed: potency testing, vaccine matching, serological assessments, field evaluations and mathematical modelling.

2.1 Potency tests

Evaluation of FMD vaccines is performed mainly through experimentally infecting vaccinated animals in a controlled setting in the context of a “potency test”. Two types of potency test are outlined in the OIE *Terrestrial Manual* (OIE, 2009). The first of these is also the recommended European Pharmacopeia (EP) test that provides a 50% protective dose (PD₅₀) value. Naïve or vaccinated cattle over six months of age are challenged by intradermolingual (IDL) virus inoculation, 21 or 28 days (for aqueous- or oil-based respectively) post vaccination with the homologous strain. Groups of five animals are vaccinated with either a full dose of vaccine, 1/4 dose or 1/10 dose to determine the PD₅₀ value. Animals are observed and examined for eight days with protection defined as no observable foot lesions. At least two statistical methods are employed for calculating the PD₅₀ value. The EP suggests maximum likelihood methods (probit analysis and logistic regression) while the OIE prefer the Spearman-Kärber statistic in assessing the dose response relationship (Reeve et al., 2011). Vaccines are recommended to contain at least 3 PD₅₀ per dose when used “for routine prophylactic use”, although 6 PD₅₀ is preferred (OIE, 2009). The other OIE approved test is the “Protection from Podal Generalisation” (PPG) which is commonly used in South America. A full dose of vaccine is given to 16 cattle that are IDL-challenged at least four weeks post-vaccination. A minimum of 12 animals must show protection for the vaccine to be approved for field use. In both tests, summarised in Table 2.1, two unvaccinated control animals must show generalised disease for the test to be declared valid. Vianna Filho et al. (1993) collated data from two different laboratories in Brazil and Argentina for three serotype (O, A, C) comparing the percentage of protection with the PD₅₀ of

the vaccine as measured by potency tests. These data are represented in Figure 2.1. Although still based on challenge studies, this graph does demonstrate the expected increase in clinical protection with increasing PD₅₀ value which appears to be a repeatable trend based on the small confidence intervals and using data from more than one laboratory.

Table 2.1. Summary of potency tests as recommended by the OIE (OIE, 2009).

Potency test	Number of animals ^a	Dose	Challenge (dpv ^b)	Follow up (dpc ^c)	Pass-mark
50% protective dose (PD ₅₀)	17	1/10, 1/4, full dose (5 animals per dose)	21 (aqueous-), 28 (oil-adjuvanted)	8 days post challenge	≥3.0
Protection from Podal Generalisation (PPG)	18	Full dose	30	8 days post challenge	12/16 protected

^a includes two unvaccinated control animals. ^bdpv = days post vaccination ^cdpc = days post challenge

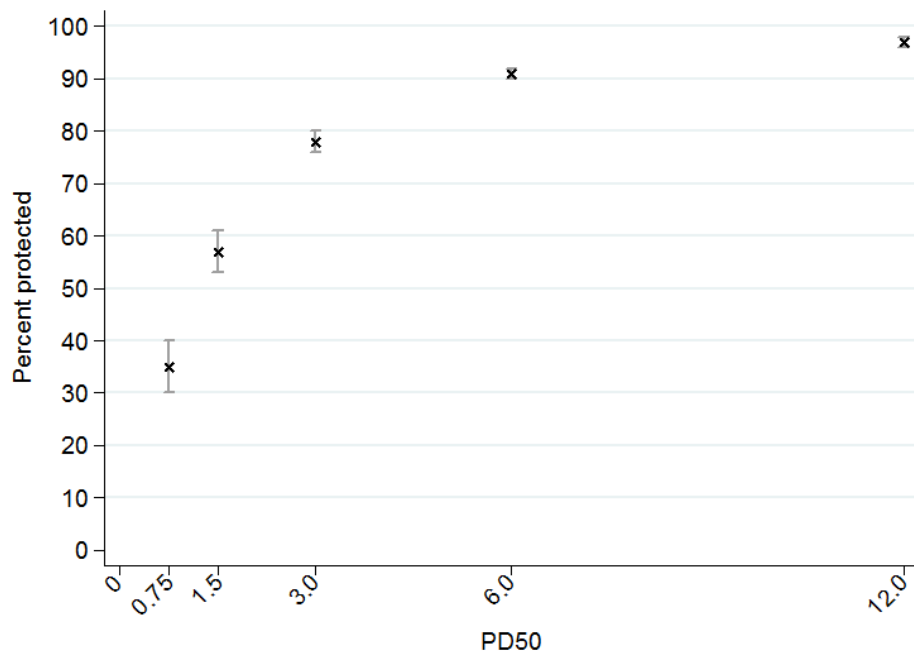


Figure 2.1. Percentage protection (with 95%CI) with different PD₅₀ levels of vaccine collated for serotypes O, A and C based on the data presented by Vianna Filho et al. (1993), Table 5.

A major limitation of potency tests is the small number of animals used leading to high degrees of statistical uncertainty. Computer simulations of potency tests have explored this uncertainty and indicated that the PPG test provides a more reliable estimate of potency than PD₅₀ calculations. A particular problem noted by Suttmoller (1986) with the PD₅₀ calculation is that the standard error becomes unacceptably high where the dose-response curve is flat. Using their PPG test simulation models, they found that if a vaccine actually protects 90% of recipients, a PPG “pass-mark” of 12/16 will approve 99% of vaccine batches. If the vaccine actually protects 70% of recipients, using this same pass-mark would approve 55% of batches. The authors note that there is a compromise between producing vaccines acceptable to the livestock industry and the cost of the tests to the manufacturers.

A more recent study reported computer simulations using archived data from 1,644 challenged individuals (Reeve et al., 2011). Based on their findings, they suggested modifying the potency tests to two groups of six animals, one receiving a 1/3 of the usual full dose of the vaccine and the other 1/6 of the dose, combined with two unvaccinated controls. They found that this regime performed better than the EP recommended PD₅₀ test at determining if a vaccine was above the 3PD₅₀ or 6PD₅₀ threshold. It was also comparable to the OIE tests for both PD₅₀ and PPG calculations despite using fewer animals (16) compared to the usual 17 or 18 with benefits to cost and animal welfare. The same study also suggested that the OIE preferred statistical method was superior to those suggested by the EP.

The issue of poor precision in the PD₅₀ calculation was assessed by Goris et al. (2007) who analysed data from 10 potency tests with the same vaccine for the O₁ Manisa strain. When combining the animals from all tests together, the overall PD₅₀ value was 10.0 (95%CI 7.5-13.3). However the results of the individual trials varied from 4.6 to 24.3. To put this in context, two of the vaccine trials had a PD₅₀ result of 6.0 but with 95% confidence intervals of 2.8-15.5 and 3.1-13.7 reflecting the uncertainty in the point estimate. They also showed that there was a 32.4% (95%CI 27.9, 36.8) probability that two animals in the same trial receiving the same dose of vaccine would have different protection level (defined as “vaccine accordance”). The between-test variability was higher with a 58.8% (95%CI 54.8-63.1) probability that animals receiving the same dose would have the same result in a different trial (defined as “vaccine concordance”).

The same research group later assessed the precision of the PPG test, analysing the results of six identical trials using a serotype A stain vaccine challenged with homologous virus (Goris et al., 2008a). Combining the results, overall 88.5% (95%CI 80.7-93.5) of vaccine recipients were

clinically protected ranging from 75 to 100% between tests. The vaccine accordance and concordance levels (using the same definitions as in the preceding paragraph) were 75.9% (95%CI 64.9-86.2) and 73.7% (95%CI 62.1-84.3) respectively. Although the results of a single PPG test was more reliable than a single PD₅₀ test, only one of the six PPG trials had a lower limit of the confidence interval above the 75% cut-off, this being where all 16 animals were protected. The authors suggested that the number of animals should be increased to provide greater statistical evidence although they considered that this might be “unfeasible and unethical from an animal welfare point of view”. Therefore they suggested that because PPG retrials were required for vaccine authorisation in Argentina, these PPG results should be combined to narrow the confidence intervals of the estimates.

Large numbers of potency test results may be combined to give a more complete account of the expected protection from a vaccine when cattle are experimentally challenged. Vianna Filho et al. (1993) combined the analysis of 658 PD₅₀ tests for serotypes A, C or O from two different institutes in Argentina and Brazil using a hydroxide-saponin adjuvanted vaccine. Analysis of the percentage of animals protected from clinical disease revealed a strong association with the PD₅₀ value ($P < 0.001$). In the groups receiving the undiluted (i.e. full) dose, 78% (95%CI 76-80) and 91% (90-93) of individuals were clinically protected for vaccines of 3.0 and 6.0 PD₅₀ respectively (Figure 2.1). However, they failed to find a “satisfactory” dose-response relationship in 145 (22%) of the potency tests performed meaning a reliable estimate could not be made, highlighting the limitations of relying on one test in evaluating a vaccine. The same study presented data from 65 PPG tests for three batches of hydroxide-saponin (n=17, n=8, n=13) and two batches of oil-based (n=20, n=7) vaccine. Except for one of the aqueous adjuvanted batches that had one test markedly different from the others (11/15 protected versus all protected in the twelve other tests), they found all results were significantly associated ($P < 0.05$) and on this basis recommended the PPG test for evaluating FMD vaccines due to greater reproducibility of the results and the absence of a dose-response requirement as with the PD₅₀ calculation (Sutmoller, 1986).

Jamal et al. (2008) combined the results of 297 potency tests performed from 1965 to 2006 to determine the relationship between the PD₅₀ level and the percentage of animals clinically protected when challenged with homologous virus. A variety of strains were used covering four FMDV serotypes (A, C, O, Asia-1). Aluminium hydroxide, aluminium hydroxide with saponin, and double oil emulsion adjuvanted vaccines were also compared. They found that there were differences seen in the relationship depending on the serotype and the adjuvant

used and that “no common level of protection can be assigned to all FMD vaccines with the same amount of PD₅₀ per dose” and so advocated alternative tests for evaluating FMD vaccines.

A meta-analysis of challenge studies was performed by Halasa et al. (2011) looking at clinical and virological protection defined as not seroconverting to NSP from IDL challenge or exposure to an infected animal in an experimental setting. Animals had been vaccinated once with a NSP-purified, high potency (>6.0 PD₅₀) vaccine as recommended to be used in an emergency setting. Serotypes O, A and Asia-1 were included. The pooled relative risk for clinical FMD comparing vaccinated and non-vaccinated individuals was 0.13 (95%CI 0.09-0.18) whilst for FMD infection it was 0.71 (0.59-0.85). No difference was seen between serotypes.

2.2 Serological assessments

The inherent variability of potency tests due to the low numbers of animals used, costs, compromised animal welfare and risks associated with facilities using live virus, have led to the search for alternative correlates so that serological tests can be used to evaluate vaccines in line with the “Replacement, Reduction and Refinement” principles of animal experimentation (Flecknell, 2002).

2.2.1 Surrogates and correlates of protection

Specific immune markers have been used for many diseases to reflect the level of protection to clinical disease when exposed to infection. Such a marker may have a direct role in conferring immunity or have no specific role but be indirectly correlated. The former is known as a “*surrogate*” of protection whilst the latter is a “*correlate*” of protection according to WHO terminology (Nguipdop Djomo et al., 2012), although only the latter term is generally used for FMD.

When considering a surrogate or correlate of protection induced by vaccine, correlations are typically sought between the use of vaccine and the marker as well as the marker with clinical protection. The overall protective effect of the vaccine may then be assessed by bypassing the marker phase. Vaccine-induced markers may have different relationships to protection compared to natural infection due to stimulating different immunological pathways.

2.2.2 Methods used in establishing correlates

The aforementioned relationships between vaccination, immune markers, and clinical protection have been assessed through a variety of methods that may be experimental or field based. The "**Prentice criteria**" have been used to assist validation of surrogates in human vaccine studies (Prentice, 1989). These include showing significant associations:

- a) between administration of a vaccine and clinical protection,
- b) between the surrogate and receiving the vaccine,
- c) between clinical protection and the surrogate, and
- d) showing that the surrogate lies on the sole causal pathway such that adjusting for the level of surrogate removes the association between receiving the vaccine and clinical protection.

Surrogates or correlates of protection have been extensively studied for a variety of human diseases. **Randomised controlled trials** offer a powerful method of assessment due to their ability to control confounding variables. Vaccine efficacy trials can provide such data if participants are sampled at appropriate time points post vaccination. For example, (Black et al., 2011) sampled a subset enrolled into an influenza vaccine trial measuring haemagglutination inhibition (HI) titres 50 days post placebo or one of two types of vaccine. Two doses were given 30 days apart and active surveillance with rtPCR confirmation used to define cases. The study related various HI titre cut-offs to clinical protection revealing the 1:40 cut-off was associated with 50% clinical protection in adults but with 22% protection in children.

Observational studies can also provide valuable insights into these relationships. An opportunistic cohort study of a measles outbreak among university students after a recent pre-exposure blood drive gave evidence that a plaque reduction neutralisation level of 120 was associated with protection from classical signs of disease (Chen et al., 1990). Of those with levels below this cut-off, 8/9 individuals developed disease compared to 0/71 with levels above.

Challenge studies have also been used in humans. Buisman et al., (2008) looked at circulating serotype specific poliovirus IgA levels in elderly people challenged with either serotype 1 or serotype 3 monovalent oral poliovirus vaccine. Inverse linear regression revealed a correlation

between pre-existing serotype specific circulating IgA and faecal virus titres for both serotypes (serotype 1, $r=0.57$; serotype 3, $r=-0.61$; $P<0.0001$).

2.2.3 Correlates of protection and FMD

Several homologous challenge experiments, including results from potency tests, provide evidence for an association between antibody titres and protection from clinical FMD. Martin & Chapman (1961) found all animals to be clinically protected above a neutralisation antibody cut-off of 1/32, representing around 60% of the 137 animals challenged, although some animals with lower levels also appeared to be protected. Van Bekkum et al. (1969) found that 424 cattle exposed to serotype C virus two weeks post vaccination were more resistant than 92 animals with similar antibody titres exposed at 9-49 months post vaccination implying vaccine-induced protection decreased over time, independent of the immunological measure.

Hamblin et al. (1987) challenged four groups of 24 cattle intradermolingually with different FMD strains after receiving different dilutions of vaccine and found overlapping titres in the protected and non-protected groups for VNT but not LPBE. Low numbers of animals in non-protected groups make interpretation difficult, but a protective cut-off of 2 logs to the base 10 was generally consistent with protection for LPBE. For serotype C, there were no non-protected animals making comparison impossible. Van Maanen and Terpstra (1989) found VNT and LPBE results to be highly correlated with each other for the serotype strains assessed (correlation coefficients A=0.84, O=0.75, C=0.74; P-values <0.0005). They found serum titres and percentage protection (probits) to be significantly associated for both tests ($P<0.01$) with the regression coefficients shown in Table 2.2 (no confidence intervals were presented). The greater reproducibility seen in LPBE results led the authors to conclude that this test provides a more reliable correlate of protection. Both of these studies reported relatively higher titres for protection when using LPBE compared to VNT in agreement with the later study by Goris et al. (2008b).

Table 2.2. Association between antibody titres measured by LPBE and VNT and clinical protection for serotypes A, O and C. Adapted from Van Maanen and Terpstra (1989).

Serotype	Number challenged	LPBE		VNT	
		PA ₅₀	Regression coefficient	PA ₅₀	Regression coefficient
A10	105	0.90	4.3	0.42	3.9
O1	79	1.23	3.4	0.99	2.7
C1	67	1.18	2.9	0.48	4.7

Note: PA₅₀ = log antibody titre that provides 50% clinical protection. LPBE = liquid phase blocking ELISA, VNT=virus neutralisation test.

Attempts have been made to establish the *expected percentage of protection (EPP)* relative to titres based on data collected from a large number of PPG tests and the generation of correlation tables (OIE, 2009). These are used extensively in South America for licensing of vaccines. Periolo et al. (1993) evaluated PPG potency tests from 1,634 animals on 102 batches of quadrivalent vaccine for serotypes A, C and O. Animals were IDL challenged at 90 days post vaccination (dpv). A close correlation between the LPBE titres and protection was observed with percentages of protection ranging between 87 and 100% for different serotypes when titres were >2.1 logs to the base 10. Although a trend was evident between the titres and protection, there were no statistical analyses or regression coefficients presented and they could not be calculated from the data shown. Based on the results of ten PPG tests with serum samples taken at 30 dpv and measured with VNT and LPBE, Robiolo et al. (2010) found the estimated EPP to have “excellent repeatability, reproducibility and concordance with PPG for vaccine potency”.

Sutmoller et al. (1980) used data from routine PPG potency tests using oil and aqueous based vaccines and were able to correlate neutralising titres at 3-4 weeks post vaccination with the expected protection. Results from 532 cattle for serotypes A, C and O found that higher titres were needed to protect cattle from the latter serotype (slope O=0.73 [SE=0.29]; slope A&C=1.402 [SE=0.096]). The overall relationship between titres and clinical protection is shown in Figure 2.2. Several other studies have demonstrated this variation between serotypes in the level of titres required for protection and also variation in protective levels from different laboratories. Van Maanen and Terpstra (1989) measured PA₅₀ (the log antibody titre that provides 50% clinical protection) values which differed between serotypes for both

VNT and LPBE assays (Table 2.2). This difference between serotypes was not seen by Barnett et al. (2003), although they found highly significant differences in protective antibody levels between laboratories, in agreement with Suttmoller and Vieira (1980) and Goris et al. (2008b) but in contrast to Maradei et al. (2008). In the study by Goris et al. (2008b), several serological tests were analysed including VNT, LPBE, SPCE, serotype O ELISA and NSP ELISA using the results of ten repeated PD₅₀ trials. They found that the accuracy of the different tests in predicting protection also depended on the laboratory performing that test. This effect was also seen by Willems et al. (2012) when analysing the results of five PD₅₀ trials for a serotype A strain.

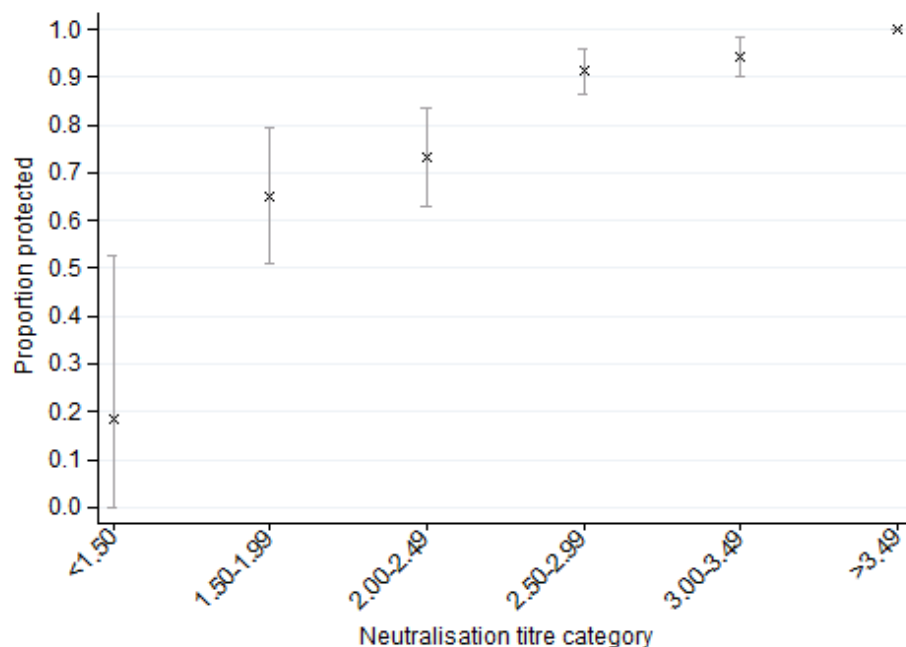


Figure 2.2. Relationship between the percentage of animals protected from clinical disease (with 95%CI) and serological titre category measured by neutralisation tests for all animals and for virus strains from serotypes O, A and C. Produced from data presented in Suttmoller et al (1980) , Table 2.

The evidence for associating antibody levels and clinical protection for FMD is based predominantly on the results of experimental potency tests. Many of the studies show a significant correlation between antibody levels and clinical protection and an EPP has been validated in South America based on the PPG test for vaccine licensure. However, small numbers of animals may be used which gives uncertainty to these estimates and there may be

variation in the protective level between the serotypes, laboratory and serological assay. Additionally, with the experimental challenge methods used, it is difficult to extrapolate protective titres to clinical protection of animals exposed in the field with differing vaccine doses, animal ages and production systems. Whereas laboratory data are required for licensing vaccines, there is a need for data on correlates in field conditions to allow serological validation of FMD vaccines that will guide vaccine strategy.

2.3 Vaccine matching

As an RNA virus, one of the characteristics of the FMD virus is that rapid mutations result from errors in RNA replication (Domingo et al., 2002). These genetic mutations may lead to antigenic changes so that the antibody induced by a particular vaccine strain is less effective for protecting against a mutated field strain. Although genetic sequencing may be useful in monitoring the emergence of these strains, the relationship between specific nucleotide changes on antigenicity is complex and poorly characterised (Paton et al., 2005).

Close match of vaccine virus to that in the field is important to ensure effectiveness. *In vitro* serological vaccine matching studies may be performed which can indicate likely effectiveness of a vaccine against a particular strain. These tests are performed by a relatively small number of laboratories around the world. Sera from cattle (known as "Bovine Vaccinal Serum" or BVS) vaccinated 21 days previously are pooled and the reactivity compared between the vaccine and field strain. A one way comparative relationship (" r_1 ") value is calculated by dividing the titre against the field isolate to that against the vaccine strain. This reactivity is usually measured by VNT or ELISA and occasionally CFT. With VNT, based on field experience a value >0.3 is considered to be indicative of sufficient match between the strains for the vaccine to confer protection against the field strain. When ELISA is used, values over 0.4 indicate a close relationship while for CFT a 0.25 cut-off is recommended (Paton et al., 2005; Rweyemamu, 1984b). Mattion et al. (2009) found VNT to be a more reliable method for estimating the r_1 values due to more reproducible inter-laboratory results compared to LPBE. There are several limitations to this *in vitro* approach including uncertainty over the identity of the precise vaccine strain and standardisation of vaccine-derived sera (Paton et al., 2005). However, the usefulness of vaccine matching tests has been demonstrated in the field. Maradei et al. (2011) were able to show a temporal decrease in r_1 -value and EPP with the emergence of a new serotype A strain consistent with PPG tests alongside an observed increase in FMD epidemics in areas using vaccination.

There have been several studies based on challenge experiments that show that if high enough neutralising titres are induced (for example by using a high potency or antigen payload vaccine) this can lead to cross-protection against heterologous strains of the same serotype. Nagendrakumar et al. (2011) compared responses to experimental challenge with either O₁ Campos or O₁ Manisa virus after administration of different payloads of O₁ Manisa vaccine. When comparing the neutralising antibody titres to O₁ Manisa for challenge with O₁ Campos or O₁ Manisa, the PA₅₀ were 1.8 (95%CI 0.44-7.7) and 0.95 (95%CI 0.19-3.5) respectively demonstrating the higher protective titre required for heterologous protection although from the confidence intervals this difference does not appear statistically significant. The cross-protective effect of high potency vaccines was also demonstrated by Brehm et al. (2008) who performed three homologous and eight heterologous PD₅₀ potency tests for a strain of serotype A (Table 2.3). Animals were vaccinated with a double oil emulsion vaccine containing 4-10µg of antigen per dose. All animals in the homologous potency tests were protected irrespective of dose. The r₁ values for the heterologous strains were all <0.3 (range 0.04 to 0.23) based on comparative VNT titres. The calculated PD₅₀ values for the heterologous challenge varied from 2 to 18, although only in two experiments was it below 6.0. Protection was associated with high neutralising titres to the challenge virus. These results indicate that protection may occur despite a poor vaccine match if the potency is sufficiently high.

Table 2.3. Summary of challenge results for homologous and heterologous viruses compared to the vaccine matching r_1 value. Adapted from Brehm et al. (2008).

Vaccine strain	Challenge strain	Challenge type	PD ₅₀	r_1 -value
A22 Iraq	A22 Iraq	Homologous	32	
A22 Iraq	A Iran 96	Heterologous	6.06	0.09
A22 Iraq	A Egypt 06	Heterologous	10.56	0.12
A22 Iraq	A Iran 99	Heterologous	3.84	0.04
A Iran 99	A Iran 99	Homologous	32	
A Iran 99	A 22 Iraq	Heterologous	13.93	0.10
A Iran 99	A Iran 96	Heterologous	18.38	0.23
A Iran 96	A Iran 96	Homologous	32	
A Iran 96	A Iran 99	Heterologous	10.56	0.12
A Iran 96	A22 Iraq	Heterologous	2.0	N/A
A Iran 96	A22 Iraq	Heterologous	8.0	0.10

Note: PD₅₀ values were determined by OIE/EP recommended methods. r_1 values were determined through virus neutralisation tests.

The EPP method (described in section 2.2) can also be used to estimate the expected protection against heterologous field strains. Tables of correlation between titres and clinical protection are formulated from multiple PPG test results using logistic regression models. The expected protection can be estimated against a field strain by measuring titres to that strain using serum from animals vaccinated with an alternative strain. Sera are taken from 16 or 30 18-24 month old cattle at 30 days post vaccination and again at 30 days post-revaccination with titres measured through VNT or LPBE. The EPP is calculated by comparing the titres to the correlation tables and calculating the proportion of animals one would expect to be protected. Using sera from the revaccinated animals, an EPP <75% (for a sera panel of 16 animals) or <70% (for a panel of 30 animals) indicates low expected protection against the field strain (OIE,

2009). Based on the results of four cross-protection trials, Robiolo et al. (2010) found the mean EPP to be closely correlated with the mean PPG percentage particularly when assessed through VNT (Table 2.4).

Table 2.4. Comparison of PPG tests and EPP estimates from four cross protection trials. Adapted from Robiolo et al. (2010).

Trial	PPG (%, 95%CI)	LPBE	VNT			
			Mean titre	EPP (%, 95%CI) ^a	EPP (%, 95%CI) ^b	Mean titre
3	56.3 (33.2-76.6)	2.01	57.9 (46.7-69.1)	76.4 (70.3-82.6)	1.17	50.6 (34.9-66.2)
4	25.0 (10.5-50.0)	1.74	41.9 (30.8-53.0)	66.0 (58.3-73.8)	0.97	29.1 (18.5-39.7)
5	12.5 (3.8-36.4)	2.15	61.9 (48.0-75.8)	78.3 (70.5-86.0)	0.85	19.8 (19.8-19.8) ^d
6	12.5 (3.9-36.6)	2.09	63.3 (50.7-75.9)	78.9 (70.5-86.0)	0.85	19.8 (19.8-19.8) ^d

Note: The vaccine strain was A24 Cruzeiro. Challenge strain for PPG and the LPBE/VNT titres was A/Arg/01. Titres are the mean of the 16 animals used in the PPG potency trial. Trial numbers are identical to that used in the published study.

^a based on SENASA's curve for A/Arg/01. ^b based in SENASA's curve for A24. ^c based on PANAFTOSA's curve for A24. ^d Confidence intervals reported in original manuscript.

Brito et al. (2014) evaluated the sensitivity and specificity of the r_1 value and other serological measures of cross-protection (including VNT, IgG1 and IgG1/IgG2 ratio) through the generation of Receiver Operator Characteristic (ROC) curves. The gold standard was clinical protection through a PPG test with samples taken 30 days post vaccination. Heterologous serotype A strains were used for vaccination (A24/Cruzeiro) and challenge (A/Arg/01). The r_1 was calculated using VNT, and all the other tests were done for both the vaccine and challenge

strains. Sixty-four animals were included in the analysis. All results were significantly different between protected and non-protected based on a P-value being <0.05 although the evidence was less for the r_1 value at $P=0.13$. Using the recommended 0.3 cut-off, a sensitivity and specificity of 0.41 (95%CI 0.22-0.64) and 0.81 (95%CI 0.67-0.90) respectively were found. For the other tests, greater specificity was achievable but the confidence intervals for the sensitivity all tended to overlap (Table 2.5). Compared to the r_1 value, the other tests tended to have greater accuracy with the most accurate test being the VNT level to the heterologous strain. Interestingly the optimal cut-off for r_1 was 0.45 based on the ROC curve analysis which had a sensitivity and specificity of 0.12 and 1.00 respectively. The authors suggested that a combination of tests be used rather than relying on a single measure of expected cross-protection. This study is limited in just considering individual animal values, when pooled sera are more often used in vaccine matching studies.

Teklegiorghis et al. (2014) also used a ROC curve analytical approach, but instead used 10 different serotype A strains with five cattle vaccinated per strain. Blood samples were taken 21 days post vaccination and tested for VNT, neutralization index test and LPBE from which r_1 values were calculated. The gold standard for ROC curve analysis was a measure of the difference in amino acid sequence in the VP1 region of the genome rather than clinical protection. They found r_1 values from LPBE tests to be most consistent in determining genetic differences. However, since r_1 values do not reflect the magnitude of the titres needed for protection, it is recommended that vaccines should be selected based on the levels of titre induced against the field virus rather than the r_1 value alone.

Table 2.5. Analysis of sensitivity and specificity obtained by different assays for predicting the protective status against a heterologous strain of FMDV. Adapted from Brito et al. (2014).

Assay	Cut-off value	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy
r ₁ VNT	0.30	0.41 (0.22, 0.64)	0.81 (0.67, 0.90)	0.70
r ₁ VNT	0.45	0.12 (0.02, 0.36)	1.00 (0.91, 1.00)	0.78
IgG1/IgG2 (A24)	19.92	0.29 (0.10, 0.56)	0.96 (0.85, 0.99)	0.79
IgG1 (A24)	2000.00	0.47 (0.23, 0.72)	0.94 (0.82, 0.99)	0.69
VNT (A24)	2.16	0.41 (0.18, 0.67)	1.00 (0.92, 1.00)	0.84
VNT (A/Arg/01)	1009	0.47 (0.26, 0.69)	1.00 (0.91, 1.00)	0.86
IgG1 (A/Arg/01)	314.33	0.53 (0.31, 0.74)	0.96 (0.85, 1.00)	0.84
IgG1/IgG2 (A/Arg/01)	9.28	0.71 (0.47, 0.87)	0.98 (0.88, 1.00)	0.91

Note: Animals were vaccinated with A24/Cruzerio with blood samples taken 30 days later. The heterologous stain under consideration was A/Arg/01. Gold standard is based on clinical protection after intradermolingual challenge at 30 days post vaccination. Optimal cut-offs established through Receiver operator characteristic (ROC) curve analysis. Accuracy is defined by the sum of the true positive and true negatives divided by the total number of samples.

2.4 Field evaluations

Although field-effectiveness studies are an essential part of ongoing evaluation of human vaccines, they are rarely conducted for veterinary vaccines, including FMD. In Israel, an outbreak on a feedlot and neighbouring dairy farm that were using non-structural protein (NSP) purified vaccines, allowed comparison of morbidity estimates and NSP seropositivity of groups with different vaccine histories. The investigators found that, despite a good vaccine match ($r_1=0.37$ compared to the recommended >0.3 for neutralising vaccine matching tests) and potency ($PD_{50}>6.0$): a) cattle were not protected from *infection* seven months post vaccination regardless of the lifetime number of doses received (100% NSP antibody positive, 18% clinically affected); b) two doses of vaccine with the second dose three months before challenge did not provide protection for all animals from infection or clinical disease (96% NSP antibody positive, 50% clinically affected) and c) vaccination two weeks prior to the outbreak with one dose of vaccine was sufficient to protect all from clinical disease with minimal NSP antibody positive animals in these groups (Elnekave et al., 2013). In Turkey, post-outbreak

investigations among smallholders revealed poor effectiveness of the Asia-1 Shamir vaccine consistent with a poor vaccine match. Higher vaccine effectiveness estimates of 69% and 63% were calculated for protection from disease and infection respectively using the alternative Sindh-08 vaccine strain (Knight-Jones et al., 2014a). Both of these studies provided information which would not have been possible to obtain through experimental studies, and both have important implications for vaccine policy.

As part of a wider study looking at the impact of maternal immunity on response to vaccination, Nicholls et al. (1984) conducted a field study on four large farms in Brazil where FMD was endemic, comparing the incidence of FMD among calves given two different schedules. The traditional vaccine schedule (dosed every four months irrespective of age) was used on half the calves on three farms and on all the animals on the remaining farm (n=9,056 calves). A different schedule was used on all remaining animals (n=7,951 calves), delaying the first dose to 5-6 months old with a second dose given one month later. The clinical disease incidence was monitored for a 12 month period after implementing the change. Among calves, the incidence on the farms using the traditional schedule was 11.0% compared to 0.09% with the revised schedule, a difference unlikely due to chance ($\chi^2 = 741, P < 0.01$). Combined with the other findings in the paper, they recommended that vaccination should be delayed in calves until 5-6 months of age. Although it is encouraging to see field data provided to support a modification to the vaccine schedule, there are several limitations to this study including: a) "calves" and "adults" not being clearly defined; b) no explanation on the likelihood of exposure of all the groups (the authors assume "the possibility of an FMD outbreak was equal in all vaccination groups"); c) no comparison groups on the one farm which just received the traditional schedule d) numbers of cases and animals were not provided at the individual farm level. No vaccine effectiveness estimate was attempted by the authors in this study and given these methodological problems it cannot be calculated from the data presented.

Another study examined the use of vaccination over a ten year period in Uganda, where ring vaccination in response to outbreaks is government policy for FMD control (Muleme et al., 2012). Vaccine was procured from a manufacturer in neighbouring Kenya, and it was estimated that an average of 10.3% of cattle were vaccinated per year although this varied between regions and was related to the reported number of outbreaks. The reported mean time between outbreak notification and commencement of vaccination was 7.5 weeks, ranging from 1 to 40 weeks which they claimed to be too long to allow control the outbreak particularly with the low coverage. This study suggests that ring vaccination was ineffective in

this setting due to the lack of resources, delayed vaccine procurement and other constraints such as regional conflicts. The authors also highlight the lack of vaccine matching data. No individual animal data or effectiveness estimates were presented in this study.

A meta-analysis performed by Cai et al. (2014) analysed the results of 28 Chinese language papers looking at field-based seroconversion post-vaccination in order to evaluate the “efficacy” of routine vaccination in China. Seventy-three studies were included in these papers, covering serotypes O and Asia-1, and included pigs, cattle, sheep and goats. Samples were tested either with LPBE or indirect haemagglutination assay (IGA). Among cattle, the overall median with “protective immunity” was 81% (90%CI 62-96%) based on a predefined protective titre. No references or data were provided to indicate the source and accuracy of the protection cut-offs used. The analysis does not consider the effect of multiple doses or previous exposure limiting its usefulness. Despite the title and objectives of this study, the efficacy or effectiveness of vaccination was not covered in this analysis based on conventional use of this terminology.

2.5 Simulation models

The potential effectiveness of vaccination as an intervention in FMD control, either alone or in combination with other measures, has also been explored with the use of mathematical models. These are mainly used by FMD-free counties to inform decision makers on control measures in the event of a disease incursion as part of a national contingency plan. Typically, various aspects of ring-vaccination have been assessed. It is outside the scope of the thesis to do a detailed appraisal of all aspects of these simulation models, but a review focussing on how models are parameterised in relation to vaccine effectiveness is worthwhile in order to highlight the limitations of using models in vaccine evaluation and reveal areas where relevant data are lacking.

Most modelling studies make assumptions that a vaccine becomes protective, either partially or completely, at three (Ferguson et al., 2001; Thornley and France, 2009), four (Bates et al., 2003; Halasa et al., 2014; Porphyre et al., 2013; Tildesley et al., 2006; Traulsen et al., 2011; Ward et al., 2009), seven (Hayama et al., 2013; Keeling et al., 2001; Morris et al., 2001), 11 (Backer et al., 2012), 14 (Dürr et al., 2014), or 21 (Martínez-López et al., 2010) days after a single dose of vaccine. Roche et al. (2014) incorporated uncertainty into this onset of immunity by having a probability distribution (triangular (4, 5, 6)) although no references were provided

to support this assumption. Boklund et al. (2013) incorporated the time to onset of immunity in their sensitivity analysis and the infectivity after vaccination was based on expert opinion. Within all these studies there are also various assumptions on vaccine efficacy and the effect of vaccination on infectiousness often at the farm level. This protection is typically assumed to be 80 to 100% after the assumed onset of immunity. Fewer studies consider vaccine efficacy at the individual animal level. Porphyre et al. (2013) assumed 90% of cattle were totally protected on vaccinated farms leaving the remaining 10% totally susceptible to infection and onward transmission. They varied the efficacy from 50 to 98% in a sensitivity analysis. Tildesley et al. (2006) similarly varied the vaccine efficacy from 50 to 100%. Some more recent models (Boklund et al., 2013; Halasa et al., 2014) based their assumptions on vaccine efficacy on the output of meta-analysis by Halasa et al. (2011) which considers protection only in an experimental setting as described previously.

The studies typically referenced for the assumptions (if given) are all based on experimental challenge studies. To appreciate the models they are informing, an understanding of those studies that are commonly cited is necessary. Doel et al. (1994) challenged groups of 2-3 cattle at different times after vaccination (4, 8, 12, 16 and 21 days) with either an aqueous or oil-based high potency (i.e. $>6.0PD_{50}$) serotype O or C vaccine. Cattle were challenged by being exposed for one hour to an experimentally inoculated pig in the acute stages of infection. Of the four animals challenged four days after vaccination (two for oil and two for aqueous), all appeared to be protected from clinical disease for both serotypes. The two non-vaccinated control animals showed generalised clinical disease. Those cattle that were tested for the serotype O strain were the subjects for later experiments with the serotype C strain but with an alternative adjuvant. This crossover design was also used for those initially challenged with the serotype C strain. Similar experiments were performed by Golde et al., (2005) using a double oil emulsion vaccine for serotype O with a PD_{50} of 3.0. Two trials using groups of either three or five cattle were IDL challenged at four and seven days post vaccination and compared to two unvaccinated controls. In the group of three, all showed clinical disease at 4 dpv but none showed disease at 7 dpv. For the group of five, those challenged at 4 dpv had reduced disease severity compared to controls. Again those at 7 dpv appeared to be protected. Cox et al. (2005) tested a high potency oil-adjuvanted serotype O vaccine by vaccinating 20 cattle and having five unvaccinated controls. Challenge was at 21 dpv by exposure to five inoculated cattle for a five day period. A different strain of serotype O was used for challenge. All animals were protected except the five unvaccinated controls. They showed that 45% of vaccinated cattle that had been exposed were PCR positive 28 days after challenge but live virus could not

be isolated so were considered low risk for transmission on this basis. A later similarly designed study by the same research group showed apparent protection at ten days post challenge for both single and 10x antigen concentration vaccine compared to unvaccinated controls although there was no significant difference between the vaccinated groups (Cox et al., 2007) . This latter study also introduced five vaccinated “sentinel” cattle to those vaccinated cattle that were challenged and demonstrated viral RNA to be present in three sentinels indicating possible transmission. However, due to the lack of clinical disease or NSP-seroconversion the risk they pose for onward transmission should be considered negligible. Orsel et al. (2005) did experiments using 24 calves that were divided into six groups of four. Three groups were vaccinated with a high potency oil-adjuvanted serotype O vaccine while the other three groups were not vaccinated. Two calves from each group were intranasally inoculated with a heterologous strain 14 days after vaccination. Disease in the two non-inoculated calves was monitored and average number of transmissions (“reproduction numbers”) were calculated for those vaccinated and non-vaccinated. The reproduction numbers were significantly different among the two groups (0.18 [95%CI 0.01-1.2] vs 2.52 [95%CI 1.13-52.1]) with the values being significantly below and above 1.0 for the respective groups based on a one sided statistical test. On this basis the authors conclude that vaccination may reduce virus transmission. A similar approach with similar conclusions was later published using adult cattle (Orsel et al., 2007).

Three modelling studies were found that used field data on vaccines to parameterise models that then evaluated their effectiveness. Cleland et al. (1994) used data from 60 villages in Thailand to parameterise a state-transition model of a village herd. This was combined with the results of serological monitoring of cattle and buffalo¹ from 21 villages performed over four six-monthly vaccination rounds. Neutralising titres were measured for serotypes A, O and Asia-1. Animals were sampled at vaccination and one month later. It was assumed that a neutralisation titre of 1/32 (or 1.5 logs to the base 10) was associated with protection. Immunity was assumed to only come from vaccination and not natural exposure. “Vaccine efficacy” was used inappropriately to describe seroconversion to vaccination rather than protection. Animals that had antibody levels <1.2 at first sampling for all three serotypes were used in the “efficacy” estimate which assumed protection if increasing in titre to over 1.5 after vaccination. Probabilities were calculated for animals being able to maintain their immunity at protective levels between rounds of vaccination which was then used to parameterise the model. The model concluded that “six-monthly vaccination with an approximate 70% coverage

¹ Species not stated but presumably Water buffalo, *Bubalus bubalis*

would never achieve the minimum acceptable level of herd immunity". Comparatively "Increasing the coverage to 90% resulted in periods of several months where more than 80% of animals were immune alternating with periods immediately prior to revaccination where the level of herd immunity dropped below an acceptable level". Although interesting, this does not evaluate the vaccine in terms of its effectiveness at preventing infection or clinical disease.

Woolhouse et al. (1996) used data from two vaccine trials and outbreak data from four Saudi Arabian dairy farms to parameterise a mathematical model. Data from two vaccine trials (n=30, n=18) with periodic sampling up to 110 days post a single dose of vaccine were used and the proportion of animals with antibody titres above an assumed protective threshold (2 logs to the base 10, measured by LPBE) was modelled over time and parameterised with these trial data. This was combined with detailed outbreak data from five outbreaks on four large (1,750-2,850 cattle) dairy farms over a five year period that were used to construct a Susceptible, Latent, Infectious, Recovered (SLIR) model. The conclusion of this paper was that currently available vaccines cannot prevent outbreaks on large-scale dairy herds due to the short duration of protection, antigenic differences between viruses and high FMDV transmissibility. A number of assumptions in this model bring these conclusions into doubt including:-

- a) The protective threshold used was based upon experimental challenge with IDL virus (Hamblin et al., 1987; Periolo et al., 1993). The variation in the protective threshold was acknowledged but not incorporated into the model.
- b) Previous vaccinations were assumed to not affect the response to vaccination
- c) Animals below the age of six months were not considered because "outbreaks among calves are delayed, typically by 20-25 days, and affect relatively few animals, typically 2-3%, and so have little or no impact on the initial course of an outbreak".
- d) Antibody titres after vaccination are independent of the number of previous doses and the time since the previous dose "consistent with available information" although the citation given was not consistent with this assumption
- e) Entry into the herd balances the loss due to mortality, although the number of doses of animals exiting the herd was likely to be much higher than an animal entering a herd (animal entering herd has 1-2 doses, 5 year old animals exiting herd would have received up to 15 doses if vaccinated every four months).

A different approach to investigating these outbreaks is required in order to more thoroughly establish the reason for vaccine ineffectiveness and suggest modifications to current practices.

Brito et al. (2011) used data from an outbreak of serotype A in Argentina to parameterise an infectious disease model and evaluate the effectiveness of vaccination. Records were available from 1,349 herds with clinically affected animals (representing 56.4% of those affected in the outbreak). The within-herd transmission coefficient (β , defined as the “average number of individuals that are newly infected from an infectious individual per unit time) was estimated for each herd using the standard mass action assumption:-

$$\beta = \frac{NC_a}{SI}$$

Where N is the number of animals in the herd, C_a the number of cases caused by a single infected animal per day (defined as the total number of cases on the farm divided by the duration of the outbreak in days), I the number of animals infected at the time of the outbreak detection and S the number of susceptible animals at the beginning of the outbreak ($S=N-I$). Cases and control herds were defined by having a β over and below the median β value. Multivariate logistic regression was used to associate putative risk factors including vaccination status and time since vaccination. The results of the model for the different vaccination categories are shown in Table 2.6. The authors conclude that “the protective effect of the vaccine was evidenced by the association between vaccination and low rate of within-herd transmission...suggesting that emergency vaccination has a protective impact on disease transmission and that there is a decreased transmission rate within the herd even if the vaccine is applied soon before or even [a] few days after initial infection in the herd”. As can be seen in Table 2.6, the P-value with herds vaccinated 0-4 days after the estimate date of first infection was 0.218, making this aspect of their conclusion questionable.

The approach used by Brito et al. (2011) is also open to a number of biases:

- a) Although cattle had been previously vaccinated with a strain of virus that had poor match with the field strain, the authors assumed it would have no protective effect and did not consider previous disease exposure.
- b) Homogenous mixing was assumed on the farm which is unlikely particularly during an outbreak when farms often instigate control measures.
- c) “ I ” is based on the number of animals affected at outbreak detection. This is likely to vary between farms as certain types of farm may be expected to detect cases earlier in the outbreak.
- d) Lesion ageing was used to establish the estimated date of FMDV introduction into the herd. There are well known limitations with lesion ageing particularly when older

lesions are found but there is no indication in the study of the lesion ages seen with this uncertainty incorporated into the statistical model.

Overall the modelling component seems unnecessary in this study. A more useful (and simpler) approach that would have more effectively evaluated the vaccine, would have been to look at the association between the individual incidence and vaccination status that could have been adjusted for other farm-level factors such as age distribution.

Table 2.6. Logistic regression output comparing the time since vaccination and the odds of having a transmission coefficient above the median value for the outbreak based on 1221 outbreaks (representing 51% of outbreak farms). Modified from Brito et al. (2011). Model is also adjusted for report date and days to detection based on lesion ageing.

Variable	Category	n	Odds ratio	95% confidence intervals	P-value
Vaccination	Non-vaccinated	823	Baseline	-	-
	0-4 days after ^a	25	0.60	(0.26, 1.36)	0.218
	1-7 days before	105	0.49	(0.32, 0.75)	0.001
	8-14 days before ^a	56	0.61	(0.35, 1.07)	0.085
	15-28 days before ^a	57	0.79	(0.45, 1.36)	0.391
	>28 days before ^a	156	0.69	(0.47, 1.01)	0.056

^a Days in relation to the estimated day of first infection in the herd

2.6 Conclusion

Evidence from potency tests show that the PPG provides a more reliable estimate than the PD₅₀ test in evaluating the vaccine's ability to protect experimentally inoculated animals. Although these potency tests are well established and extensively studied, they are all based on artificially inoculated animals in controlled settings making their relevance to conditions of field exposure uncertain. Although often used, serological correlates of protection also have their limitations with differences seen between tests, serotypes and laboratories. Like potency tests, they are also primarily based on experimental conditions that have questionable relevance to the field. Although useful to give an indication of likely protection, vaccine

matching also has several limitations and has not been thoroughly validated in the field being again mainly based on experimental evidence or anecdote.

Mathematical models are important to policy makers as they can rapidly, and cheaply, explore the potential impact of putative control strategies. This is particularly important for FMD in free countries where outbreaks are devastating in naïve populations and so they are extensively used and published in the literature. A review of the assumptions used on vaccination has revealed that the data used to parameterise them are typically based on challenge experiments. Extrapolating the results of these experiments to a broader “real-life” population is likely to be highly inaccurate due to the contrast in conditions and heterogeneity in individuals present in a typical animal population. Unfortunately those models that do use field data make a number of other assumptions that limit their ability to inform policy. The dangerous limitations that models have was highlighted in reviews after the devastating UK 2001 outbreak when “severely flawed” models supported the contiguous cull policy that used “highly improbable biological assumptions” with a “culling policy driven by unvalidated predictive models” (Kitching et al., 2006; Mansley et al., 2011). James and Rushton (2002) highlighted the lack of available published data to inform a ring vaccination policy so that its effect cannot be satisfactorily predicted and an economic model cannot be formulated with confidence envisaged. These experiences should highlight the need for collection of field data to improve the accuracy of these models. However, this has not been the case and even models published this year tend to use the same limited experimental studies to inform their parameters. Although most countries that published these models are FMD-free and field trials are inappropriate, several other countries have used vaccination to control disease incursions and lessons can be learnt (or appropriate studies may be financially or logistically supported) in endemic countries that use a ring vaccination policy.

In recent years, field data have begun being published relating to vaccine effectiveness although this is still relatively minor compared to the literature on experimental studies. The bias towards the latter is likely for a number of reasons including the greater level of control in experimental studies that makes their conclusions appear to be more scientifically rigorous and do not suffer from such issues as confounding to the same extent. Additionally, such experiments are easier to justify on ethical grounds compared to challenge studies in humans which are comparatively rare and so observational studies have been more typical. The advantages and disadvantages of experimental and field approaches are summarised in Table 2.7. Challenge models based on serological correlates or through measurement of direct

clinical protection are the norms for assessment of veterinary vaccines prior to licensure including for FMD. These approaches are limited as they do not account for such issues as vaccine coverage, cold chain problems, multiple doses, differing schedules and maternal immunity. They also don't account for field level exposure to a pathogen which may be very different to that used in the experimental model. However, for human vaccines field based evaluation is an essential part of the licensure process with well validated epidemiological methods that should be applied equally to veterinary vaccine evaluation.

Table 2.7. Comparison of the issues related to experimental and field based methods for evaluating FMD vaccines.

Issue	Experimental	Field
Population	Homogenous ages and breeds	Mixed ages and breeds
Precision	Small numbers lead to statistical uncertainty.	Larger numbers possible
Cost	Expensive due to facilities needed	Relatively cheap. Prospective studies can be expensive.
Location	Only at bio-secure facilities	Anywhere with endemic disease using vaccination
Comparability	Controlled conditions facilitate comparisons between tests and laboratories	Each evaluation may be specific to particular setting
Confounding	Homogenous population and randomisation means this is less of a problem	Needs to be adjusted for if observational study. Field trials can use randomisation.
Bias	Homogenous sample makes it difficult to apply the results to a heterogeneous population	Selection bias (e.g. farms with vaccine failures may not be representative “outbreak bias”) Recall bias in retrospective studies Loss to follow-up in cohort studies
Ethics	Ethical issues with experimenting on animals	Making use of animals infected in field means no experimental inoculations needed. Ethical issues if using non-vaccinated controls or placebos.
Data availability	Detailed data collection on individuals possible	May be lack of data on affected population (e.g. vaccination histories)
Length of follow up	Usually short	Can be long term
Experience	Long and established. Have been used successfully in past as part of control programmes	Limited experience in veterinary field. Lots of experience with human vaccines.
Virus exposure	Un-natural exposure route and uncertainty over which route is most representative of the field	Real-life, variable exposure In prospective cohort studies, animals may not be exposed to virus
Strain selection	Can control	Less control
Policy relevance	Greater for FMD-free settings	Greater for FMD endemic settings, or FMD free with vaccination.
Vaccine selection	Can choose specific vaccine	May not have ability to choose. May have problems interpreting serology if using a non-NSP purified vaccine.
Vaccine delivery	Can control how vaccine is given and timing	Limited control over how vaccine is delivered and can’t predict timing
Resources available	More resources due to mainly being used by richer, FMD-free countries	Limited resources in endemic settings to perform studies

Chapter 3. Research questions and field work

3.1 Research aims

The literature on FMD vaccine evaluation demonstrates the global lack of field-based evaluations of these vaccines and the propensity for relying upon experimental methods. The heavy reliance on mathematical models based upon questionable assumptions further highlights the need for the collection and analysis of field data of vaccine effectiveness. As later detailed in the introduction sections of the research papers in Chapter 5 and Chapter 6, there is also a dearth of information related to the impact of disease in endemic settings.

The aim of this thesis is to address these shortfalls through the collection of relevant data from a FMD endemic country. As shown in the introduction to the thesis, there is also a lack of data on FMD epidemiology among Kenyan dairy farms which limits the capacity to offer advice to these farms relating to prevention, containment and reducing the impact of FMD outbreaks.

3.2 Field work and funding

Through the European Commission for the Control of Foot-and-Mouth disease (EuFMD) based at the Food and Agriculture Organisation (FAO), Kenya was suggested as a suitable location for these studies, in particular the area surrounding Nakuru in what was then the Rift Valley Province, now known as Nakuru County (Figure 3.1). There were several reasons for this recommendation:-

1. FMD is endemic in Kenya with many outbreaks occurring each year
2. Kenya produces its own FMD vaccine which is used in its control strategy
3. Kenya is looking to move forward with disease control for FMD under the “Kenya 2030 vision” and is supportive of relevant research to meet these aims
4. The area surrounding Nakuru has a large population of FMD susceptible species evidenced by the 2009 national census, including a large number of dairy farms and European breeds of cattle appropriate for studies on FMD
5. Ongoing EuFMD training courses in Nakuru provide opportunities for access to the field, to attend ongoing FMD outbreaks on a variety of farm types, and to provide local contacts to assist with data collection
6. There are good transport links in and out of Nakuru for attending outbreaks of disease



Figure 3.1. Map showing the location of Nakuru County within Kenya. Nakuru County is circled in red (Source: Google Maps, accessed 22/10/2014).

Over the initial 12 months of the PhD, several field trips were made to Kenya, facilitated by EuFMD. During this time, field experience of FMD was gained and opportunities to explore the feasibility of various research projects were taken. Through contacts made, two outbreaks on large-scale dairy farms were attended that provided the data for Part A of this thesis. Both of these outbreaks were used by EuFMD as part of their training courses. The first outbreak was in August-September 2012 (“Farm 1”) whilst the second was in October-December 2013 (“Farm 2”). Both farms were willing to share their data for the purposes of this research and signed a consent form (Appendix A). The data from Farm 1 were available electronically and with an appropriate follow-up time within the time-frame of the thesis to make a disease impact assessment possible.

A meeting was held with KEVEVAPI in February 2012, which revealed the existence of four quality assurance (QA) farms. Vaccines are supplied to these farms for free in exchange for KEVEVAPI being allowed to take blood samples at vaccination and 21 days later. Data collected over 20 years were available for one farm of which several were available electronically. KEVEVAPI agreed to share these data for analysis and this farm was conveniently located to allow further data collection on the animals present. This analysis forms Part B of the thesis, with specific aims and objectives informed by Part A.

A Memorandum of Understanding was signed between the FAO and the Department of Veterinary Services (DVS) which formed the basis of a research permit application to study FMD epidemiology, disease impact and vaccine effectiveness (Appendix B).

Funding for field work was from a combination of sources including EuFMD, the Royal Veterinary College and MSD Animal Health. A Bloomsbury Scholarship provided the stipend and tuition fees for a three year period. All field work was led by myself (Nick Lyons). I was assisted by veterinary officers at the National FMD Laboratory, Embakasi, in particular Drs Abraham Sangula, Eunice Chepkwony, and Kenneth Ketter as well as regional government staff (veterinary officers or animal health assistants) initially under the authority of the Provincial Director of Veterinary Services for Rift Valley Province, Dr Nathan Songok, and later the County Director of Veterinary Services, Dr Cleophas Kogo. Centrally, field work was supported by the Deputy Director of Veterinary Services, Dr Thomas Dulu working under the authority of the Director of Veterinary Services for Kenya. Data were made available from KEVEVAPI under the authority of the Managing Director, Dr Geoffrey Muttai assisted by the QA manager, Mr Stephen Njeu.

3.3 Research objectives

This thesis addresses the shortage of field evidence supporting vaccine effectiveness for FMD and the lack of evidence behind the impact of disease on large-scale dairy farms in Kenya. The research is presented in two parts, based on the epidemiological evidence gathered (Part A) and the serological evidence as provided by the vaccine manufacturer (Part B). The specific objectives are presented in Table 3.1.

Table 3.1 Research objectives, research questions, hypotheses and methodological approaches used to address the study aims. The chapters denote where in the thesis the different sections are addressed. Continued on next page.

Research Objective	Research question	Hypothesis	Methodological approach	Chapters
Understand how FMD vaccines are currently evaluated.	How are vaccines for FMD currently evaluated? What are the limitations of these approaches?	Current methods for the evaluation of FMD vaccines are inadequate and a greater emphasis on field evaluation is needed.	Comprehensive literature review	2
Part A – Epidemiology				
Understand typical outbreaks of FMD on large-scale dairy farms in Kenya.	How do typical outbreaks on large-scale dairy farms develop, and what are some of the potential routes of introduction and intra-farm spread?	Detailed outbreak investigations increase our understanding of FMD in these settings and can inform the development of generic and farm-specific contingency plans for large-scale dairy farms in Kenya.	Outbreak investigation, cohort study with univariable analysis and spatial representation methods (Farms 1 and 2)	4, 7, 8, 11
	What are the risk indicators for FMD in individual cattle on large-scale dairy farms in Kenya?			
	What control measures may reduce the intra-farm spread and subsequent disease impact?			

Table 3.1. Continued from previous page.

Research Objective	Research question	Hypothesis	Methodological approach	Chapters
Part A- Epidemiology				
Evaluate the effectiveness of FMD vaccines on large-scale dairy farms using regular prophylactic vaccination.	What is the effectiveness of routine vaccination on large-scale farms?	Patterns of the incidence by the number of doses received indicate vaccine effectiveness.	Cohort study	4, 7, 8
Quantify the impact of FMD on clinical mastitis, culling and milk yield.	What is the impact of FMD on the rate of clinical mastitis?	Animals with FMD are at a risk of developing clinical mastitis.	Survival analysis (Farm 1)	5
	What is the impact of FMD on culling rate?	Animals with FMD are at risk of culling during and for a period of time after the outbreak period.	Survival analysis (Farm 1)	5
	What is the impact of FMD on milk yield?	Animals with FMD produce a significantly lower amount of milk compared to non-diseased individuals.	Generalised Estimating Equations (Farm 1)	6

Table 3.1. Continued from previous page.

Research Objective	Research question	Hypothesis	Methodological approach	Chapters
Part B – Serology*				
Understand the decay of maternally derived antibody and the impact this has on the response to vaccination.	What is the published evidence for the half life in the decline of maternal antibodies for FMD?	Published half lives are mainly based on experiments and are comparable with the literature for antibodies to other diseases.	Comprehensive literature review	9
	What published evidence supports the interference of maternally derived antibody with response to FMD vaccines and how may this be circumvented?	Limited field evidence exists behind this reputed interference.		
	What are the half-lives for SP antibodies of the four serotypes present in the Kenyan FMD vaccine?	Half-lives for these titres are consistent with the evidence in the literature.	Tobit regression, correlating VNTs with age at the first dose of vaccine	10
	How does the titre at first dose affect the response to vaccination?	Higher titres at first vaccination are associated with a reduced increase in titre as measured 21 days later.	Linear regression	10

*The analysis of Part B was informed by the vaccine evaluations performed in Part A of the thesis.

Part A – Epidemiology

Chapter 4. Farm 1 – Outbreak description and vaccine evaluation **(Research Paper 1)**

In September 2012 whilst on location in Kenya, a notification was received directly from a farmer of an outbreak of FMD that had begun a few days before. The farmer was asked to record the date and identification number of each animal as they became affected. Although a sample had already been taken by an animal health assistant (AHA) representing the local government veterinary officer, a further visit was made to the farm shortly afterwards with a veterinarian from the National FMD laboratory to confirm the presence of disease and to discuss data availability for research purposes.

Due to other commitments and the possibility of infection spreading to other farms, it was not possible to remain on the farm for the full duration of the outbreak period. Therefore farm staff assisted with data collection as described in section 4.3.3 of this chapter. The farm was visited on several occasions during and after the outbreak for data collection purposes and interviewing the farm owner and staff.

This chapter gives a detailed account of the outbreak and evaluation of the vaccine performance. It includes necessary background for the impact analysis presented in Chapter 5 and Chapter 6. It has been accepted for publication by Acta Tropica in September 2014 (DOI: 10.1016/j.actatropica.2014.09.010). Supplementary materials are presented at the end of this chapter. The formatting of this paper has been adapted so that it is compatible with the rest of this thesis. Further details on data management are in Appendix C and photographs of clinical cases can be found in Appendix E.



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Epidemiological analysis of an outbreak of foot-and-mouth disease (serotype SAT2) on a large dairy farm in Kenya using regular vaccination

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4.1 Abstract

During August-September 2012, an outbreak of Foot-and-mouth Disease (FMD) due to serotype Southern African Territories-2 (SAT2) occurred on a large, extensively grazed dairy farm in Nakuru County, Kenya. Over 29 days, 400/644 (62.1%) cattle were recorded as displaying clinical signs consistent with FMD. Out of the 18 management groups present, 17 had clinical cases (weighted mean incidence rate 3.5 per 100 cattle-days, 95% CI 2.4, 5.1; range 0.064 to 10.9). Transmission may have been encouraged when an infected group was moved to a designated isolation paddock. A four to five day minimum incubation period was apparent in five groups for which a point source exposure was evident. Further transmission was associated with the movement of individual animals incubating infection, use of a common dip and milking parlour, and grazing of susceptible groups in paddocks neighbouring to infectious cases. Animals over 18 months old appeared to be at highest risk of disease possibly due to milder clinical signs seen among younger animals resulting in reduced transmission or cases not being recorded. Cows with a breeding pedigree containing a greater proportion of zebu appeared to be at lower risk of disease. The outbreak occurred despite regular vaccination (three times per year) last performed approximately three months before the index case. Incidence risk by the lifetime number of doses received indicated limited or no vaccine effectiveness against clinical disease. Reasons for poor vaccine effectiveness are discussed with antigenic diversity of the SAT2 serotype and poor match between the field and vaccine strain as a likely explanation. Detailed field-derived epidemiological data based on individual animals are rarely presented in the literature for FMD, particularly in East-Africa and with the SAT2 serotype. This study provides a detailed account and therefore provides a greater understanding of FMD outbreaks in this setting. Additionally, this is the first study to provide field-derived evidence of poor vaccine effectiveness using a SAT2 vaccine. Further field-based measures of vaccine effectiveness in line with evaluation of human vaccines are needed to inform FMD control policy which has previously relied heavily upon experimental data and anecdotal experience.

Keywords: foot-and-mouth disease; vaccine effectiveness; Kenya; outbreak; cattle; dairy; epidemiology

4.2 Introduction

Foot-and-mouth disease (FMD) is a highly contagious infectious disease of cloven-hooved animals caused by a Picornavirus of the genus Aphthovirus. Seven distinct serotypes with limited immunological cross protection exist, all of which are known to be highly transmissible and with devastating impacts when introduced into FMD-free countries. Transmission of FMD virus (FMDV) occurs mostly by direct contact or aerosol droplets although indirect transmission through animal products, fomites and wind is also possible (Alexandersen et al., 2003). Different viral strains may differ in terms of excretion levels, virulence and transmissibility (Alexandersen et al., 2003).

In recent years, outbreaks have been described in several previously FMD-free countries including the UK (Gibbens and Wilesmith, 2002; Gibbens et al., 2001), the Netherlands (Bouma et al., 2003), Japan (Muroga et al., 2012) and South Korea (Park et al., 2013). These descriptions and subsequent risk factor studies have focussed primarily on farm-to-farm level transmission, since control policies have emphasised preventing the virus moving to non-infected holdings (Ellis-Iversen et al., 2011; Muroga et al., 2013; Wilesmith et al., 2003). In endemic countries like those in sub-Saharan Africa, studies of FMD have tended to focus on genetic sequencing with phylogenetic tree construction (Sheila N Balinda et al., 2010; Sahle et al., 2007; Sangula et al., 2010a; Wekesa et al., 2013), ecological studies on spatiotemporal distribution of reported outbreaks (Allepuz et al., 2013; Ayelet et al., 2012), seroprevalence (Bayissa et al., 2011; Kibore et al., 2013), and the role of wildlife (Caron et al., 2013).

In both FMD-free and endemic settings, few field data are available at the individual animal level. In “FMD-free” settings, culling is likely to begin immediately, preventing the observation of all cases and related data collection. Data are limited from endemic settings due to a combination of under-reporting, lack of resources and poor farm records. Hutber and Kitching (2000) described the temporospatial spread of FMD due to serotype O between livestock groups on a large-scale dairy farm in Saudi Arabia. This analysis indicated that physical barriers between pens slowed transmission and that direct contact was the main reason for spread. In Thailand, an outbreak of FMD serotype A on two related dairy units also indicated the importance of direct animal contact, with virus not spreading to susceptible cattle 20 metres away from an infected group (Gleeson et al., 1995).

Much of our knowledge on virus transmission has been based upon animal experiments (Charleston et al., 2011; Mardones et al., 2010; Orsel et al., 2009, 2007). These experiments

cannot fully account for the heterogeneity of conditions in the field. Additionally, due to high cost, a small number of animals are often used leading to large degrees of statistical uncertainty (Alexandersen et al., 2003). Caution should be taken when extrapolating experimental results to mathematical models at a local, national or wider regional level (e.g. East Africa). There is therefore a need for data from real outbreaks to be reported to increase our understanding of FMDV behaviour in a variety of field settings.

Data are particularly lacking for the field evaluation of vaccines. The OIE-approved test for vaccine potency involves intradermolingual challenge of immunologically naive cattle after receiving varying doses of vaccine of the homologous strain (OIE, 2009). Subsequent disease monitoring allows the calculation of a dose that protects 50% of recipients (PD_{50}). A PD_{50} value over 3.0 is considered acceptable for use in a routine prophylactic setting though a value over 6.0 is preferred (OIE, 2009). Low repeatability and low reproducibility of this test have been reported and large confidence intervals exist due to small numbers of animals used (Goris et al., 2007; Jamal et al., 2008). Various alternative approaches have been advocated, based primarily on serological correlates (Barnett et al., 2003; Goris et al., 2008b). In the field, there are many determinants of vaccine effectiveness in addition to potency, including vaccine coverage, cold chain quality and vaccination schedules. *In-vitro* vaccine matching tests based on the reaction of serum from vaccinated animals to field and homologous vaccine strains provides an “r-value” (r_1) that indicates antigenic similarity and expected protection but does not necessarily correlate with field performance because of the many other factors that can limit vaccine effectiveness (Paton et al., 2005). For example a study from Thailand reported disease among animals that had been vaccinated 2-3 months before despite a r_1 value of 0.61 (Gleeson et al., 1995), a value that indicates a close relationship between the field and vaccine strains and expected cross-protection (Paton et al., 2005). Therefore detailed evaluation of FMD vaccines through collection and analysis of field data is essential to understand performance and limiting factors. The latter will help inform policy on FMD control using vaccination which is particularly important in regions committed to the Progressive Control Pathway (PCP) (Sumption, 2012).

Although field effectiveness studies are an essential part of ongoing evaluation of human vaccines, they are rarely conducted for veterinary vaccines, including FMD. In Israel, an outbreak on a feedlot and neighbouring dairy farm was analysed. Comparison of morbidity estimates and non-structural protein (NSP) seropositivity of groups with different vaccine histories revealed that despite a good vaccine match ($r_1=0.37$ compared to the recommended

>0.3 for neutralising vaccine matching tests) and potency ($PD_{50} \Rightarrow >6.0$): a) cattle were poorly protected from infection seven months post vaccination regardless of the lifetime number of doses received; b) two doses of vaccine with the second dose three months before challenge provided poor protection from infection or clinical disease and c) vaccination two weeks prior to the outbreak with one dose of vaccine was sufficient to provide some protection from clinical disease (Elnekave et al., 2013). In Turkey, post-outbreak investigations among smallholders revealed poor effectiveness of the Asia-1 Shamir vaccine consistent with a poor vaccine match. Higher vaccine effectiveness estimates of 69% and 63% were calculated for protection from disease and infection respectively using the alternative Sindh-08 vaccine strain (Knight-Jones et al., 2014a). Both of these studies provided information which would not have been possible to obtain through experimental studies, and both have important implications for vaccine policy.

The objectives of this study were to conduct a detailed descriptive analysis of an FMD outbreak using individual animal data in an endemic setting and to quantify risk indicators for disease including an assessment of vaccine performance. The broader aim is to use field-derived data to inform FMD control at the national and East Africa regional level in particular where vaccination is used and indicate areas for further evaluation. The analysis of this outbreak will also provide a background for further studies on FMD impact during the same outbreak.

4.3 Materials and methods

4.3.1 Farm background

The outbreak occurred at a 1,600 hectare mixed arable and large-scale commercial cattle dairy farm located in Rongai subcounty of Nakuru County. An estimated 25% of the total farm area was used for livestock. Several residential properties and other businesses unrelated to the dairy herd were present on the farm with around 150 employees coming onto the farm each day. Of these, approximately 25 people had direct contact with livestock. Apart from segregation from neighbouring farms, the presence of a perimeter fence, and a policy of not purchasing replacement stock, there were no specific biosecurity measures in place. Dairy farm income was mainly through milk sales and selling in-calf or freshly calved heifers to other dairy farms. Milk was also purchased from local small-holders for onward sale to a dairy.

4.3.2 Study population

Numbers of FMD susceptible livestock kept on the farm were approximately 600 cattle, 100 sheep and 300 goats. Small ruminants were kept in separate grazing areas a few kilometres from the cattle, preventing direct contact between them (Figure 4.1). No pigs were owned and minimal wildlife was present due to low numbers in the local area and the presence of perimeter fencing.

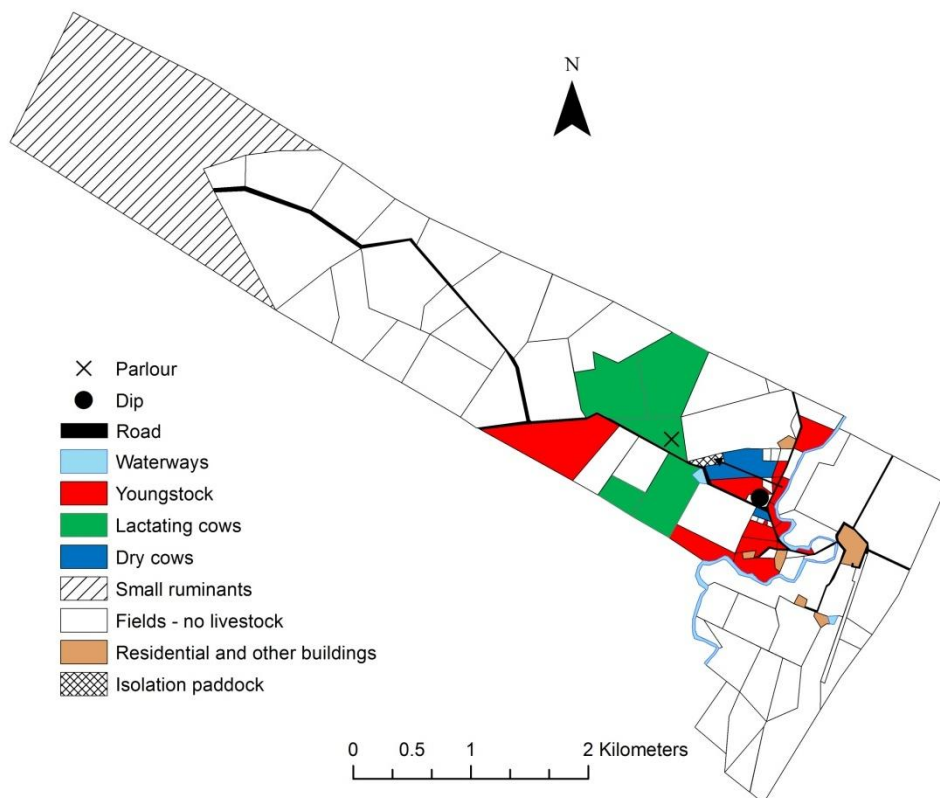


Figure 4.1. Overall map of farm and locations of livestock groups on the first day of the FMD outbreak on Farm 1 (31st August 2012). The arrow indicates the movement of the group containing the index case to the isolation paddock.

Cattle were managed in 18 different groups based on age, weight, production and pregnancy status (Table 4.1) and kept in separate paddocks. All groups were kept outside and supervised 24 hours a day by at least one stockman for purposes of security and to monitor animal health and oestrus events. As soon as possible after birth, each calf was placed into an individual hutch up to the age of around eight weeks when they were weaned. After receiving four litres

of pooled colostrum, calves were fed fermented milk known locally as “maziwa lala”. There were five separately grazed lactating cow groups including three that tended to contain cows of lower parity. All cattle were uniquely identified with a number visible on an ear tag which was placed shortly after birth. The location of animal groups was recorded and movement between paddocks was by within-farm roads and cross-paddock movements as appropriate (Figure 4.1).

Calving occurred all year round and all breeding was through artificial insemination utilising sexed semen. The breeds were predominantly European pedigree (Holstein-Friesian, Ayrshire and Jersey), with a variable genetic contribution from zebu breeds (predominantly Boran). No bulls were present on the farm, and any male calves were sold within a few days of birth. Cows were milked twice daily through a single mobile milking parlour which had a semi-fixed location depending on the paddocks grazed by the lactating cow groups. During milking, the lactating animals were fed at common feed troughs. Over the 12 month period prior to the outbreak, the average number of lactating cows was 183 resulting in an average daily milk yield of 17.0kg. All cattle were dipped once a week through a single permanent dip located centrally in the farm. Dip and parlour locations at the start of the outbreak are shown in Figure 4.1.

Table 4.1. Descriptive data and FMD incidence rate by management group during the FMD outbreak on Farm 1 (31st August-28th September 2012). Age and parity at start of outbreak. Table continued on following page.

Group ID	Cattle-days	N ^a	Age ^b (mean, range)	Parity (mean, range)	Lifetime vaccine doses (mean, median, range) ^c	Incidence rate per 100 cattle-days (95% CI)	Onset date
Youngstock 1 (Y1)	1569	69	17.4 (-28, 121)	-	0.01 (0, 0-1)	0.064 (0.0090-0.45)	19/9/12
Youngstock 2 (Y2)	517	19	93.3 (69, 168)	-	0.26 (0, 0-1)	0.19 (0.027-1.4)	13/9/12
Youngstock 3 (Y3)	242	9	137.4 (110, 204)	-	1 (1, -)	0	-
Youngstock 4 (Y4)	901	38	216.6 (161, 515)	-	1.1 (1, 1-3)	1.0 (0.52-1.9)	6/9/12
Youngstock 5 (Y5)	55	6	530.3 (469-608)	-	3.2 (3, 3-4)	10.9 (0.49-24.3)	7/9/12
Youngstock 6 (Y6)	380	32	328.2 (227-401)	-	2.2 (2, 1-3)	4.2 (2.6-6.9)	7/9/12
Youngstock 7 (Y7)	407	27	309.8 (227-572)	-	2.0 (2, 1-3)	2.5 (1.3-4.6)	5/9/12
Youngstock 8 (Y8)	574	47	463.1 (311-628)	-	3.0 (3, 2-4)	5.9 (4.2-8.3)	31/8/12
Youngstock 9 (Y9)	947	50	598.0 (404-913)	-	3.6 (3, 3-6)	2.9 (2.0-4.2)	6/9/12
Youngstock 10 (Y10)	1147	67	785.4 (568-1431)	-	5.0 (5, 3-9)	4.1 (3.1-5.5)	5/9/12

^a For groups, total number of individual cattle that were in the group at some stage of the outbreak. ^b Days for youngstock and "All groups", years for other groups.

^c Full vaccine histories not available for all cattle (Dry cows =27, Close-up dry cows=31, Lactating 4=52, Lactating 5=52)

Table 4.1. Continued from previous page.

Group ID	Cattle-days	N ^a	Age ^b (mean, range)	Parity (mean, range)	Lifetime vaccine doses (mean, median, range) ^c	Incidence rate per 100 cattle-days (95% CI)	Onset date
In-calf heifers (H)	467	55	2.4 (1.6-3.7)	-	5.7 (6, 3-9)	9.2 (6.8-12.4)	5/9/12
Dry cows (D)	421	29	4.4 (1.0-8.1)	1.6 (0-4)	9.1 (9, 2-13)	3.6 (2.1-5.9)	6/9/12
Close-up dry cows (C)	379	32	3.7 (2.3-9.0)	1.1 (0-5)	7.8 (7, 5-13)	3.7 (2.2-6.2)	5/9/12
Lactating 1 (L1)	578	38	3.3 (2.6-4.3)	1.1 (0-2)	7.7 (7, 6-10)	5.2 (3.6-7.4)	7/9/12
Lactating 2 (L2)	592	30	3.2 (2.4-4.5)	1.1 (1-2)	7.5 (7, 6-10)	3.4 (2.2-5.2)	6/9/12
Lactating 3 (L3)	672	36	3.3 (2.6-5.6)	1.1 (1-3)	7.7 (7.5, 6-11)	4.0 (2.8-5.9)	11/9/12
Lactating 4 (L4)	917	56	5.0 (3.4-9.4)	2.6 (1-6)	10.1 (10, 8-13)	5.5 (4.1-7.2)	12/9/12
Lactating 5 (L5)	983	55	4.7 (3.4-9.7)	2.5 (1-6)	9.8 (10, 8-13)	5.0 (3.8-6.6)	6/9/12
All groups	11748	644	850.5 (-28-3543)	1.7 (1-6)	5.0 (5, 0-13)	3.4 (3.1-3.7)	31/8/12

^a For groups, total number of individual cattle that were in the group at some stage of the outbreak. ^b Days for youngstock and "All groups", years for other groups.

^c Full vaccine histories not available for all cattle (Dry cows =27, Close-up dry cows=31, Lactating 4=52, Lactating 5=52)

4.3.3 FMD history and outbreak

The last recorded outbreak on the farm occurred in July 2004 although the sample submitted to the Kenyan National FMD Laboratory was negative on antigen detection ELISA. Only five animals present during the outbreak described here were on the farm in July 2004, and no detailed records were available from the earlier outbreak.

The recent FMD outbreak began in late August 2012. On 7th September, four epithelium samples were taken from four affected animals in the isolation paddock and sent to the National FMD Laboratory in Embakasi, Kenya. On 10th September, FMD virus was confirmed by antigen detection ELISA as due to serotype SAT2 in all four samples. This was subsequently confirmed by the World Reference Laboratory (WRL), Pirbright, UK as SAT2 topotype IV from VP1 sequencing (WRL batch number 2013/00019; sample KEN/4/2012). Daily recording of FMD cases was made by the livestock manager in consultation with individual group stockmen using lists of cattle ear tag identification numbers for each group. FMD cases were defined by the presence of hyperptyalism with at least one other clinical sign consistent with FMDV infection (decreased milk yield, decreased feed intake, oral lesions, interdigital lesions, pyrexia), although not all cases received a physical examination by farm staff due to the large numbers affected and limited resources. No clinical cases of FMD were seen among small ruminants. This recording system resulted in the number of cattle in the following categories: clinical cases, non-clinical cases in affected group, non-affected group.

Prior to the outbreak, the farm routinely vaccinated cattle against FMDV with an aqueous quadrivalent vaccine (O, A, SAT1, SAT2) approximately every four months. All animals present on the allotted date were vaccinated irrespective of age. No additional doses were given to those after receiving their first dose as part of a primary course. The date of the last vaccination was 22nd May 2012. Sheep and goats were not vaccinated.

4.3.4 Data analysis

All data pertaining to animal details, health events, breeding, individual animal movements, and farm exit were recorded using InterHerd software (InterAgri, School of Agriculture, Reading, UK) using the animal's unique ear tag identification number. Due to the large number of cases of FMD, they were not individually entered into InterHerd but instead manually recorded on paper before being entered into MS Excel and imported to Stata 12.0 where they were merged with appropriate data from InterHerd/Microsoft Access 2007.

A map of the farm was created by identifying fields and generating polygons using Google Earth 7.1 in consultation with the farm owner and livestock managers prior to being imported into ArcGIS 10.2 (ESRI, Redlands, California, USA). Putative exposure dates at the group level were made based on group locations, group movements and individual animal movements.

For descriptive analysis, a cohort study approach was utilised. The case definition was based on observations conducted and recorded by farm staff. Due to the complexities of genetic backgrounds, breeds were classified according to the proportion of the pedigree ascribed to zebu breeds based on breeding history (100% exotic, <25% zebu, 25-49% zebu, ≥50% zebu). Days in milk for lactating cows was defined as early (0-100 days), mid (101-250 days) and late (>250 days). Those calving during the outbreak were included in the “early” category. A mixed effects Poisson regression model was utilised to generate rate ratios, incorporating group ID as random effect to account for the correlation within groups. Likelihood ratio tests were used to assess model fit including variables as linear or categorical.

4.4 Results

4.4.1 Demography

A total of 644 cattle were present on the farm during the outbreak period (31st August – 28th September) totalling 11,748 cattle-days at risk (Table 4.1). Twenty-seven cows calved during the outbreak period producing 26 live calves of which one was male; one cow had a stillbirth and no twins were recorded. Of all female cattle on the farm, most were nulliparous when the outbreak began (380/643, 59.1%) with the majority of non-nulliparous cows being primiparous (138/263, 52.5%). Of those cows calving during the outbreak, eight were calving for the first time and for those lactating during the outbreak, most were in the early lactation period (0-100 days in milk; 107/271; 39.5%). Eleven cows were dried-off during the outbreak whilst 42 were dry for the entire outbreak period.

Most cows (551/644, 85.6%) were of a European pedigree with the predominant breed being Holstein-Friesian (HF): 237/644 (36.8%) were purebred HF and 248/644 (38.5%) had HF make up more than half of the animals pedigree. Other European breeds used include Ayrshire, Guernsey, Simmental and Jersey. The zebu breeds present were Boran and Sahiwal.

Four animals exited the herd during the outbreak. The male calf that was born was sold to another farm at nine days of age. Two female calves died at five and six days of age during the

outbreak with no clinical signs of disease having been recorded. Both were 100% European pedigree. One purebred Holstein-Friesian 3.5 year old cow died six days after being recorded as a case of FMD. This represents an overall mortality rate of 3/644 (0.47%). No specific reasons for any deaths were recorded nor post-mortem examinations conducted. Two further deaths occurred in the two week period after the outbreak (one yearling heifer and one 12 day old calf) although neither had been recorded as clinically affected with FMD.

4.4.2 FMD outbreak description

The outbreak occurred over a 29-day period with the first case recorded on 31st August and the last on the 29th September 2012 with an overall attack rate of (400/644) 62.1%. Only one management group had no recorded cases (Y3). The index case was an 18-month old Holstein-Friesian cross Guernsey heifer located in group Y8. After the index case, three successive generations of cases can be seen as the outbreak spread around the farm (Figure 4.2). Within affected management groups, the weighted mean incidence rate was 3.5 per 100 cattle-days (95% CI 2.4, 5.1) varying from 0.064 to 10.9 (Table 4.1).

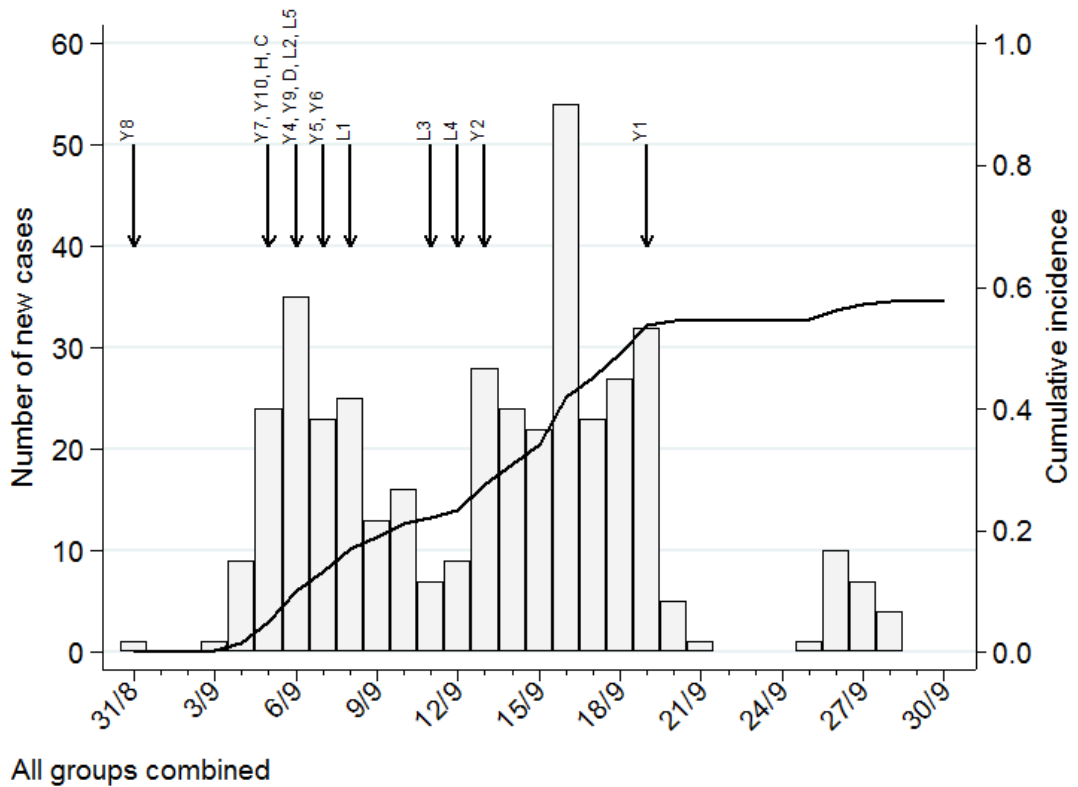


Figure 4.2. Epidemic curve and cumulative incidence of FMD for all management groups combined on Farm 1. Arrows indicate the dates of FMD onset within each group. The denominator for the cumulative incidence is the mean daily number of cattle present on the farm over the outbreak period (31st August-28th September).

The location of the index case was in the centre of the farm (Figure 4.1). The origin of infection was unknown, but FMD was present in the local area making introduction by workers who keep their own susceptible livestock the most likely pathway of introduction. No small ruminants were recently purchased prior to the outbreak and their distance from the cattle makes it very unlikely that they were the source of infection. Although milk was purchased by the farm, it did not enter animal holding areas with the milk tank being located remotely from the mobile parlour. The one bull calf that was born during the outbreak was sold to another farm and did not contact any other groups on the study farm. The affected group was moved to the designated isolation paddock the day after the index case was identified together with another youngstock group (both termed Y8, Supplementary material 4.1). The latter had not had any recorded cases at the time of the mixing and the reason for their mixing was not recorded and therefore likely to have been an error. The dry cow group (“D”) was moved into the paddock that the index case was in directly afterwards, presumably due to a lack of awareness of the potential transmissibility of infection, and developed its first cases five days

later. Cases also appeared in the following days along the road down which the group with the index case moved (Groups Y7, Y10, H and C, Figure 4.3). Local spread was apparent around the initial location of the index case, whilst simultaneously appearing in the south west corner of the farm (groups L2 and L5) which appeared to be then spread in a northerly direction (Figure 4.3). Both foci continued their spread towards the centre of the farm (Figure 4.3).



Figure 4.3. Dates and location of FMD onset and movements of groups subsequent to becoming affected on Farm 1. Movement of group Y3 which had no cases is also shown. The group with the index case is shown in the isolation paddock after being moved on 1st September (see Figure 4.1 & Supplementary material 4.1). The milking parlour was moved from location 1 to location 2 on 1st September.

Most likely exposure dates and routes were established for each group. In five groups, a point exposure is likely (Supplementary material 4.2). In groups Y4, Y7, Y9, C and L2 it is thought to be related to the movement of the infected group to the isolation paddock along the road on 1st September. The time from exposure to the first case was 4 or 5 days consistent with field derived incubation periods (Hugh-Jones and Tinline, 1976). For groups L1 and L5 exposure can be ascribed to a single freshly calved animal having been moved from the close-up group which had cases two days after the move. In both cases the animal that was moved was the index case in the new group and was believed to have been incubating virus when moved. The time from this animal becoming a case to other cases seen in the group was 4 and 3 days for groups L1 and L5 respectively (Supplementary material 4.3). The remaining groups appear to have been affected due to spread from neighbouring paddocks or possibly to exposure at the milking parlour or dip.

4.4.3 Farm response to FMD outbreak

On seeing the first suspected case of FMD on the 31st August 2012 the local government district veterinary officer was notified and an appropriate sample subsequently taken. The farm management responded the following day by isolating the affected group and moving the lactating cow groups and mobile parlour to another part of the farm in an attempt to protect the milking herd (Supplementary material 4.1). Individual animals were subsequently added to the isolation groups as they became clinically affected in an attempt to limit spread and so individual treatment could be efficiently delivered. On day 14 of the outbreak, isolation was stopped due to widespread disease and practical difficulties. The farm took no other specific biosecurity measures although stockmen were assigned to a single group for the outbreak period.

Affected cattle were treated with an oral application of sodium carbonate powder. This alkali is a commonly used treatment in this region that is believed to kill the virus through an increased pH in the oral cavity. Where deemed appropriate by the livestock managers, cattle were also treated with intramuscular antibiotics and topical antibiotic spray to the inter-digital areas. Those with teat lesions may have had teat cannulas used to allow milk let down due to the lesions preventing effective milking. No reactive vaccination took place.

4.4.4 Patterns by age, parity, lactation and breed

Examination of incidence risk by age showed a dramatic increase in FMD risk by age category appearing to plateau by 12-18 months of age (Table 4.2). No effect was seen when looking at parity or lactation stage although of lactating groups, those in mid-lactation (101-250 days) had the highest incidence risk (Table 4.2). When considering the effect of zebu genetics on the risk of FMD, there was a negative correlation between FMD risk and the percentage of zebu breeding pedigree (Table 4.2).

Table 4.2. Descriptive analysis evaluating associations between various risk factors and being a case of FMD on Farm 1.

Variable	N	FMD cases (row %)	Rate ratio (95% CI)	P-value ^a
Age category (n=644)				
Born during outbreak	26	1 (3.8)	0.061 (0.0078, 0.48)	0.008
<6 months	78	4 (5.1)	0.040 (0.014, 0.47)	<0.0001
6m-<1y	57	18 (31.6)	0.26 (0.14, 0.48)	<0.0001
1-<1.5y	79	58 (73.4)	1.0 (0.66, 1.6)	0.92
1.5-<2y	75	49 (65.3)	0.79 (0.54, 1.1)	0.22
2-<3y	120	93 (77.5)	Baseline ^b	-
3-<4y	111	97 (87.4)	1.1 (0.76, 1.5)	0.76
4-<5y	55	47 (85.5)	0.82 (0.53, 1.3)	0.36
5-<6y	22	17 (77.3)	0.73 (0.41, 1.3)	0.29
6-<7y	9	6 (66.7)	0.61 (0.26, 1.4)	0.26
7-<8y	6	6 (100)	1.2 (0.50, 2.8)	0.71
8-<9y	3	2 (66.7)	0.59 (0.14, 2.5)	0.47
9-<10y	3	2 (66.7)	0.65 (0.16, 2.7)	0.56
Parity (n=643)				
0	380	184 (48.3)		
1	138	110 (79.7)		
2	77	67 (87.0)	0.89 (0.74, 1.1)	0.18
3	29	22 (75.9)		
≥4	19	17 (89.5)		
Lactation stage (n=271)				
Early-lactation (<0-100d)	101	83 (82.2)		
Mid-lactation (101-250d)	85	75 (88.2)		
Late-lactation (>250d)	43	34 (79.1)	1.0 (0.89, 1.1)	0.99
Dry	42	29 (69.1)		
Breed (n=644)				
100% Exotic breed	551	356 (64.6)		
<25% Zebu	53	24 (45.3)		
25-49% Zebu	28	12 (42.9)	0.79 (0.66, 0.95)	0.011
≥50% Zebu	12	8 (66.7)		

Note: For age and breed, n=644. For other risk indicators n=643 as the male calf born during the outbreak was excluded from the analysis. Rate ratios are generated from Poisson regression incorporating the group ID as a random effect. Parity, breed and lactation stage were included as linear variables based on the result of likelihood ratio tests comparing categorical and linear models.

^a Wald test. ^b Baseline category assigned to group with highest number of individuals.

4.4.5 Patterns by lifetime vaccine doses received

The risk of FMD appeared to increase with increasing total lifetime doses of vaccine (Figure 4.4). The point estimates plateau from 5 doses onwards although the confidence intervals overlap for all groups with 3 doses or more. This represents a very similar pattern to the incidence risk by age due to the routine administration of vaccine every approximately 4 months to all animals on the farm leading to strong collinearity between these variables.

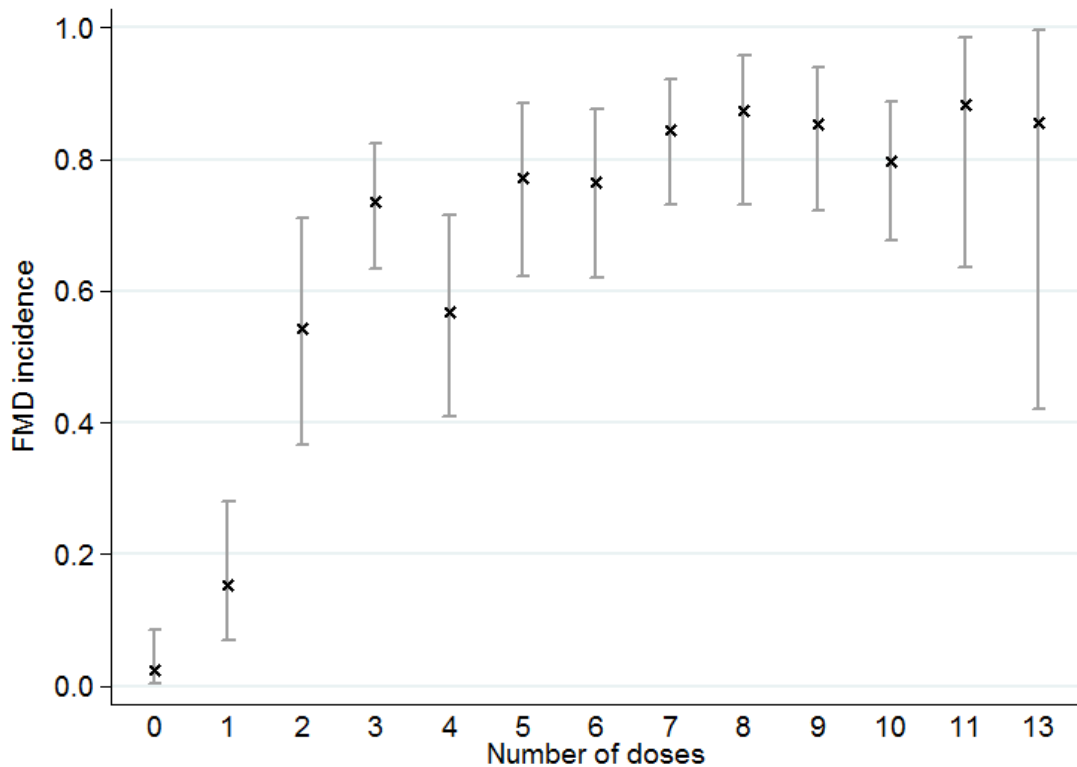


Figure 4.4. Incidence risk (with 95% confidence intervals) of FMD by the lifetime number of FMD vaccine doses given to all animals present on Farm 1 during the outbreak period (31st August-28th September).

4.5 Discussion

This study provides a detailed analysis of an FMD outbreak due to SAT2 on a large-scale dairy farm in an endemic setting. The outbreak appeared to spread through the movement of incubating animals, the movement of an infected group along a road, the use of a single milking parlour and dip, and through neighbouring paddocks. Key findings include the low

incidence rate seen among younger animals and the apparent lack of vaccine effectiveness. Animals with a zebu pedigree tended to be at lower risk of disease.

In an attempt to limit the spread of the outbreak, the farmer moved the group with the index case (the “index group”) to an isolation paddock towards the west side of the farm, whilst the lactating cow groups were moved to the eastern part. This action likely led to exposure of other groups particularly since the distance between animals on the road and neighbouring paddocks is short (Supplementary material 4.4). Moving a dry cow group to a pasture in which clinically affected animals had been present earlier the same day was also likely to cause exposure in this group. An alternative isolation and group movement strategy may have led to a more restricted outbreak and prevented exposure of the lactating groups although it is likely that extended biosecurity measures would also have been required. The latter is important since the extensive use of shared equipment among lactating groups led to widespread exposure and negatively impacted milk production and short-term farm income. All large-scale farms should have individual contingency plans developed with their veterinary surgeon for actions to be taken when FMDV and other infectious disease incursions occur. Such plans should pre-identify isolation paddock locations based on the possible location of the index case and also should restrict the movements of workers between groups.

The incidence risk among youngstock was low in comparison to older animals. This is surprising since these animals had not left the farm so had not been exposed elsewhere, and they had received fewer doses of vaccine. Immunity is passed to calves solely through colostrum and it thought to wane by approximately 4-5 months of age (Nicholls et al., 1984). The last outbreak of FMD was in 2004 and only five animals on the farm were still present in 2012; passive immunity would not be expected to have a great impact even if the same strain had been present in 2004. All youngstock groups except one (Y3) had at least one clinical case. One explanation is that this one group was not exposed to virus. Although the group had no neighbouring paddocks with clinically affected animals up until day 20 of the outbreak (group L3 was located on the other side of a river and wooded area), on day 20 it moved past paddocks with clinically affected animals. In addition the weekly dipping increased the probability that this group was also exposed to infection. All youngstock groups except Y1 were in free grazing paddocks and therefore any infection would be expected to spread once in a group. In group Y1, the calves were in individual hutches in rows of three. Infection would be expected to spread more slowly as there is little direct contact, although given the relatively close proximity of the hutches combined with the communal use of feeding equipment and

movement of personnel, it seems likely that most calves would still have been exposed to significant quantities of virus. Despite this, only one case was seen in this group.

As all groups were likely to be exposed to infection, the low incidence risk among younger animals needs explanation. It is possible that records were poorly kept among the youngstock as they are less regularly inspected. However, every group was permanently supervised by individuals who liaised with the livestock manager each day and reported any sick animals. The farm manager does not believe youngstock were less observed or reported compared to other groups during the outbreak. Alternatively it is possible that the severity of disease was less in calves, or there was some innate immune mechanism in place that reduced their susceptibility to infection. This hypothesis could not be formally tested as the severity of clinical signs were not recorded in this outbreak to support this theory. The lower risk among cows with zebu genetics has also been reported elsewhere and is consistent with anecdotal reports (Singh et al., 1981).

It is interesting that an outbreak of FMD was reported in the Netherlands in 2001 which was traced to a group of calves that passed through an infected location in France connected to the serotype O outbreak in the UK (Bouma et al., 2003). In the farm that had the index case, calves were housed together but in individual pens restricting direct contact. Limited transmissibility was demonstrated based on NSP seroconversion and no clinical disease observed despite their being immunologically naïve. Experiments later performed on 4-9 week calves with the same strain supported these findings (Bouma et al., 2004). Additionally severe clinical signs were seen in adult cows (Orsel et al., 2010). In the outbreak reported here, a similar clinical pattern indicates that clinical signs of FMD may be relatively mild among youngstock in different settings and serotypes and therefore less readily detected in passive surveillance. No physical examinations of the animals were performed so it is possible mild signs were present but not detected. Follow up serological investigations might have provided further information but with the regular use of a non NSP-purified vaccine, the results would have been difficult to interpret due to the presence of maternal antibodies that may contain anti-NSP antibodies and calves receiving their first dose at less than 4 months of age. There is a need to evaluate the NSP antibody induction with the Kenyan vaccine so that serosurveys can be interpreted more effectively in the evaluation of vaccine effectiveness.

An absolute estimate of vaccine effectiveness was not possible in this investigation because of the lack of appropriate comparison groups. Vaccine effectiveness is defined as 1 minus the relative risk of disease comparing vaccinated and unvaccinated groups, measured in the field

(rather than in controlled conditions) and assuming equal exposure (Halloran et al., 1997). The situation on this farm is complicated by all animals having been vaccinated at the same time meaning there is an absolute correlation between the number of doses and age so that age cannot be adjusted for when investigating incidence by the number of doses received. Despite being unable to produce an estimate, the vaccine appeared to have had limited or no effect in reducing the risk of clinical disease in this outbreak evidenced by the high risk in multivaccinated animals.

There are a variety of reasons why the vaccine may not have worked in this outbreak, including low potency, inability to maintain the cold chain, poor match between the field and vaccine strain, and waning immunity since the last dose. In the field, reasons for poor effectiveness can be complex and multifactorial so specific reasons may not necessarily be elucidated. Vaccines for SAT2 are known to be difficult to produce and a higher concentration of antigen per dose is usually required, possibly because the immunogenic antigen is less stable (Parida, 2009). For the vaccines used in this farm, the manufacturer performs vaccine potency tests according to the OIE guidelines and only markets vaccines with a PD_{50} over 6.0 per dose according to OIE recommendations for routine prophylactic use (OIE, 2009). In this outbreak, the last vaccination was just over three months before the index case. Generally, FMD vaccines are expected to provide immunity to the homologous strain for six months after an initial two dose regimen (Doel, 2003) although in the author's (NL) experience most large-scale farms in Kenya use the vaccine every four months. No second dose was included in the primary course although this is often recommended for FMD vaccines. However, the absence of a second boosting dose is unlikely to have been responsible for the high incidence risk in this outbreak as animals that had received several doses also had high risk of disease. Problems with the cold chain cannot be ruled out in this outbreak, but are considered unlikely as many animals had received a large number of doses with no evidence of cumulative protection observed. Also, repeated problems with the cold chain on this farm are considered unlikely given the farm's vaccination practices. SAT2 virus is known to have a high sequence variability and therefore likely antigenic diversity (Sahle et al., 2007; Vosloo et al., 2009). Two distinct lineages were observed to be circulating in Kenya during the same time period (Sangula et al., 2010b). Furthermore the VP1 sequencing performed at the WRL found the strain to be topotype IV which although the same as the vaccine strain (Hall et al., 2013; Sangula et al., 2010b) had a 13% difference in the nucleotide sequence (Knowles, N., personal communication, 2014) suggesting possible issues with the matching of these strains. Vaccine matching test results for the strain isolated from this outbreak are not available but it is clear that with such known

diversity, such testing should be regularly performed particularly for SAT2 strains. The inclusion of multiple strains in the vaccine may also be necessary for field effectiveness.

Care must be taken when generalising the results of this single outbreak to other scenarios where FMD vaccines are used, as the effectiveness may differ. From a broader perspective, endemic countries that use vaccination should develop standard protocols to investigate apparent vaccine failures. Part of this includes notification of the vaccine manufacturer, though for transparency independent epidemiologists should perform subsequent investigations. When outbreaks occur on large-scale farms, farmers should be encouraged to record the date and identification of animals affected. This information should be supplemented by individual animal data to include a minimum of age, sex, and number of doses of vaccine received. Where resources allow, specific clinical signs should be individually recorded to indicate disease severity as this may be reduced by vaccination. A standard clinical case definition should be utilised such as the one in this study and information on how the cold chain is maintained should be collected. Where outbreaks occur in multiple farms such as in smallholder dairy areas, clinical data on individual animals are less likely to be available so farm level data may be more appropriate. In both scenarios, where comparison groups are available, for example if different groups received the latest dose at different times, these groups should be compared. Additionally, where a NSP purified vaccine is used, these studies may be supplemented by performing a structured post-outbreak serosurvey to identify infected animals. Vaccine matching may also be used alongside these analyses although the accuracy of vaccine matching may be low (Brito et al., 2014). As demonstrated in this study useful data can still be collected without this information.

In summary, this study is the first to describe an outbreak of FMD due to serotype SAT2 at the individual animal level in a field setting. More effective internal control measures might have limited the spread of infection and it is recommended that all large-scale farms should develop individual contingency plans with their veterinarian. Evidence is presented that indicates poor vaccine effectiveness and suggests further investigations should be conducted to elucidate the reasons behind the poor performance. This study demonstrates that field evaluations can reveal circumstances in which the vaccine may be underperforming despite being associated with a high PD_{50} . National control strategies in FMD endemic countries where vaccination is used should have routine field evaluations as part of their control policy and standardised practices to investigate apparent vaccine failures including notification of the vaccine manufacturer. Such evaluation is even more essential where government subsidised vaccines

are used so that cost-effectiveness of the control programme can be ensured. Given that outbreaks of FMD due to SAT2 have been reported outside of sub-Saharan Africa in recent years, the evidence presented in this study have broad implications for FMD control at an international level where SAT2 vaccines may be used and indicate how vaccine evaluations may be performed.

4.6 Conclusion

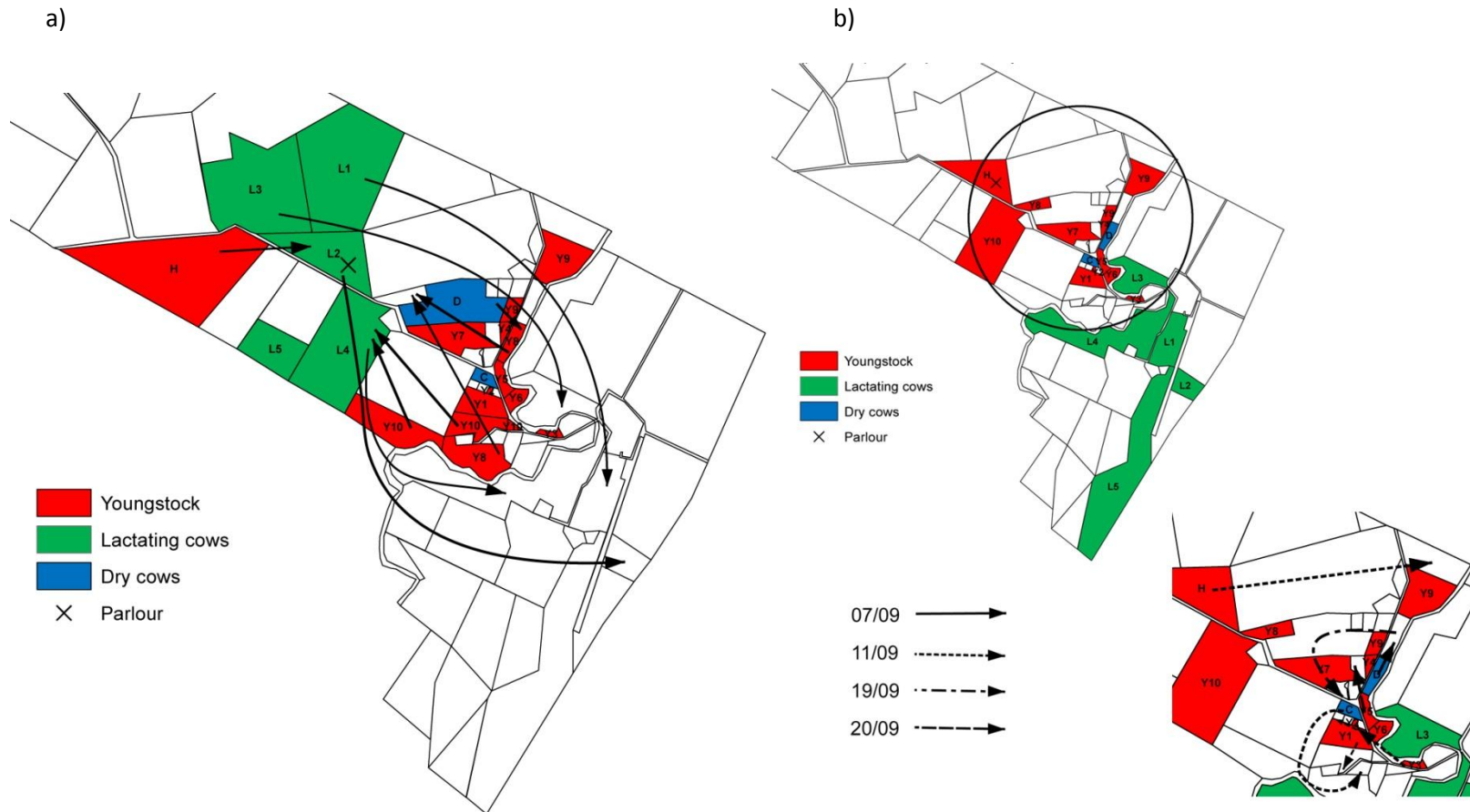
Detailed accounts of FMD are rarely presented in the literature from endemic settings and this is the first known account for serotype SAT2. The findings have implications for FMD control in a national and broader regional context. Problems were found with the field performance of the SAT2 vaccine. This has been suspected based on molecular studies and experience from potency tests, but has not to date been reported in detail in the field. The lower incidence or milder clinical signs among youngstock has implications for surveillance as it may take longer for infection to be detected in these groups leading to further onward spread. This phenomenon has not previously been reported with a SAT serotype but is similar to that seen with a serotype O strain in the Netherlands. Although the reason for poor vaccine effectiveness was not determined, and care must be taken with generalising the results from only one outbreak, this study does indicate the potential and procedure for analysis of field data to reveal problems with FMD vaccination in endemic settings. This approach should be applied to similar scenarios worldwide and is useful even where advanced laboratory techniques like vaccine matching are not available. It is suggested that such evaluations be encouraged in similar and different settings to build up a more complete picture of actual vaccine effectiveness.

4.7 Acknowledgements

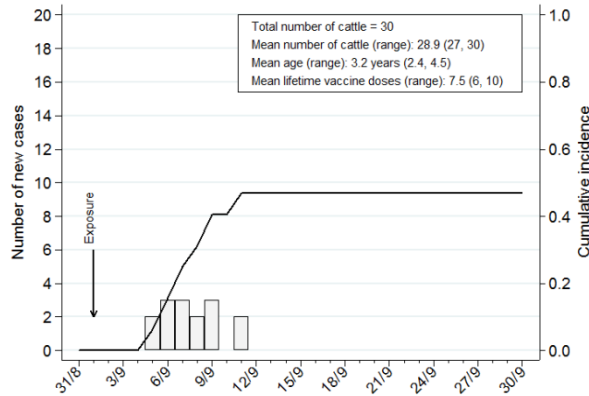
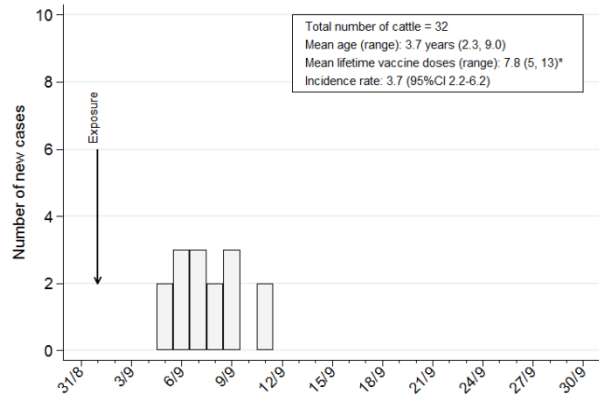
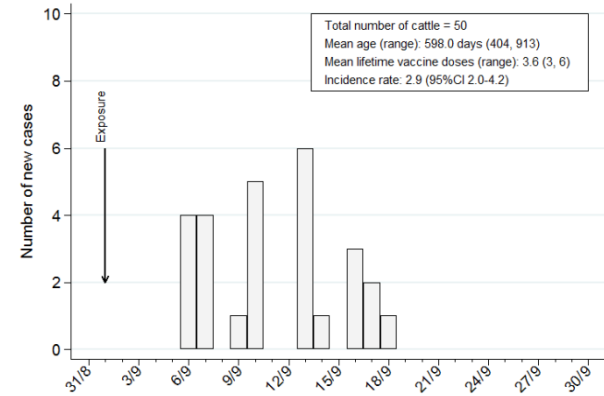
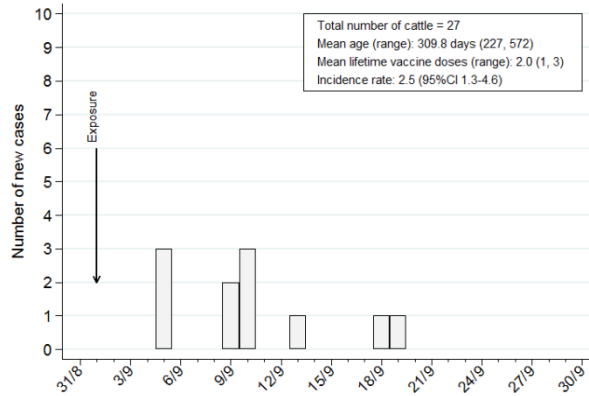
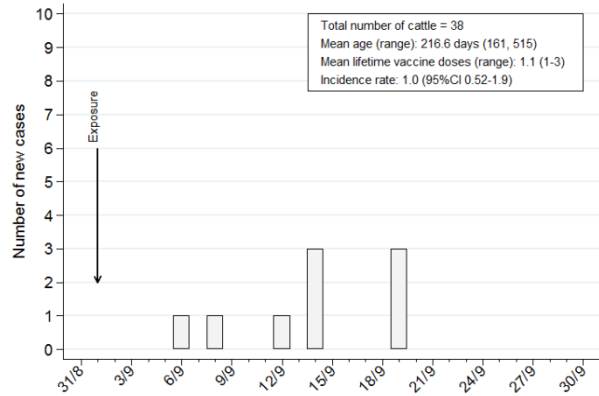
Special thanks go to the farm owner, Mr Hamish Grant and all his farm staff for their ongoing assistance and cooperation with the project. Thanks also go to Keith Sumption, Eoin Ryan and Nadia Rumich at the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) and Jonathan Rushton at the Royal Veterinary College (RVC) for facilitating the project. Thanks also to Richard Booth at the RVC for advice on data extraction and Chris Grundy at the LSHTM for advice on aspects of mapping. Nick Lyons was supported by a Bloomsbury Colleges scholarship with additional funding to support field work from the RVC and MSD Animal Health. Dr Sangula and Dr Chepkwony's employment involves work on

aspects of vaccine quality control for the Kenyan Veterinary Vaccine Production Institute (KEVEVAPI) although are not employed by this company. This research was authorised by the Kenyan National Council for Science and Technology.

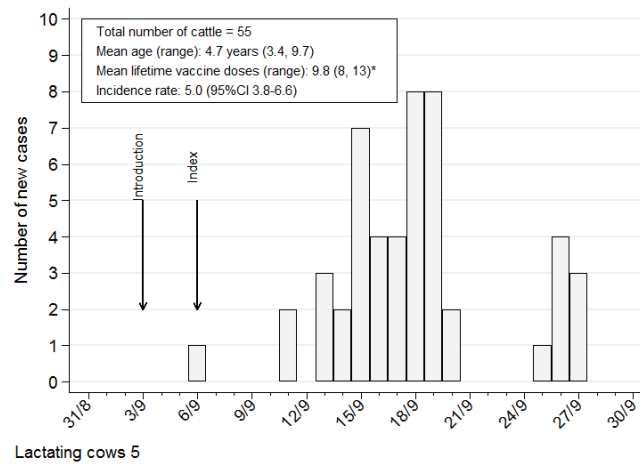
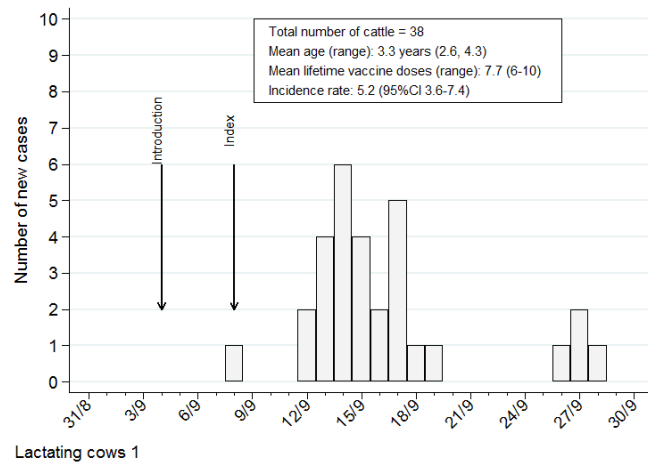
Supplementary material 4.1. Cattle group locations and movements during the outbreak on Farm 1 up to the date that the last group became affected. a) Day 1 locations and day 2 group movements b) Day 2 locations and subsequent group movements during outbreak (see arrow legend). The circle represents the area enlarged below. In “a” Groups Y9 and Y10 were moved to the more centrally located paddock each evening before returning the following morning. After 1st September, this was only done for group Y9.



Supplementary material 4.2. Epidemic curves for management groups with putative point exposures to FMDV on Farm 1. The arrows indicate dates when exposure is likely to have occurred due to the group with the index case having been walked along the road neighbouring paddock.



Supplementary material 4.3. Epidemic curves for management groups with putative exposure to FMDV through the introduction of an incubating animal on Farm 1. Arrows indicate the dates of introduction and the date of onset for the index case in that group. In both groups, the introduced animal was also the index case in that group.



Supplementary material 4.4. Photograph showing the movement of animals along the road on Farm 1 demonstrating the fencing used and proximity to the fields containing other livestock groups.



Chapter 5. Impact of FMD on mastitis and culling (Research Paper 2)

The data recorded on Farm 1 provided the opportunity to do an analysis of the impact of FMD on clinical mastitis, culling and milk yield using individual animal records. As of August 2013, 12 months of data were available allowing data extraction, cleaning and analysis to begin. Data were routinely backed-up every day by the farm to a Dropbox account to which access was permitted. Further details on data management are in Appendix C.

The first analysis looks at clinical mastitis and culling for which a survival analysis approach was utilised. This paper has been submitted to the journal "Veterinary Research". Supplementary materials are included at the end of this chapter.



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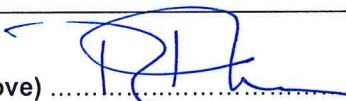
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Conceived the study, performed the statistical analysis, wrote the paper
.....

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Impact of foot-and-mouth disease on mastitis and culling

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5.1 Abstract

Foot and mouth disease (FMD) is a highly transmissible viral infection of cloven hooved animals associated with severe economic losses when introduced into FMD-free countries. Information on the impact of the disease in FMDV-endemic countries is poorly characterised yet essential for the prioritisation of scarce resources for disease control programmes. A FMD (virus serotype SAT2) outbreak on a large-scale dairy farm in Nakuru County, Kenya provided an opportunity to evaluate the impact of FMD on clinical mastitis and culling rate. A historical cohort approach followed animals over a 12-month period after the commencement of the outbreak. For culling, all animals were included; for mastitis, those over 18 months of age. FMD was recorded in 400/644 cattle over a 29-day period. During the follow-up period 74 animals were culled or died whilst in the over 18 month old cohort 63 developed clinical mastitis. Hazard ratios (HR) were generated using Cox regression accounting for non-proportional hazards by inclusion of time-varying effects. Univariable analysis showed FMD cases were culled sooner but there was no effect on clinical mastitis. After adjusting for possible confounders and inclusion of time-varying effects there was weak evidence to support the effect of FMD on culling (HR=1.7, 95% confidence intervals [CI] 0.90-3.4, P=0.10). Conversely for mastitis, there was stronger evidence of an increased rate in the first month after the onset of the outbreak (HR=2.9, 95%CI 0.97-8.9, P=0.057).

Keywords: foot-and-mouth disease; Kenya; cattle; dairy; epidemiology; economics; mastitis; culling

5.2 Introduction

Any disease among livestock creates inefficiency in a production system with negative economic impact to farmers. This impact can be divided into direct and indirect losses (Rushton, 2009). Direct losses are associated with an animal having a disease whose consequences may be immediately visible (e.g. death, abortion) or latent (e.g. reduced fertility). Indirect losses can be divided into additional costs, such as through the use of vaccines for disease prevention, or lost revenue which may occur if a farm is under quarantine, restricting access to local markets (Rushton, 2009). For many animal diseases an accurate estimation of disease impact is difficult due to a lack of available data and the variability of production systems seen around the world.

Foot and mouth disease (FMD) is a viral condition of ruminants characterised by initial pyrexia followed by the development of vesicles on the tongue, hard palate, coronary band and interdigital region. Lesions are also commonly seen on the teats in lactating cows and a sudden milk drop is typically seen (Kitching, 2002). Sudden death may also occur in young calves secondary to an acute myocarditis (Alexandersen et al., 2003). FMD virus is well known for being highly transmissible, made evident by widespread outbreaks seen when introduced to disease-free susceptible populations (Gibbens and Wilesmith, 2002; Muroga et al., 2012; Yang et al., 1999); the virus is prevalent to varying degrees throughout Africa, South America and Asia (Sumption et al., 2008).

The annual global economic impact of FMD has recently been estimated at US\$11 billion (90% range US\$6.5-21 billion) in endemic settings and an additional minimum of US\$1.5 billion has been ascribed to virus incursions into FMD-free countries (Knight-Jones and Rushton, 2013). The latter impact may be considerably more, given for example that in the UK in 2001, where the outbreak has been estimated to have cost \$US9 billion (Thompson et al., 2002). Moreover, the direct impact due to production loss in endemic areas is likely to be considerably underestimated as this is based on data from studies only considering losses through deaths, decreases in weight gain, milk production and draught power (Barasa et al., 2008; Ferrari et al., 2013; Şentürk and Yalçın, 2008; Shankar et al., 2012). A study from Turkey also considered fertility and culling related losses, but was based on an economic model utilising evidence gained through a survey of expert opinion rather than objective data (Şentürk and Yalçın, 2008, 2005). A Kenyan field study of a SAT1 outbreak on four commercial dairy farms in 1999 did consider a broader range of direct and indirect impacts and estimated these losses to total around US\$468,000 (range 15,000-225,000) (Kimani et al., 2005). This study is limited by only considering losses occurring during the outbreak period, analysing herd level losses retrospectively through a post-outbreak survey and not considering background levels of disease and culling. Ultimately, poor characterisation of these effects and a lack of available data preclude a more accurate estimate of economic impact in endemic areas. It is important that data from real outbreaks in the field are collected to gain a more accurate depiction of FMD impact and so inform resource allocation by governments and individual farmers. This is particularly necessary in endemic settings where many infectious diseases are present and competing for resources to implement control.

In developed countries, mastitis is frequently referred to as the most economically important disease in dairy herds (Seegers et al., 2003) and is also reported as a major cause of morbidity

among smallholders in Eastern and Southern Africa (Phiri et al., 2010). Farm profitability is reduced through decreased milk production, increase in milk discard, treatment costs, and associated culling. In animals affected with FMD, viral infection and replication within the udder may occur and teat lesions are likely to increase the risk of bacterial infection leading to clinical and subclinical mastitis (Wellenberg et al., 2002). Mastitis is one of many factors important in determining herd culling or replacement rate which have major implications for herd profitability (Gröhn et al., 2003; Lehenbauer and Oltjen, 1998).

Kenya has the largest population of dairy cattle in East Africa (Thorpe et al., 2000) and is endemic for four serotypes of FMD virus (A, O, SAT1, SAT2) (Namatovu et al., 2013b). Although smallholder dairies are estimated to supply over 70% of the marketable milk in Kenya (Muriuki, 2011), large-scale farms are still an important part of the Kenyan dairy industry tending to be more resistant to seasonal changes in milk production due to adoption of fodder storage technologies (Karanja, 2003). Despite the clear impacts of mastitis and culling on dairy herd profitability, these parameters are poorly characterised among farms affected with FMD in endemic settings, and this may lead to underestimation of disease impact.

In August and September 2012, an outbreak of FMD occurred on a large-scale dairy farm in Nakuru County, Kenya (Lyons et al., 2015). The aim of this study was to use the data from this outbreak to estimate the impact of being a case of FMD on risk of developing clinical mastitis and of subsequent culling, utilising survival analysis methods to provide objective evidence for these parameters in an endemic setting.

5.3 Materials and Methods

5.3.1 Study area and population

The study area and population have been described in detail elsewhere (Lyons et al., 2015). In brief, the data were from a 1600 hectare mixed arable and large-scale commercial cattle dairy farm. Normal numbers of FMD susceptible livestock on the farm are approximately 600 cattle, 100 sheep and 300 goats. The farm had no pigs, and perimeter fencing ensured minimal wildlife. Small ruminants were kept in separate paddocks a few kilometres from the cattle preventing direct contact between them. Cattle were all extensively grazed in 18 different groups based on age, weight, production and pregnancy status. As soon as possible after birth, the calf is placed into an individual hutch up to the age of around eight weeks. There are five separately grazed lactating cow groups including three that tend to contain cows of lower

parity. All groups are supervised 24 hours a day by at least one stockman for purposes of security and to monitor animal health and oestrus events. All cattle are uniquely identified with a number visible on an ear tag which is placed shortly after birth.

Dairy farm income is mainly through milk sales and selling in-calf or freshly calved heifers to other dairy farms. Cattle give birth all year around and all breeding is through artificial insemination utilising sexed semen. No bulls are present on the farm, and any male calves are sold within a few days of birth. All data pertaining to health events, breeding, and farm exit are recorded using InterHerd software (InterAgri, School of Agriculture, Reading, UK).

5.3.2 FMD outbreak

The outbreak of FMD on this farm has been described in detail elsewhere (Lyons et al., 2015). In brief, serotype SAT2 was detected by antigen ELISA by the National FMD Laboratory in Embakasi, Kenya. This was subsequently confirmed by the World Reference Laboratory, Pirbright, UK. The index case was reported on the 31st August 2012. FMD cases were defined by demonstrating hyperptyalism with at least one other clinical sign consistent with FMDV infection (decreased milk yield, decreased feed intake, oral lesions, interdigital lesions, pyrexia), although not all recorded cases received a physical examination by farm staff due to the large numbers affected. Daily recording of FMD cases was made by the livestock manager in consultation with individual group stockmen using lists of cattle ear tag identification numbers for each group. No clinical cases of FMD were seen among small ruminants.

The last recorded case in the study outbreak had onset on the 28th September 2012. The last previous outbreak on the farm occurred in July 2004 although the sample submitted on that occasion to the Kenyan National FMD Laboratory failed to detect any viral antigen. Only five animals present during the current outbreak were on the farm in July 2004 but no detailed records were available from the earlier outbreak.

All cattle on the farm were vaccinated with the locally available quadrivalent vaccine (O, A, SAT1, SAT2) approximately every four months. The date of the last vaccination was 22nd May 2012. Sheep and goats were not vaccinated. Previous analysis found very limited or no vaccine effectiveness in preventing clinical disease (Lyons et al., 2015).

5.3.3 Study design

In this historical cohort study of disease impact, the primary risk factor under consideration was being a clinical case of FMD. The primary outcomes were whether a cow developed clinical mastitis (defined by having a swollen quarter or the presence of visible changes in the milk) or were culled (defined by leaving the herd due to any disease or death). The study population was all cattle present on the farm at some point during the outbreak period (31st August – 28th September 2012). The date of the index case (31st August 2012) was the date of entry into the study unless animals were born during the outbreak in which case the date of birth was used. All animals were followed until exit from the herd or the end of the study period (22nd August 2013). Reasons for herd exit are routinely recorded by the farm including the primary disease responsible for culling. If an animal was sold for breeding or meat with no associated health reason for exit, it was censored at the date of herd exit. For the clinical mastitis analysis, the study population was restricted to animals over the age of 18 months at the start of the outbreak, considered the age when clinical mastitis becomes a possibility, and animals exited the cohort at their first clinical episode of clinical mastitis and did not re-enter the cohort.

Potential confounders for the association between being a case of FMD and culling or developing clinical mastitis included age, parity, stage of lactation, breed, and suffering another disease in the 12 months prior to the beginning of the outbreak. Several breeds and cross-breeds are present on the farm. Breed classification was based on the proportion of pedigree from indigenous breeds compared to non-indigenous exotic varieties. Binary variables were created for whether an animal was recorded as being affected with a previous disease in the last 12 months. Although the farm vaccinates against FMD, previous analysis demonstrated limited or no effectiveness against clinical disease and the schedule used means this is highly co-linear with age so vaccination was not considered as a separate variable in the analysis. Age was categorised according to quintiles, and the number of days in milk was classified at the start of the outbreak as pre-lactation, early-lactation (<0-100 days), mid-lactation (101-250 days), late-lactation (251+ days) and dry. Cows calving during the outbreak were included in the early lactation category (<0-100days).

5.3.4 Statistical analysis

Hazard ratios were generated through Cox proportional hazard regression models to estimate the effect of being a case of FMD on the primary outcomes. Assessment of confounders was

made through their association with the risk factor (being a case of FMD) and outcome (culling or developing clinical mastitis), changes in the hazard ratio when added to the multivariable Cox regression model as well as not being present on any putative causal pathway. Associations between variables and being a case of FMD were assessed through chi-square tests whilst association with subsequent culling and developing clinical mastitis was through calculation of rate ratios and likelihood ratio tests. Associations with P-values of <0.1 were retained for multivariable model building using a backward fitting approach. Age was included in all models as an a priori confounder. Prior to model building, likelihood ratio tests were used to assess for linear trends of risk factor variables where appropriate. The proportional hazards assumption was assessed through examination of a combination of Nelson-Aalen plots and global Schoenfeld residual tests. Where evidence for non-proportional hazards was observed, Schoenfeld residuals for each indicator variable were generated alongside scaled Schoenfeld residuals plots to explore the non-proportionality. Based on these observations, variables were incorporated as time-varying effects with a choice of multiplier function based on the observed plots and Akaike information criterion (AIC) values. Repeated examination of Schoenfeld residuals and scaled residual plots were conducted to ensure the time varying effect was accounted for in the model incorporating the time-varying effect. Interaction between terms in the final model were tested through likelihood ratio tests.

All data were extracted from Interherd through Microsoft Access and imported into Stata 13.0 (Statacorp, Texas, USA) for analysis.

5.4 Results

A total of 644 cattle were present at some point during the outbreak including 26 born during this period of which one was male. Four hundred and nine animals were at least 18 months of age at the start of the outbreak period and hence included in the mastitis analysis. Of all cattle present during the outbreak, 400 (62.1%) were recorded as clinical cases of FMD. Total follow-up time for mastitis was 3683.0 cattle-months (mean 9.0, range 0.36-11.7 per animal) whereas for culling it was 6669.6 (mean 10.4, range 0.16-11.7). During the follow-up period, 63 cattle developed clinical mastitis (incidence rate 17.1 per 1000 cattle-months, 95%CI 13.4-21.9). The total number of animals exiting the herd during the follow up period was 166, of which 74 left the herd due to disease or death. The most common reason for culling was infertility (Table 5.1). The overall incidence rate for culling was 11.1 per 1000 cattle months (95%CI 8.8-13.9).

Examination of Kaplan-Meier plots showed differences between FMD cases and non-cases for both outcomes with strong statistical evidence for a difference provided by the log rank tests for culling alone (Figure 5.1). For clinical mastitis, it can be seen among FMD cases that there is a large increase in hazard in the 1-2 months after the outbreak before appearing equivalent to the non-cases. The HR ultimately becomes higher among non-cases seven months after the outbreak started although with the confidence intervals overlapping this is unlikely to be a significant difference (Figure 5.1). For culling, the hazards grow progressively apart with animals appearing to exit in groups at 3, 5 and 9 months after the beginning of the follow-up period (Figure 5.2). After nine months the confidence intervals do not overlap.

Table 5.1. Reasons for exit and culling after a foot-and-mouth disease outbreak on Farm 1.

Exit category	n	Column %			
Exit herd	166	25.8			
Not exit herd	478	74.2			
Total	644				
Reasons for exiting herd (n=166)					
Culling	52	31.3	Reasons for culling	n	Column %
			Infertility	31	59.6
			Mastitis	5	9.6
			Lameness	5	9.6
			Tick-borne disease	2	3.9
			Mastitis and fertility	2	3.9
			Poor condition	4	7.7
			Other illness	3	5.8
Death	22	13.3			
Low production	2	1.2			
Old age	2	1.2			
Behavioural	1	0.6			
Sold for meat	5	3.0			
Sold as breeding stock	82	49.4			
Total	166				

Note: Dairy farm is located in Nakuru County, Kenya. Follow-up period is 12 months following the beginning of the foot-and-mouth disease outbreak. Animals are included if present on the farm at some point during the outbreak period (31st August-28th September). Culling is defined as exiting the herd due to any disease or death.

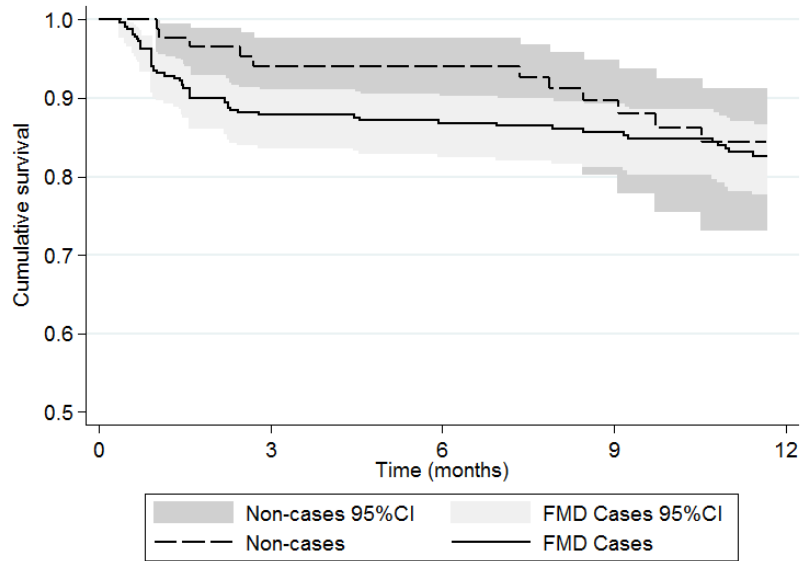


Figure 5.1. Unadjusted Kaplan-Meier survival curve for FMD cases and non-cases related to developing clinical mastitis on Farm 1. Dairy farm was located in Nakuru County, Kenya. Animals were included in the analysis if present on the farm during the outbreak period (31st August-28th September 2012) and were followed for 12 months after the commencement of the outbreak. Cattle are included if over the age of 18 months at the start of outbreak, considered as the age when the outcome becomes a possibility. Log-rank test for equality of survivor function, $P=0.43$.

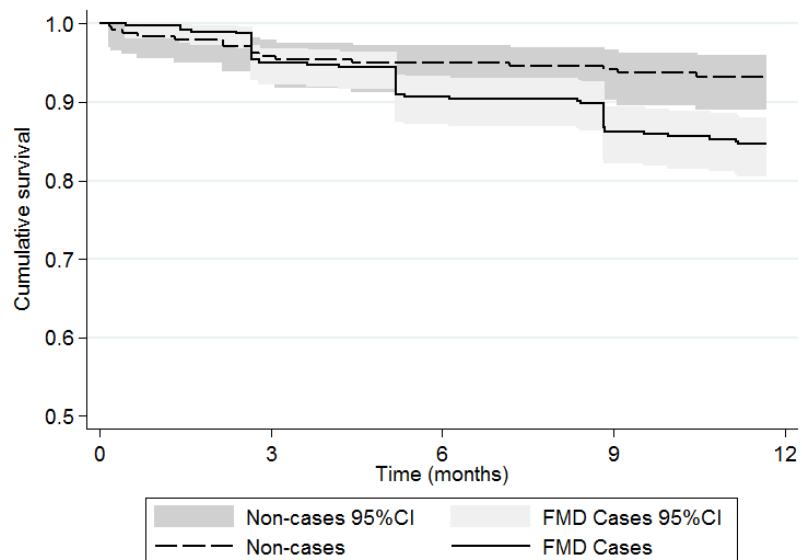


Figure 5.2. Unadjusted Kaplan-Meier survival curves for FMD cases and non-cases related to culling on Farm 1. Animals were included in the analysis if present on the farm during the outbreak period (31st August-28th September 2012) and were followed for 12 months after the commencement of the outbreak. Culling is defined as exiting the herd due to any disease or death. Log-rank test for equality of survivor function, $P=0.0028$.

Previous analysis of the entire study population (n=644) indicated that older animals and those with a more exotic breed pedigree were at higher risk of clinical disease (Lyons et al., 2015). Cattle that had any disease event in the 12 months preceding the outbreak were also at increased risk of clinical FMD with individual associated conditions including abortion, eye disease, lameness, clinical mastitis, and tick borne-disease (Supplementary material 5.1). Conversely having diarrhoea appeared to be protective.

Older cows in later stages of lactation had an increased rate of clinical mastitis, which was also the case for cows with increasing indigenous breed pedigrees (Table 5.2). Previous lameness was also associated with increased mastitis incidence rate (Supplementary material 5.2). Similarly older cows in later stages of lactation had an increased rate of culling, which was also associated with dystocia, clinical mastitis and tick-borne disease in the previous 12 months (Table 5.3, Supplementary material 5.1). Having had any disease in the previous 12 months was similarly associated with likelihood of being culled.

Table 5.2. Clinical mastitis - characteristics of the study population (n=409) and univariable analysis for Farm 1. Continued on following page.

Variable			FMD		Clinical mastitis		
	N	(col %)	N (row %)	P-value	Rate per 1000 cattle-months (95% CI)	HR (95% CI)	P-value
FMD							
Yes	323	79.0	-	-	18.0 (13.7, 23.7)	1.3 (0.68, 2.5)	0.43
No	86	21.0	-		13.8 (7.6, 24.9)		
Age (quintiles)							
1.5-<2.0y	81	19.8	54 (66.7)	0.0073 ^a	3.4 (1.1, 10.6)	1.8 ^b (1.5, 2.2)	<0.0001
2.0-<2.8y	82	20.1	61 (74.4)		7.5 (3.3, 16.6)		
2.8-<3.5y	82	20.1	71 (86.6)		17.8 (10.3, 30.7)		
3.5-<4.3y	83	20.3	73 (88.0)		18.1 (10.5, 31.2)		
4.3-9.7y	81	19.3	64 (79.0)		50.8 (35.1, 73.6)		
Parity							
0	146	35.7	107 (73.3)	0.042 ^a	1.9 (0.6, 6.0)	1.7 ^b (1.4, 2.1)	<0.0001
1	138	33.7	110 (79.7)		22.4 (15.3, 32.9)		
2	77	18.8	67 (87.0)		33.2 (21.6, 50.9)		
3	29	7.1	22 (75.9)		29.1 (13.1, 64.9)		
≥4	19	4.7	17 (89.5)		55.6 (26.5, 126.0)		
Days in milk ^c							
Pre-lactating	138	33.7	102 (73.9)	0.027	1.4 (0.34, 5.4)	1.7 ^b (1.4, 2.0)	<0.0001
Early-lactation (<0-100d)	107	26.2	88 (82.2)		18.5 (11.5, 29.7)		
Mid-lactation (101-250d)	85	20.8	75 (88.2)		27.7 (17.7, 43.5)		
Late-lactation (>250d)	44	10.8	35 (79.6)		45.2 (26.8, 76.3)		
Dry	35	8.6	23 (65.7)		37.8 (20.9, 68.2)		

Note: Univariable analysis examines the associations with the primary risk factor (being a case of clinical FMD) and primary outcome (clinical mastitis) for cattle present during an outbreak of foot-and-mouth disease (FMD) on a dairy farm in Nakuru County, Kenya. Cattle are included in the analysis if over the age of 18 months at the start of outbreak, considered as the age when clinical mastitis becomes a possibility. HR = Hazard ratio.

^a Chi-square test for trend. ^b Included as linear variables based on likelihood ratio tests ^c Defined at the beginning of the outbreak period.

Table 5.2. Continued from previous page.

Variable			FMD		Clinical mastitis		
	N	(col %)	N (row %)	P-value	Rate per 1000 cattle-months (95% CI)	HR (95% CI)	P-value
100% Exotic breed	351	85.8	285 (81.2)	0.0046 ^a	15.7 (11.9, 20.7)	1.3 ^b (1.0, 1.8)	0.053
<25% Indigenous	27	6.6	20 (74.1)		23.5 (10.6, 52.3)		
25% Indigenous	19	4.7	10 (52.6)		12.8 (3.2, 51.1)		
50% indigenous	12	2.9	8 (66.7)		54.8 (22.8, 131.6)		

Note: Univariable analysis examines the associations with the primary risk factor (being a case of clinical FMD) and primary outcome (clinical mastitis) for cattle present during an outbreak of foot-and-mouth disease (FMD) on a dairy farm in Nakuru County, Kenya. Cattle are included in the analysis if over the age of 18 months at the start of outbreak, considered as the age when clinical mastitis becomes a possibility. HR = Hazard ratio.

^a Chi-square test for trend. ^b Included as linear variables based on likelihood ratio tests ^c Defined at the beginning of the outbreak period.

Table 5.3. Culling - characteristics of the study population (n=644) and univariable analysis for Farm 1.

Variable	N	(col %)	Culling		
			Rate per 1000 cattle-months (95% CI)	HR (95% CI)	P-value
FMD					
Yes	400	62.1	14.2 (11.0, 18.4)	2.3 (1.3, 3.9)	0.002
No	244	37.9	6.2 (3.8, 10.1)		
Age (quintiles) ^a					
-28- <227d	128	19.9	5.0 (2.4, 10.5)	1.6 ^b (1.4, 2.0)	<0.0001
227-<577d	129	20.0	2.0 (0.66, 6.3)		
577-<974d	129	20.0	11.1 (6.7, 18.4)		
974-<1363d	129	20.0	17.1 (11.2, 25.9)		
1364-3543d	129	20.0	23.2 (15.9, 33.8)		
Parity					
0	381	59.2	4.8 (3.7, 7.4)	1.5 ^a (1.3, 1.8)	<0.0001
1	138	21.4	25.6 (18.1, 36.0)		
2	77	12.0	11 (6.1, 22.4)		
3	29	4.5	36.8 (19.2, 70.7)		
≥4	19	3.0	18.2 (5.9, 26.6)		
Days in milk					
Pre-lactating	373	57.9	4.9 (3.1, 7.6)	1.6 ^a (1.4, 1.9)	<0.0001
Early-lactation (<0-100d)	107	16.6	15.2 (9.3, 24.9)		
Mid-lactation (101-250d)	85	13.2	18.8 (11.3, 31.2)		
Late-lactation (>250d)	44	6.8	25.4 (13.7, 47.2)		
Dry	35	5.4	39.9 (23.1, 68.6)		
Breed					
100% Exotic breed	551	85.6	5.7 (9.0, 14.6)	0.8 ^a (0.5, 1.3)	0.30
<25% Indigenous	53	8.2	10.9 (4.9, 24.4)		
25% Indigenous	28	4.4	3.7 (0.5, 26.0)		
50% indigenous	12	1.9	8.8 (1.2, 62.7)		

Note: Univariable analysis examines putative associations with the primary outcome (culling) for cattle present during an outbreak of foot-and-mouth disease (FMD) on a dairy farm in Nakuru County, Kenya. Culling is defined as exiting the herd due to any disease or death. HR = Hazard ratio. ^a Negative values reflect animals born during the outbreak period ^b Included as linear variables based on likelihood ratio tests

Global Schoenfeld residual tests performed prior to backward model fitting revealed strong evidence of a departure from the proportional hazards assumption in both mastitis and culling models (Supplementary material 5.3). In the mastitis model, tests for individual variables and an examination of the scaled Schoenfeld residual plot showed the FMD variable to be mostly responsible for this deviation particularly in the 2 month period after the onset of the outbreak. This lack of proportionality is consistent with the Kaplan-Meier (Figure 5.1) and Nelson-Aalen plots. AIC tests indicated a logarithmic multiplier function to provide the best model fit when the FMD variable was included as a time-varying effect which led to a more stable scaled Schoenfeld residual plot and decreased the global test statistic. In the culling model, there was strong evidence that the lactation stage showed significant departure from the proportional hazards assumption which was similarly rectified by the inclusion of a logarithmic multiplier function with time based on AIC tests (Supplementary material 5.3).

The final multivariate model for mastitis incorporating the time-varying effect of FMD was also adjusted for age, lactation stage, and breed (Table 5.4). There was evidence that the hazard ratio was significantly greater than 1.0, one month after the beginning of the outbreak with the effect disappearing in the subsequent follow up period as confidence intervals continually overlap with 1.0 (Figure 5.3). For culling, the estimate was adjusted for age, parity, and the presence of tick-borne disease in the previous 12 months with lactation stage as the time varying effect (Table 5.4). Although there was a trend for cattle affected by FMD to be culled sooner during the 12 month follow-up period, the statistical evidence was weak (HR=1.7, 95% CI 0.86-3.2, P=0.13).

Table 5.4. Final multivariate Cox-regression model examining the association of FMD with clinical mastitis and culling on Farm 1.

Variable	Clinical mastitis		Culling			
	HR (95%CI)	P-value	HR (95% CI)	P-value		
FMD						
Case	2.9 (0.97, 8.9)	0.057	1.7 (0.90, 3.4)	0.10		
Non-case	Baseline	-	Reference	-		
Age^a						
	Age category		Age category			
	1.5-<2.0y	Baseline	-	-28- <227d	Baseline	-
	2.0-<2.8y	0.61 (0.12, 3.0)	0.54	227-<577d	0.29 (0.07, 1.2)	0.083
	2.8-<3.5y	0.77 (0.16, 3.7)	0.75	577-<974d	0.87 (0.29, 2.6)	0.80
	3.5-<4.3y	0.64 (0.12, 3.3)	0.60	974-<1363d	0.86 (0.24, 3.1)	0.82
	4.3-9.7y	2.6 (0.45, 15.1)	0.29	1364-3543d	1.7 (0.38, 7.4)	0.50
Lactation stage						
	Non-lactating	-	-			
	Early lactation (<0-100d)	-	-			
	Mid lactation (101-250d)	-	-	0.46 (0.25, 0.85)	0.013	
	Late lactation (>250d)	-	-			
	Dry	-	-			
Breed^b						
		1.4	0.031	-	-	
Tick borne disease (last 12 months)						
	Yes	-	-	3.2 (1.7, 5.9)	<0.0001	
	No	-	-	Baseline		
Parity						
	0	Baseline	-	Baseline		
	1	11.3 (2.5, 51.2)	0.002	2.4 (0.79, 7.4)	0.12	
	2	9.8 (1.7, 55.9)	0.010	0.58 (0.14, 2.4)	0.45	
	3	4.6 (0.65, 32.6)	0.13	2.1 (0.53, 8.6)	0.29	
	≥4	8.1 (1.2, 55.8)	0.033	0.78 (0.14, 4.4)	0.78	
Time varying interactions						
FMD						
		0.43	0.016	-	-	
Lactation stage						
		-	-	1.9 (1.4, 2.6)	<0.0001	

Note: Animals were included in the analysis if present on the farm during the outbreak period (31st August-28th September 2012) and were followed for 12 months after the commencement of the outbreak. For the analysis of clinical mastitis, cattle are included if over the age of 18 months at the start of outbreak, considered as the age when the outcome becomes a possibility. Culling is defined as exiting the herd due to any disease or death. Hazard ratios (HR) incorporate time varying effects with logarithmic multiplier functions to account for non-proportional hazards. ^a Age categories based on quintiles. ^b Included as a linear effect. Categories are 100% exotic breed, <25% indigenous, 25% indigenous, 50% indigenous.

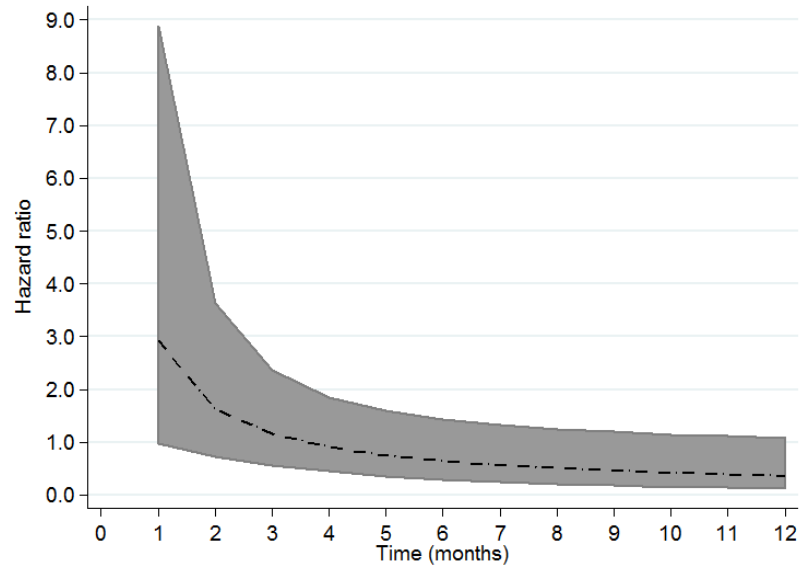


Figure 5.3. Variation in hazard ratio over time for cases of FMD developing clinical mastitis on Farm 1. Animals were included in the analysis if present on the farm during the outbreak period (31st August-28th September 2012) and were followed for 12 months after the commencement of the outbreak. Cattle are included if over the age of 18 months at the start of outbreak, considered as the age when the outcome becomes a possibility.

5.5 Discussion

During this outbreak on a large-scale dairy farm in Kenya, 400/644 (62.1%) of cattle were affected with FMD found due to serotype SAT2. The outbreak lasted for 29 days with the index case identified on 31st August 2012 and the last case on the 28th September 2012. In the 12-month follow up period commencing on the day of the index case, 74 were culled or died. For cattle aged 18 months or greater at the start of the outbreak, 63 developed clinical mastitis. Although in the univariable analysis FMD cases tended to be culled sooner after the outbreak onset, after adjusting for possible confounders there was weak evidence to support this observation. For clinical mastitis, univariable analysis showed no effect of FMD on rate of mastitis but after adjusting for the time varying effect of being a case there was good evidence of an increased rate in the first month after the onset of the outbreak.

For correct interpretation of the Kaplan-Meier curves for clinical mastitis and culling, one must consider the expected timing of disease impact and farm management. The association between FMD and clinical mastitis is mainly related to the FMD lesions that develop on the teats increasing the susceptibility to secondary bacterial infection. Since this is likely to occur soon after the appearance of lesions, seeing non-proportional hazards in the early stages of the follow-up period is expected. The reason behind the increased mastitis rate in non-FMD cases that occurs several months after the outbreak is unknown but this difference was not statistically significant. Conversely the rate of cows exiting the herd due to culling appears to increase throughout the follow-up period. This is because FMD typically has a low mortality rate and farm exit will generally occur once a cow has ceased producing milk and after an appropriate period of time to fatten if appropriate. So the overall effect of any non-fatal disease on culling rate will only become apparent around a year later when cows in early lactation are reaching the end of their lactation, presuming they continue to lactate after recovering from the disease. Lactation stage has been previously shown to be associated with culling in a survival in a study of Holstein-Friesian cattle in Kenya with cows in later lactation more likely to be culled (Ojango et al., 2005). As a consequence, lactation stage at outbreak onset was included as a time-varying effect in the multivariable model. The decreases seen in the culling survival curve around 3, 5 and 9 months are likely due to management reasons whereby cattle are removed from the farm in groups once a decision has been made to cull.

Despite the Kaplan-Meier curve and log-rank test revealing a trend indicating increased culling with cases of FMD (Figure 5.2), in the multivariate model there was only very weak evidence of a statistical association (Table 5.4). In this outbreak, older cows appeared at greater risk of

FMD and due to their advanced age, were at increased risk of culling. Therefore age was a strong confounder of the association between being a case of FMD and being culled. In contrast, the effect of FMD on clinical mastitis was strong enough that even after adjustment for age there was still a pronounced effect in the early follow-up period.

Animals that had suffered from a tick-borne disease (TBD) prior to the FMD outbreak were more likely to develop FMD and also be culled (Supplementary material 5.1). This confounder was present after adjusting for age which has been shown to be associated with the incidence of TBD (Phiri et al., 2010). Tick-borne diseases have been identified as a major cause of cow death and culling among smallholder dairy farms in Kenya and neighbouring Tanzania (Lyimo et al., 2004; Maloo et al., 2001). The major tick-borne diseases of cattle in the area are theileriosis (East Coast Fever, ECF), babesiosis and anaplasmosis. The farm does not consistently record which disease was encountered hence they were all included as one disease condition. In Kenya, ECF has been identified as the disease with the highest impact on livelihoods among pastoralists in Kenya, marginally ahead of FMD (Onono et al., 2013). The impact of ECF on culling has been less well characterised on large-scale dairy farms although an outbreak of ECF on a large-scale farm in Tanzania due to a breakdown in dipping regime led to severe economic losses (Msami, 2001). Additionally, acaricide dips are in wide use among large-scale farms in Kenya. It has been anecdotally suggested that previous exposure to other infectious diseases may increased the susceptibility to FMD although this is the first study to the authors' knowledge that provides evidence for such an association. Although no definitive diagnosis of the condition was made, the clinical signs associated with these conditions are easily observed and commonly encountered.

The majority of cattle present on the farm were affected with FMD despite vaccination being performed in all cattle approximately every 4 months. Although previous analysis indicated limited or no vaccine effectiveness in preventing clinical disease (Lyons et al., 2015), it cannot be ruled out that the vaccine provided some protection leading to an underestimate of the impact if the outbreak had occurred in an immunologically naive herd. However, this study reflects a "real-life" impact of a FMD outbreak under field conditions. Vaccination for FMD is common among large-scale herds in Kenya therefore making these results very relevant to this population and useful when considering the impact of a vaccination programme. Similar estimates from outbreaks on other dairy farms with other serotypes would be useful to demonstrate the range of impacts obtained in the field.

The study was on a large-scale dairy farm which is among the minority of dairy farm types in Kenya so care must be taken when generalising these results to the broader dairy cow population although there are similarities to the local smallholder cattle population that need emphasising. The breeds of cattle on the study farm are predominantly exotic (mainly Holstein-Friesian) with some indigenous cross-breeding. Among smallholder dairy farms in Kenya, Holstein-Friesian is also the most common breed due to the higher milk yields (Bebe et al., 2000). Indeed the study farm does sell cattle to local smallholder farmers. Additionally, the nutritional management is often very similar particularly in organised smallholder dairy regions where farmers have access to concentrated feeds as is the case in the study area. Collecting data from surrounding affected and non-affected smallholder farms would add weight to the findings. The routine recording of data on various production parameters meant the data were easily extracted and analysed in this study. Such routine recording of data is rarely performed on smallholder farms making retrospective studies more challenging. Overall, it would not be surprising to see a similar impact among smallholder dairy cattle although the overall socio-economic impact is likely to be different in these production systems. Experience from the field reveals that smallholder farmers tend to use less FMD vaccine so the impact on mastitis and culling may be higher.

The statistical modelling for culling suggested that including age and parity improved model fit. There is strong collinearity between these two variables. Since the objective of the study is to look only at the impact of FMD on the primary outcomes, it was decided to include both of these covariates although this restricts the interpretation of their associated effect estimates in the final multivariate regression model.

Survival analysis utilising a Cox proportional hazards regression model relies upon the fundamental assumption that the hazards for comparison groups are proportional over the follow-up period (Collett, 2003). If a model covariate has different effects at different time periods, this can violate this assumption and lead to biased statistical associations. Despite the importance of this assumption many published studies do not provide evidence of the assessment. In one review of clinical trials for human cancer, only 5/64 studies included any form of test for proportionality (Mathoulin-Pelissier et al., 2008). The importance of ensuring the validity of the assumption was particularly clear in this study as adjusting for a time varying effect led to a large difference in the effect of FMD on the two primary outcomes.

In conclusion, this study is the first to utilise survival analysis methods to estimate the effect of FMD on subsequent clinical mastitis and culling. These results offer a detailed assessment of

disease impact that can inform future cost analyses that are currently over-reliant on expert opinion, assumptions and limited use of field data. It is only through performing such studies in different settings that the real impact of FMD can be estimated in endemic countries and inform the cost-effectiveness of national and international disease control programmes.

5.6 Competing interests

The authors declare that they have no competing interests.

5.7 Authors' contributions

NL conceived the study, carried out the field work, led the study design and statistical analysis and wrote the manuscript.

NA participated in the study design and statistical analysis.

KS helped conceive the study and participated in study design and data interpretation.

TD helped conceive the study and facilitated and co-ordinated the field work.

JR helped conceive the study and participated in study design and data interpretation.

PF helped conceive the study, participated in study design and data interpretation and helped draft the manuscript.

All authors read and approved the final manuscript.

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Supplementary material 5.1. Culling – univariable associations with other diseases on Farm 1. Continued on next page.

Disease event	N	Col %	FMD N (row %)	P-value	Culling Rate per 1000 cattle-months (95%CI)	HR (95%CI)	P-value ^b
Abortion							
Yes	16	3.5	14 (87.5)	0.034	25.0 (9.4, 66.5)	2.3 (0.84, 6.3)	0.15
No	628	96.5	386 (61.5)		10.8 (5.8, 13.6)		
Abscess							
Yes	6	0.9	3 (50.0)	0.54	0 (-)	-	-
No	638	99.1	397 (62.2)		11.2 (8.9, 14.1)		
Broken leg							
Yes	1	0.2	1 (100.0)	0.99 ^a	0(-)	-	-
No	643	99.8	399 (62.1)		11.1 (8.9, 14.0)		
Dystocia							
Yes	4	0.6	2 (50.0)	0.62	84.0 (21.0, 336.0)	7.6 (1.9, 31.2)	0.032
No	640	99.4	398 (62.2)		10.8 (8.6, 13.6)		
Eye disease							
Yes	114	17.7	84 (73.7)	0.005	10.2 (5.8, 17.9)	0.9 (0.5, 1.7)	0.74
No	530	82.3	316 (59.6)		11.3 (8.8, 14.5)		
Lameness							
Yes	40	6.2	32 (80.0)	0.016	9.8 (3.7, 26.1)	0.9 (0.3, 2.4)	0.78
No	604	93.8	368 (60.9)		11.2 (8.8, 14.1)		
Dislocated hip							
Yes	1	0.2	1 (100.0)	0.99 ^a	0(-)	-	-
No	643	99.8	399 (62.1)		11.1 (8.9, 14.0)		
Clinical mastitis							
Yes	35	5.4	29 (82.9)	0.009	32.9 (17.7, 61.2)	3.2 (1.6, 6.2)	0.0031
No	609	94.6	371 (60.9)		10.1 (7.9, 12.8)		

Note: Previous disease experienced in the 12 months prior to the commencement of the outbreak and the association with being a case of FMD and culling rate.

^a Fisher's exact test ^b Likelihood ratio test

Supplementary material 5.1. Continued from previous page.

Disease event	N	Col %	FMD N (row %)	P-value	Culling Rate per 1000 cattle-months (95%CI)	HR (95%CI)	P-value ^b
Pneumonia							
Yes	22	3.4	13 (59.1)	0.77	12.3 (4.0, 38.2)	1.1 (0.35, 3.5)	0.85
No	622	96.6	387 (62.2)		11.0 (8.8, 13.9)		
Retained foetal membranes							
Yes	4	0.6	4 (100.0)	0.12	28.1 (4.0, 199.2)	2.4 (0.34, 17.6)	0.38
No	640	99.4	396 (61.9)		11.0 (8.7, 13.8)		
Diarrhoea							
Yes	49	7.6	17 (34.7)	<0.0001	5.5 (1.8, 17.1)	0.48 (0.15, 1.5)	0.17
No	595	92.4	383 (64.4)		11.6 (9.2, 14.6)		
Snake bite							
Yes	1	0.2	0 (0)	0.38 ^a	0 (-)	-	-
No	643	99.8	400 (62.2)		11.1 (8.9, 14.0)		
Three-day sickness							
Yes	3	0.5	3 (100.0)	0.18	0 (-)	-	-
No	641	99.5	397 (61.9)		11.1 (8.9, 14.0)		
Tick-borne disease							
Yes	46	7.1	36 (78.3)	0.019	38.0 (22.9, 63.0)	3.9 (2.2, 6.9)	<0.0001
No	596	92.9	364 (60.9)		9.4 (7.3, 12.1)		
Vulval discharge							
Yes	8	1.2	7 (87.5)	0.14	26.5 (6.6, 106.1)	2.4 (0.59, 9.8)	0.22
No	636	98.8	393 (61.8)		10.9 (8.7, 13.8)		
Wound							
Yes	17	2.6	7 (41.2)	0.071	0 (-)	-	-
No	627	97.4	393 (62.7)		11.4 (9.1, 14.3)		
Any disease							
Yes	269	41.8	185 (68.8)	0.003	14.8 (10.8, 20.1)	1.7 (1.1, 2.7)	0.022
No	375	58.2	215 (57.3)		8.6 (6.1, 12.0)		

Note: Previous disease experienced in the 12 months prior to the commencement of the outbreak and the association with being a case of FMD and culling rate. ^a Fisher's exact test ^b Likelihood ratio test

Supplementary material 5.2. Clinical mastitis - univariable associations with other diseases on Farm 1. Continued on next page.

Disease event	N	Col %	FMD N (row %)	P-value	Clinical mastitis Rate per 1000 cattle-months (95%CI)	HR (95%CI)	P-value ^b
Abortion							
Yes	16	3.9	14 (87.5)	0.39	6.3 (0.9, 44.7)	0.37 (0.05, 2.7)	0.24
No	393	96.1	309 (78.6)		17.6 (13.7, 22.6)		
Abscess							
Yes	5	1.2	2 (40.0)	0.031	0 (-)	-	-
No	404	98.8	321 (79.5)		17.4 (13.6, 22.2)		
Broken leg							
Yes	0	0.0	-	-	0 (-)	-	-
No	409	100.0	323 (79.0)		17.1 (13.4, 21.9)		
Dystocia							
Yes	4	1.0	2 (50.0)	0.15	44.3 (6.2, 314.7)	2.1 (0.29, 15.1)	0.51
No	405	99.0	321 (79.3)		16.9 (13.2, 21.7)		
Eye disease							
Yes	80	19.6	59 (73.8)	0.20	22.8 (14.0, 37.2)	1.4 (0.82, 2.6)	0.22
No	329	80.4	264 (80.2)		15.8 (11.8, 21.0)		
Lameness							
Yes	31	7.6	26 (83.9)	0.49	37.6 (19.6, 72.2)	2.3 (1.1, 4.6)	0.024
No	378	92.4	297 (78.6)		15.7 (12.0, 20.5)		
Dislocated hip							
Yes	1	0.2	1 (100.0)	0.61	0 (-)	-	-
No	408	99.8	322 (78.9)		17.2 (13.4, 22.0)		
Clinical mastitis							
Yes	35	8.6	29 (82.9)	0.56	22.1 (9.9, 49.1)	1.2 (0.53, 2.9)	0.64
No	374	91.4	294 (78.6)		16.7 (12.9, 21.7)		

Note: Previous disease experienced in the 12 months prior to the commencement of the outbreak and the association with being a case of FMD and mastitis rate. ^a Fisher's exact test ^b Likelihood ratio test

Supplementary material 5.2. Continued from previous page.

Disease event	N	Col %	FMD N (row %)	P-value	Clinical mastitis Rate per 1000 cattle-months (95%CI)	HR (95%CI)	P-value^b
Pneumonia							
Yes	13	3.2	10 (76.9)	0.85	22.7 (7.3, 70.5)	1.4 (0.44, 4.4)	0.59
No	396	96.8	313 (79.0)		16.9 (13.1, 21.8)		
Retained foetal membranes							
Yes	4	1.0	4 (100.0)	0.30	68.9 (17.2, 275.6)	3.9 (0.96, 16.1)	0.11
No	405	99.0	319 (78.8)		16.7 (13.0, 21.5)		
Diarrhoea							
Yes	3	0.7	2 (66.7)	0.60	0 (-)	-	-
No	406	99.3	321 (79.1)		17.3 (13.5, 22.1)		
Snake bite							
Yes	1	0.2	0 (0.0)	0.052	0 (-)	-	-
No	409	99.8	323 (79.2)		17.2 (13.4, 22.0)		
Three-day sickness							
Yes	3	0.7	3 (100.0)	0.37	0 (-)	-	-
No	406	99.3	320 (78.8)		17.2 (13.5, 22.1)		
Tick-borne disease							
Yes	42	10.3	35 (83.3)	0.46	29.7 (15.4, 57.0)	1.7 (0.83, 3.4)	0.18
No	367	89.7	288 (78.5)		16.0 (12.2, 20.9)		
Vulval discharge							
Yes	8	2.0	7 (87.5)	0.55	31.1 (7.8, 124.3)	1.7 (0.42, 7.0)	0.49
No	401	98.0	316 (78.8)		16.9 (13.1, 21.7)		
Wound							
Yes	8	2.0	5 (62.5)	0.25	11.9 (1.7, 84.6)	0.74 (0.10, 5.3)	0.75
No	401	98.0	318 (79.3)		17.2 (13.4, 22.1)		
Any disease							
Yes	185	45.2	145 (78.4)	0.79	19.4 (13.7, 27.6)	1.2 (0.75, 2.0)	0.41
No	224	54.8	178 (79.5)		15.3 (10.8, 21.7)		

Note: Previous disease experienced in the 12 months prior to the commencement of the outbreak and the association with being a case of FMD and mastitis rate. ^a Fisher's exact test ^b Likelihood ratio test

Supplementary material 5.3. Schoenfeld residual tests for non-proportionality of each indicator variable prior to model backward fitting.

Variable	Clinical mastitis		Culling	
	χ^2 statistic	P-value	χ^2 statistic	P-value
FMD	2.0	0.16	1.6	0.20
Age (quintiles)				
Quintile 1	-	-	-	-
Quintile 2	0.87	0.35	0.4	0.53
Quintile 3	3.1	0.078	2.2	0.14
Quintile 4	3.7	0.054	0.00	0.96
Quintile 5	3.0	0.089	0.00	0.97
Parity				
0	-	-	-	-
1	0.37	0.54	0.04	0.84
2	0.25	0.62	0.04	0.84
3	0.47	0.50	0.40	0.53
4+	0.35	0.55	0.14	0.71
Lactation stage ^a				
Non-lactating	-	-	10.6	0.0011
Early lactation (<0-100d)	0.53	0.46		
Mid lactation (101-250d)	0.03	0.86		
Late lactation (>250d)	0.07	0.79		
Dry	0.11	0.74		
Tick bone disease ^b	-	-	2.1	0.15
Clinical mastitis ^b	-	-	2.9	0.089
Breed	1.5	0.23		
Any disease ^b			0.2	0.68
Global	32.0	0.0041	39.7	0.0002

^aLRT indicated lactation stage to be categorical in the mastitis model and linear in the culling model. ^bIn previous 12 months prior to commencement of outbreak

Chapter 6. Impact of FMD on milk yield (Research Paper 3)

The following chapter is a research paper including the results of the milk yield analysis from Farm 1. This paper has been submitted to Preventive Veterinary Medicine. The supplementary material is included at the end of the chapter. Further details on data management are in Appendix C.

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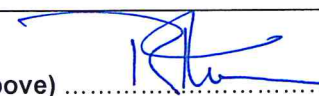
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Impact of foot-and-mouth disease on milk yield in large-scale dairy farming

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6.1 Abstract

The economic impact of FMD has been poorly characterised particularly in endemic settings where such knowledge is essential for making decisions on disease control with limited resources. In order to address this, a study was designed using individual animal data from a large-scale dairy farm in Kenya to estimate the impact of an FMD outbreak due to SAT2 on milk yield. Daily milk yields from 218 mainly European breeds of cattle that were lactating during the 29-day outbreak period were considered in the analysis. At the herd level, the average daily yields decreased from around 20kg to 13kg per cow, recovering approximately 2 months after the commencement of the outbreak. Generalised estimating equations (GEE) and an

autoregressive correlation matrix were used to compare yields of reported clinical FMD cases and non-cases. No difference was found between reported clinical and non-clinical cases suggesting inaccurate case recording, poor sensitivity of the case definition and subclinical infections being present. To further investigate the impact of FMD, yields were predicted for each individual animal based on historic data from the same herd using a similar GEE approach. Comparisons were made between actual and predicted yields from the commencement of the outbreak for cattle lactating during the outbreak using a linear regression model. Animals produced significantly less than predicted if in parity 3 or greater and between 0 and 200 days in milk (DIM). The maximum effect was seen among animals in parity ≥ 4 being between 101 and 200 DIM at the start of the outbreak, producing on average 547.4 kg (95%CI 295.6, 799.1) less milk than predicted for their remaining lactation. Generalisation of the results requires caution as the majority of Kenyan milk is produced in smallholder farms. However, such farms use similar genetics and feeding practices to the one the study is based on and such systems are increasingly important in the supply of milk globally. Therefore the results make an important and unique contribution to the evidence base on FMD impact among dairy cattle in an endemic setting.

Keywords: foot-and-mouth disease; economics; dairy cattle; Kenya; milk yield

6.2 Introduction

Foot-and-mouth Disease (FMD) is caused by a highly transmissible viral infection of cloven-hooved animals responsible for economically devastating outbreaks when introduced into FMD-free countries. Its impact in endemic countries is poorly characterised. Most of the world is endemic except for Europe, North America, Australia and New Zealand. The disease is characterised by lesions on the dental pad, tongue, muzzle, interdigital space and teats. These appear alongside initial pyrexia, dramatic reduction in milk yield and occasional abortion. Animals typically recover clinically within a couple of weeks after onset although secondary bacterial infection particularly in the feet and udder may lead to more protracted disease. It has been claimed that an animal will not recover to their full lactating potential for the remainder of the lactation (Kitching, 2002).

Quantifying the reduction in milk output due to FMD is fundamental to understanding its economic impact in dairy herds and therefore resource allocation for control in endemic settings. This loss has been poorly documented and is often based on farm and expert opinion (James and Rushton, 2002; Knight-Jones and Rushton, 2013). Table 6.1 summarises all the

relevant available studies that could be found in the literature. Studies have estimated milk loss in a variety of different settings and different approaches. In an FMD free setting, a cost-benefit evaluation in the UK estimated that the output of milk would be reduced by 25% in the first four years should the UK become endemic reducing to 12.5% in subsequent years (Power and Harris, 1973). In endemic settings, a survey of expert opinions in Turkey estimated on average a 22% milk yield loss per lactation in Holstein cattle if affected with FMD incurring a financial loss of 266USD (Şentürk and Yalçın, 2008, 2005). The estimated loss was lower for local breeds at 10% and 47USD respectively. Using participatory epidemiological methods, studies among pastoralists have estimated yield reductions of 53% and 62% during the period of illness in Kenya and South Sudan respectively (Barasa et al., 2008; Onono et al., 2013). Post-outbreak surveys in Ethiopia among pastoralist farmers owning Borana cows estimated an average drop in milk yield of 77.3% for an average 25.5 day period representing a 7.7% reduction for the whole lactation (Bayissa et al., 2011). In a chronic form of the condition where a heat intolerance syndrome as a sequela to acute infection has been described (Catley et al., 2004), the loss was estimated at 78% for the lactation. In another Ethiopian post-outbreak survey of pastoral and crop-livestock mixed farming, households reported an average loss of 1.8 litres/cow/day (Jemberu et al., 2014). This was based on farmer estimates prior to and during the outbreak and represented a 75% reduction that lasted for a mean of 23 days (7-35) and 33.6 days (range 7-90) in the respective farming systems. In India, post outbreak surveys estimated FMD to cause a reduction of the national milk output of 6.5% with a 14-19% reduction in the annual yield of an affected animal (Saxena, 1994).

Table 6.1 Summary of studies reporting the impact of FMD on milk yield in cattle.

Country	Study period	Farming systems	Breeds	Type of study	Serotype	Estimated loss	Reference
UK	N/A ^a	UK based dairy farms	Not specified	Expert opinion	Not specified	25% in first four years nationally after incursion into FMD free UK; 12.5% thereafter if becomes endemic	Power and Harris, 1973
Pakistan	1976	Large-scale	Sahiwal	Longitudinal	Not specified	74.4 litres of milk lost per affected lactation	Kazimi and Shah, 1980
Bangladesh	1988-1991	Not specified	Not specified	Post-outbreak farm surveys	Not specified	66% reduction in average daily yield	Chowdbury et al., 1993
India	1991	Smallholders	Indigenous and cross-breed	Post-outbreak farm surveys	Not specified	14-19% reduction in the annual yield of an affected animal	Saxena, 1994
Turkey	N/A	Turkish dairy farms	Holstein-Friesian	Expert opinion	Not specified	22% and 10% milk yield loss in current lactation for Holstein Friesian and local breeds respectively	Şentürk and Yalçın, 2005
South Sudan	2005	Agropastoralists	Indigenous	Post-outbreak interviews (PE ^b methodology)	Not specified	62% reduction while sick. Average 14 day illness. Mean daily loss 1.6 litres per cow compared to normal 2.6 litres	Barasa et al., 2008
Ethiopia	2008	Large-scale	Fogera	Longitudinal	Not specified	50% of pre-outbreak level	Mazengia et al., 2010
Ethiopia	2008	Pastoral and agro-pastoral	Borana	Post-outbreak farm surveys	Not specified	Acute phase: 1.37 litres/cow/day for average 25.5 days (73.3% reduction while sick; 7.7% reduction per lactation). Chronic phase: 0.67 litres/cow/day for 3.8 months (78% reduction per lactation)	Bayissa et al., 2011
Kenya	Not specified	Pastoralists	Indigenous	Farmer surveys (PE methodology)	Not specified	53% reduction in a herd during outbreak period	Onono et al., 2013
Pakistan	Not specified	Smallholder	Not specified	Longitudinal	Not specified	51.8% of potential during outbreak	Ferrari et al., 2013
Ethiopia	2012-2013	Pastoral and smallholder	Indigenous	Post-outbreak farm surveys	O	75% of pre-outbreak level (average loss of 1.8 litres/cow/day)	Jemberu et al., 2014

^a N/A not applicable

^b PE Participatory epidemiology

Empirical data on milk yield impact have been presented in few settings. In Pakistan, examination of milk records for 77 Sahiwal cows revealed the average duration of milk loss to be 17.9 days with 74.4 litres of milk lost per affected lactation (Kazimi and Shah, 1980). Also in Pakistan, Ferrari et al (2013) performed a longitudinal study following clinically affected cows and buffalo² on 50 smallholder farms. A 60-day follow-up period post disease onset was evaluated and included 72 milking cows and 125 milking buffalo. They estimated that cows and buffalo produced only 51.8% and 55.4% of their milk yield potential during this period. A study among Bangladesh cattle found a reduction of average daily yields of 66% but the time period for this reduction was not clearly specified (Chowdbury et al., 1993). An investigation into an outbreak at a government owned farm in Ethiopia examined the daily yields of 14 indigenous Fogera cows affected with clinical FMD. A comparison of the yields produced 10 days prior to disease with the 10 days after revealed an approximate 50% reduction between these two periods (Mazengia et al., 2010).

Kenya is endemic for FMDV with serotypes A, O, SAT1 and SAT2 being present in domestic species (Paton et al., 2009). A nationwide serosurvey performed in 2010 revealed a seroprevalence of 52.5% (Kibore et al., 2013). Kenyans are among the largest consumers of milk in the developing world and consume four times the average for sub-Saharan Africa (Smallholder Dairy Project, 2004). Despite the importance of the Kenyan dairy industry, the impact of FMD has been poorly characterised in the country. A study among Kenyan pastoralists ranked FMD and East coast fever as diseases with the highest impact on livelihoods (Onono et al., 2013). A case-report from a large-scale farm in Kenya estimated milk loss to contribute 42% of the total cost of the outbreak in the 95 day period from the beginning of the outbreak although the exact methodology used in this calculation is not clear (Mulei et al., 2001).

In August/September 2012, an outbreak of FMD due to serotype SAT2 occurred on a large-scale dairy farm in Nakuru County as described elsewhere (Lyons et al., 2015). The regular recording of individual milk yields on this farm presented the opportunity to quantify objectively the impact of disease on milk yields. The objectives of the current study were to estimate the economic impact of FMD infection in commercial dairies by comparing the milk production between clinical FMD cases and non-cases using a linear regression approach and to make comparisons with predicted yields generated through analysis of historic herd data.

² Species not given but presumably Water buffalo, *Bubalus bubalis*

6.3 Materials and methods

6.3.1 Study population

The study farm was a 1600 hectare mixed arable/livestock enterprise located in Nakuru County, Kenya possessing approximately 650 cattle, 100 sheep and 300 goats. The farm typically milked around 200 cattle and had an all year around calving system. Cattle were kept in 18 different management groups, with five of these containing lactating cows. Each group had continuous (24 hour) surveillance by 1-2 farm staff to guard against potential predators and theft in addition to monitoring animal health.

6.3.2 FMD outbreak summary

Details of the outbreak being examined have been published elsewhere (Lyons et al., 2015). Briefly, all new clinical cases were reported daily by the livestock guardians to the farm's livestock manager, who manually recorded the identification number and date. One of the current authors interviewed farm personnel shortly after the outbreak to confirm the case definition. Cases were recorded as having FMD based on visible clinical signs only. Cases were defined as FMD if the cow was reported as having hyperptyalism during the outbreak period with one other clinical sign consistent with FMD. Other clinical signs were not specifically recorded. The index case was recorded on 31st August 2012 with the last clinical case on the 28th September, which defines the outbreak period. Epithelium samples were taken by the government veterinary services and the FMDV serotype confirmed by antigen ELISA as Southern African Territories 2 (SAT2) by the Kenyan National FMD Laboratory, Embakasi. This was later confirmed at the World Reference Laboratory, Pirbright, UK. The farm uses locally produced quadrivalent (O, A, SAT1, SAT2) FMD vaccine (FotivaxTM, Kenya Veterinary Vaccine Production Institute, Embakasi, Nairobi) administered to all animals on the farm irrespective of age every four to six months. The vaccine had very limited or no effectiveness at preventing clinical disease in this outbreak (Lyons et al., 2015).

6.3.3 Milk yield recording

Lactating cows are kept in five groups in separate locations, three of which tend to have more cows in lower parities. Each group is brought to the milking parlour separately twice daily. The parlour is automated and mobile with a semi-fixed location depending on the location of the lactating cow groups. Each individual cow's yield from every milking is weighed by farm personnel and recorded manually before being entered into Microsoft Excel spreadsheets. A

weekly mean is calculated and entered into the Interherd (InterAgri) database associated with the unique animal identification number. The milk yield value for each milk recording date therefore refers to the mean daily yield since the previous recording (approximately seven days). If a cow stops lactating during the week, the denominator is the number of days that week the cow was lactating.

Only cows that were lactating during the outbreak period were considered in this analysis, defined as having at least one milk recording during this period. In order to capture the impact of short lactations, for all cows that were dried off before 305 days and which were either subsequently culled or had a dry period over the farm target of 60 days, zero yield entries were entered up until 305 days. Cows culled before 305 days were similarly treated. This did not apply for cows sold for breeding during lactation.

6.3.4 Statistical analysis

Data were extracted from Interherd (InterAgri) using Access (Microsoft) and imported into Stata 13.0 (Statacorp) for data cleaning and analysis. For comparing the yields of reported clinical FMD cases versus non-cases, due to the expected correlation of repeated measures of milk yields over time for each cow, a generalised estimating equations (GEE) model was utilised with a first order autoregressive correlation matrix. This method has been shown to produce the most appropriate model fit for bovine lactation curves (Wilson et al., 2004). Based on previous analysis, variables for inclusion in the model that may confound the association include the number of days in milk (DIM), breed of cow, parity, and calving date. Number of days in milk at each recording entry was considered as a linear, quadratic and cubic term as well as a categorical variable (50 day categories, 100 day categories, quartiles, and quintiles). Breeds were classified according to the proportion of the genotype ascribed to zebu breeds based on breeding history (100% exotic, <25% zebu, 25-49% zebu, ≥50% zebu). Model fit was assessed using the “quasilikelihood under the independence model information criterion” (QIC) whereby the model with the smallest QIC_u measure is preferred (Hardin and Hilbe, 2003).

In order to compare the actual milk production to what is predicted based on previous lactations by cows in the same herd, available data from all whole lactations (i.e. ≥305 days) prior to the FMD outbreak were extracted from Interherd and a GEE model based on that used by Schukken et al (2009) used for the prediction. The model used was:-

$$\text{Yield} = \text{Parity} + \text{Days in milk} + \text{Season} + e$$

where *Parity* is grouped into 1, 2, 3 and ≥ 4 ; *Days in milk* was the number of days since calving at each recording; *Season* is categorised into four (January/February = 1st dry season, March-May = Long rains, June-September = 2nd dry season, October-December = Short rains); *e* represents the error incorporating the autoregressive (AR1) variance structure. After running this model, an out of sample prediction was made on the dataset from cows lactating during the FMD outbreak. Each animal's total cumulative yield (predicted and actual) from the beginning of the outbreak period to the end of the concomitant lactation was calculated through summing the area under the curve. The difference between the predicted and actual yield was used as the dependent variable in a multiple linear regression model. Parity, days in milk and breed were considered for inclusion in the model based on the result of univariable ANOVA tests, while the Akaike information criterion (AIC) was used to assess the inclusion of variables as linear or categorical. Heteroskedasticity was evaluated through examination of residual plots.

6.4 Results

Two hundred and seventy one cows were eligible for the study having had at least one calf prior to or during the FMD outbreak. Forty-two were dry during the whole outbreak period so were excluded. Seven were dried off on 3rd September 2012 and were excluded from the analysis. Two of these animals subsequently developed clinical FMD but this was not the reason they were dried off. One cow gave birth to a full term calf during the outbreak period having been a clinical FMD case four days before. This cow died two days after calving and did not produce any recordable milk so was excluded from the analysis. Records were absent for three cows leaving 218 cows included for analysis. One cow had two parities during the outbreak period as a management error led to the cow not being dried off at the correct time and she calved whilst still being milked. Each of these parities was treated separately in the analysis so that 219 lactations were included in the analysis.

The study population is described in Table 6.2. At the beginning of the outbreak, the mean age was 4.1 years (median 3.8; range 2.4, 9.7) and the mean number of days in milk was 142.3 (median 130; range -28, 581) with negative values reflecting cows calving during the outbreak period. Seventeen cows had lactations that terminated before 305 days and failed to re-calve. This was either due to early drying off or culling/death. Clinical FMD was recorded in 188/219 (85.8%) of parities considered in the analysis. The demographics and FMD incidence rates for each management group are shown in Table 6.3.

Table 6.2. Description of study population for analysing the impact of FMD on milk yields on a large-scale dairy farm in Nakuru County, Kenya.

Variable	Category	N	Clinical FMD (row %)
Age category	2-<3y	42	31 (73.8)
	3-<4y	90	81 (90.0)
	4-<5y	53	46 (86.8)
	5-<6y	18	16 (88.9)
	6-<7y	7	6 (85.7)
	7-<8y	4	4 (100.0)
	8-<9y	2	2 (100.0)
	9-<10y	3	2 (66.7)
	Parity	1	98
2		74	69 (93.2)
3		28	23 (82.1)
4		12	11 (91.7)
5		4	4 (100.0)
6		3	2 (66.7)
Days in milk	Calved during outbreak	26	21 (80.8)
	1-50	48	40 (83.3)
	51-100	25	21 (84.0)
	101-150	16	14 (87.5)
	151-200	32	26 (81.3)
	201-250	35	34 (97.1)
	251-300	14	12 (85.7)
	301-350	7	7 (100.0)
	>350	16	13 (81.3)
Breed	100% Exotic	183	161 (88.0)
	<25% Zebu	12	11 (91.7)
	25% Zebu	13	9 (69.2)
	50% Zebu	11	7 (63.6)

Note: Cows were included if they had at least one milk recording during the outbreak period (31st August 2012 to 28th September 2012). Two hundred and eighteen cows were included in the analysis; one cow had two parities occurring during the outbreak which were considered separately.

Table 6.3. Summary of management groups containing lactating cattle for analysing the impact of FMD on milk yield on a large-scale dairy farm in Nakuru County, Kenya.

Group ID	Number of cows (range)	Age (mean, range)	Parity (mean, range)	FMD incidence rate (cases per 100 cattle days; 95%CI)
L1	29, 37	3.3 (2.6-4.3)	1.1 (0-2)	5.3 (3.7, 7.5)
L2	28, 30	3.2 (2.4-4.5)	1.1 (1-2)	3.4 (2.2, 5.2)
L3	30, 38	3.3 (2.6-5.6)	1.1 (1-3)	4.1 (2.8, 6.0)
L4	51, 58	5.0 (3.4-9.4)	2.6 (1-6)	5.5 (4.2, 7.3)
L5	50, 57	4.7 (3.4-9.7)	2.5 (1-6)	5.0 (3.8, 6.6)

Note: Cows were included if they had at least one milk recording during the outbreak period (31st August 2012 to 28th September 2012).

The mean daily milk yield for reported FMD cases compared with non-cases is shown in Figure 6.1. Prior to the outbreak, the overall mean daily milk yield was around 20 litres per cow per day, dropping to a low of 13 litres during the outbreak period. After the outbreak, there was an apparent recovery of the yields rising to around 17 litres around 2 months after the beginning of the outbreak before another decrease in mid-December to 10 litres per day. There is also a clear decrease in milk yield in the week prior to observed onset of the outbreak; yields per group alongside the epidemic curves are shown in Supplementary material 6.1. Throughout the analysis period, there appears to have been no significant difference between reported clinical FMD cases and non-cases which is supported by the results of the GEE model (Table 6.4).

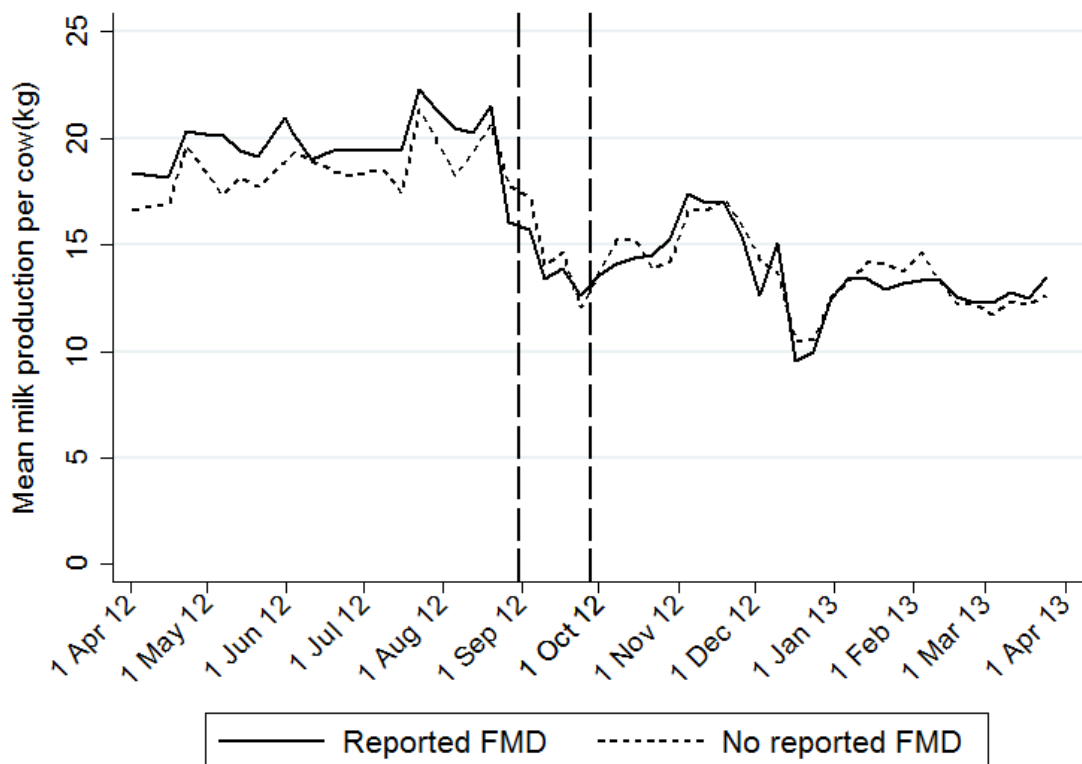


Figure 6.1 Weekly calculated mean daily milk yields for cattle that were lactating during an outbreak of foot-and-mouth disease (FMD) on a large-scale dairy farm in Nakuru County, Kenya. The outbreak period was between 31st August and 28th September 2012 and is represented by vertical dashed lines in the figure. The graph compares reported clinical FMD cases and non-cases for six month periods either side of the outbreak period.

Table 6.4. Results of a generalised estimating equation (GEE) model comparing milk yields of reported clinical FMD cases and non-cases on a large-scale dairy farm in Nakuru County, Kenya.

Variable	Category	Coefficient (95% CI)	SE ^a	P-value
Clinical FMD	Yes	-0.14 (-1.1, 0.82)	0.49	0.77
	No	Reference		
Parity	1	Reference		
	2	0.82 (0.051, 1.6)	0.39	0.037
	3	0.21 (-0.81, 1.2)	0.69	0.69
	≥4	1.1 (-0.13, 2.3)	0.63	0.080
Days in milk	Calved during outbreak	1.5 (0.23, 2.8)	0.39	0.037
	0-50d	Reference		
	51-100d	1.6 (0.39, 2.7)	0.60	0.009
	101-150d	1.3 (-0.0079, 2.6)	0.67	0.051
	151-200d	1.8 (0.78, 2.9)	0.54	0.001
	201-250d	2.5 (1.4, 3.6)	0.55	<0.0001
	251-300d	2.2 (0.72, 3.6)	0.75	0.003
	301-350d	2.0 (-0.083, 4.0)	1.0	0.060
>350d	1.0 (-0.36, 2.5)	0.72	0.15	
Breed	100% Exotic breed	Reference		
	<25% Zebu	-1.1 (-2.5, 0.26)	0.71	0.11
	25-49% Zebu	-1.2 (-2.6, 0.22)	0.73	0.098
	≥50% Zebu	-2.2 (-3.7, -0.75)	0.75	0.003
Constant	-	14.2 (13.0, 15.3)	0.57	<0.0001

Note: An autoregressive (AR1) variance structure was used to account for the correlation of yields for individual animals through time. ^a Standard error

The frequency distribution of actual yields produced from the beginning of the outbreak period is shown in Figure 6.2. Predictions of yields were based on 383 complete lactations finishing prior to the onset of FMD outbreak commencing from May 2005 onwards. A comparison made between predicted and actual yields from the beginning of the outbreak to the end of that animal's lactation revealed no difference based on a paired t-test (mean yields: actual 2156.1 versus predicted 2010.0 litres, mean difference 56.1 litres [95%CI -31.6, 143.9]; P=0.21). Based on ANOVA tests, parity and days in milk at the start of the outbreak were associated with the difference between subsequent predicted and actual milk yields so were included in a multiple linear regression model (Table 6.5). The combinations of the predictors with confidence intervals are shown in Figure 6.3. For parity 1 and 2 animals, there was no effect of lactating during the outbreak on the animal's production compared to what was predicted for the remainder of that animal's lactation regardless of the stage of lactation.

Some animals, notably parity 1 animals at 0-100 and 201-300 DIM, produced significantly more than what was predicted. Parity 3 animals significantly underperformed when in the earlier stages of lactation (0-200 DIM) but no effect was seen for those >200 DIM. For animals in parity 4 or greater, all stages of lactation underperformed even those who were over 300 days in milk at the beginning of the outbreak period. The greatest impact was among older animals in parity 4 or greater at 101-200 days in milk at the beginning of the outbreak. This group produced on average 547.4 litres (95%CI 295.6, 799.1) less than predicted.

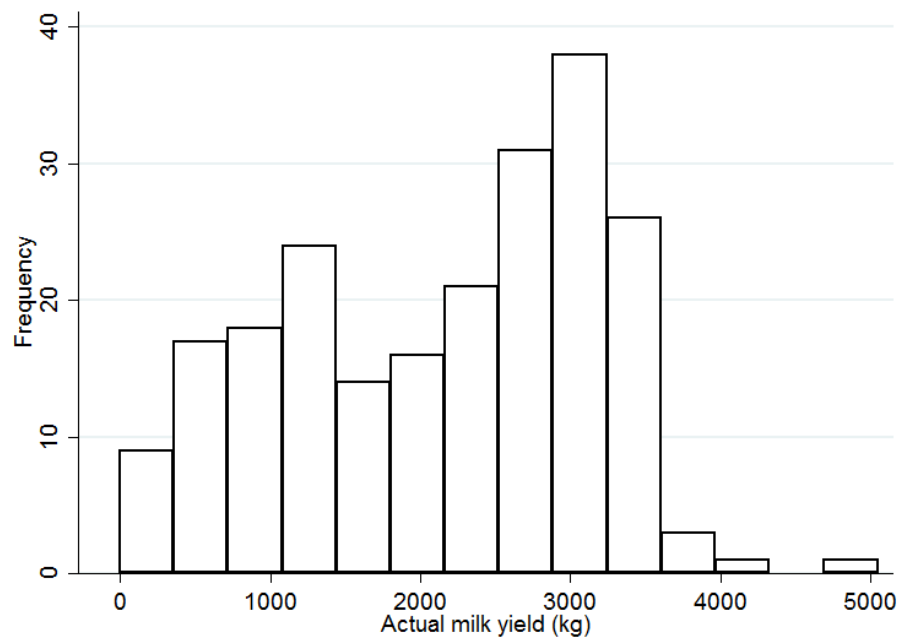


Figure 6.2. Frequency distribution of actual yields produced from the beginning of the outbreak to the end of the animal's lactation for all cows present during an outbreak of foot-and-mouth disease on a large-scale dairy farm in Nakuru County, Kenya. Cows are included if at least one milk recording is made during the outbreak period (31st August-28th September 2012). The average lactation length under consideration was 154.4 days (range 4-344).

Table 6.5. Results of a multiple linear regression model comparing the actual and predicted milk yields for cows lactating during an outbreak of foot-and-mouth disease on a large-scale dairy farm in Nakuru County, Kenya.

Variable	Category	Coefficient (95% CI)	SE ^c	P-value
Parity ^a	-	-214.1 (-304.9, -123.2)	46.1	<0.0001
Days in milk ^b	0-100	Reference	-	-
	101-200	-139.2 (-359.7, 81.2)	111.9	0.22
	201-300	130.8 (-87.6, 349.1)	110.8	0.24
	>300	-53.9 (-339.5, 231.8)	144.9	0.71
Constant	-	448.2 (256.0, 640.4)	97.5	<0.0001

Note: The outcome in the model is the difference between actual and predicted milk yield from the beginning of the outbreak. Predictions are based on historic lactation data from cows in the same herd. Negative coefficient values reflect underperformance producing less milk than predicted. Predicted yields are based on historic data from the same herd. ^a Parity included as an ordered categorical variable, grouped as 1, 2, 3 and ≥4. ^b Includes cows calving during the outbreak period. ^c Standard error

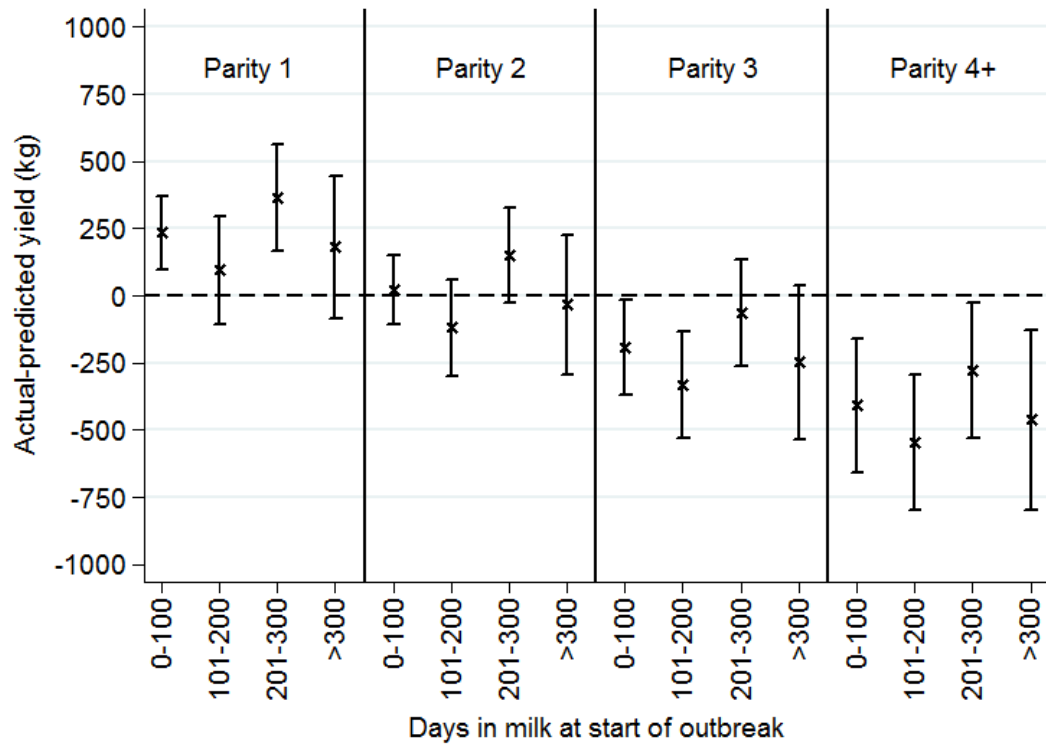


Figure 6.3. Mean difference (with 95% CI) between actual and predicted milk yields for different combinations of predictors based on a multiple linear regression model for cows lactating during an outbreak of foot-and-mouth disease on a large-scale dairy farm in Nakuru County, Kenya. Cows were included in the analysis if they had at least one milk recording during the outbreak period (31st August-28th September 2012). Yields are based on the amount produced from the beginning of the outbreak to the end of the animal's current lactation. Predictions are based on historic lactation data from cows in the same herd. X-axis categories represent the number of days in milk at the beginning of the outbreak (those calving during the outbreak are in the 0-100 category).

6.5 Discussion

The study used an empirical milk yield dataset from a large-scale dairy farm to estimate the impact of a FMD outbreak on subsequent production. A negative effect on milk production was seen at the herd level with a decrease in production from an average of around 20 litres per cow per day decreasing to around 13 litres. On further analysis utilising a GEE model and adjusting for confounding variables, there was no statistical evidence that cows reportedly affected with FMD produced less milk than non-cases. Additionally, no effect on milk production was evident when comparing the actual milk production to that predicted from historic production data for the herd when considering all animals irrespective of reported FMD status. Further analysis revealed that animals in parity 3 or greater did show evidence of poor milk production, particularly if between 0 and 200 days in milk at the beginning of the outbreak period. Possible reasons for lack of difference between reported cases and non-cases include inaccurate case recording, poor sensitivity of the case definition, subclinical infections or inadequate statistical power.

A reduction in milk yield was apparent at the herd level in the week prior to the outbreak being detected which may indicate a drop in yield prior to the onset of clinical signs and therefore may have implications for disease surveillance. The overall length of the milk yield depression is around two month's duration from the beginning of the outbreak period. Despite this clear herd level reduction concomitant with the outbreak onset, the lack of difference between recorded cases and non-cases at the individual level is surprising. Possible reasons for this include inaccurate case recording, poor sensitivity of the case definition and subclinical infections. Poor recording is possible given the large number of cases affected, although each group was attended by herdsman at all times and with the maximum group size of 58 cows, one would expect most affected cows to be detected. Additionally, all reported clinical cases of FMD were treated with oral sodium carbonate necessitating physical handling of the animals, so they could be readily identified and recorded in this context compared to merely observing them from a distance. Every group had clinical cases and it is therefore highly likely every animal was exposed to infection. The case definition for this outbreak was a cow exhibiting hyperptyalism during the outbreak period with any other clinical sign. Hyperptyalism is a useful clinical sign for recording cases of FMD because it is very commonly found, is easy to identify, and does not involve physical examination of the animal. In milder cases of disease, it may be possible for a cow to be "sick" with FMD without showing signs of hyperptyalism and therefore not be recorded as a case. Additionally, vaccination may have attenuated the disease impact

or severity although previous analysis of this outbreak revealed that the vaccine had limited or no effectiveness in preventing clinical disease (Lyons et al., 2015). There could also be a possible genetic reason with animals not showing clinical symptoms being less susceptible to the disease, yet obviously experiencing infection.

Due to the lack of any difference detected between cases and non-cases despite a clear herd-level impact during the outbreak period, the actual and predicted yields of all animals present during the outbreak were compared to explore this further. The results of this analysis revealed a significant impact among older animals of parity 3 and greater. This may be due to a greater degree of hardiness among younger cattle so that they recover more quickly but also because cows in their first and second parity tend to produce less milk over the course of lactation. In this herd, historic milk yield data prior to the outbreak showed parity three and greater cows were the highest yielders in the herd, which is consistent with observations elsewhere for Holstein-Friesian cows (Friggens et al., 1999). In fact, these results show first parity cows tended to produce more milk than predicted for reasons that are unclear but may reflect poor predictive ability of the method especially as milk production in the tropics can be so heavily influenced by seasonal differences in feed and water supply. However, the use of data from 2005-2012 and adjusting for the season when calving commenced should account for some of the variation seen. Parity one animals make up the relative majority of cows in this herd and so the lower impact seen among this group is likely to contribute to the lack of overall effect between reported clinical cases and non-cases apparent in the analysis.

The stage of lactation the animal was in during the outbreak also impacted subsequent production. The normal lactation curve for a cow typically rises to a peak early in lactation before decreasing at a relatively constant rate. It has been shown in Kenyan Holstein-Friesian cows that this peak typically occurs in the third or fourth month of lactation (Njubi et al., 2010). It is therefore logical that the stage at which an animal is affected by disease will influence the impact on milk production. If a cow is affected during its period of peak yield, then the effect may be greater in terms of total lactation yield. This is reflected in the results of this study as animals between 100 and 200 days in milk had the biggest impact when comparing predicted and actual yields. Alternatively, if a cow is affected by a disease event early in lactation, then there is greater potential to recover the 305 day yield, and being affected in late lactation the daily milk yield may be relatively quite low and therefore the impact on the 305 day milk yield would be correspondingly low. The results from this study

indicate that animals may recover their potential lactation contrary to previously published anecdotal evidence (Kitching, 2002).

In other diseases, in which higher yielding animals have been shown to be at higher disease risk, it has been observed previously that when higher yielding animals become sick, the lactation milk yield could still be comparable with that of lower yielding healthy herd mates (Gröhn et al., 1995). Although it cannot be said that higher yielding cows are at increased risk of FMD, particularly as the cows in this study population are lower yielding than typical European based Holstein-Friesians for which the phenomenon has been documented, this cannot be ruled out as a contributory factor.

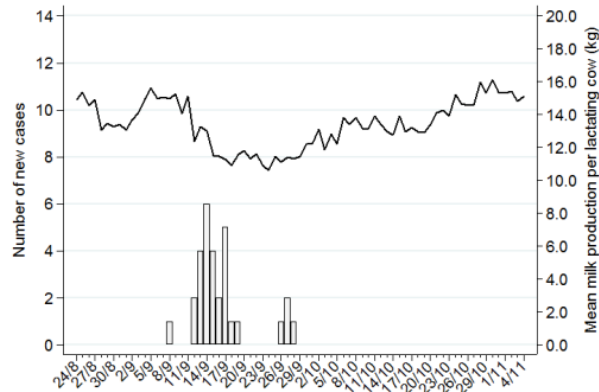
Care must be taken when generalising the study's results to other farms in Kenya as this is only one example from a farm type in a particular area and with a specific serotype and strain of virus. However, in this part of Kenya it is common for smallholder farmers to purchase cows from large-scale farms, and therefore dairy cow genetics is likely to be similar among local smallholder farms. This combined with the common practice of purchasing supplementary feeds through local co-operative organisations means the difference in yields between smallholder and large-scale farms in this region of Kenya may not be as great as one may initially assume. At a global level, these types of systems are increasingly common in milk supply. Further studies collecting objective data on milk yields and other aspects of disease impact from the local smallholder dairy population is needed in order to fully appreciate the impact of FMD in this region of Kenya and in other parts of Africa.

In summary, the impact of FMD is poorly characterised in endemic areas and it is important to publish results based on empirical data in order to contribute to the evidence base rather than relying on post-outbreak estimates or anecdote. The results of this study address this shortfall by quantifying the impact of FMD on a large-scale dairy farm in Kenya. Although there was a clear impact at a herd level, no difference was detectable between reported clinical cases and non-cases. Deeper analysis revealed that cows in parity 3 or greater particularly in the first 200 days of lactation had a significant reduction in milk yield compared to what was predicted. The most severely affected group was cattle of parity 4 or greater at 101-200 days in milk at the start of the outbreak that produced on average 547.4 kg (95%CI 295.6, 799.1) less milk than predicted.

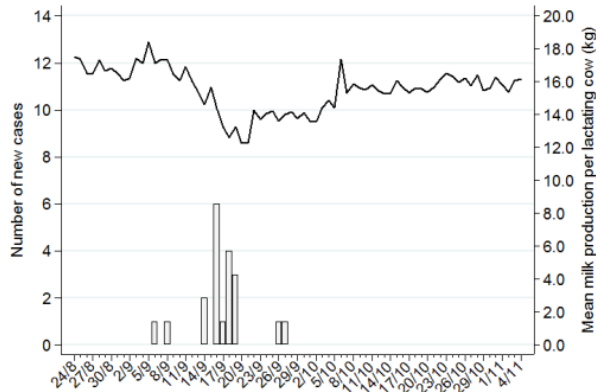
6.6 Acknowledgements

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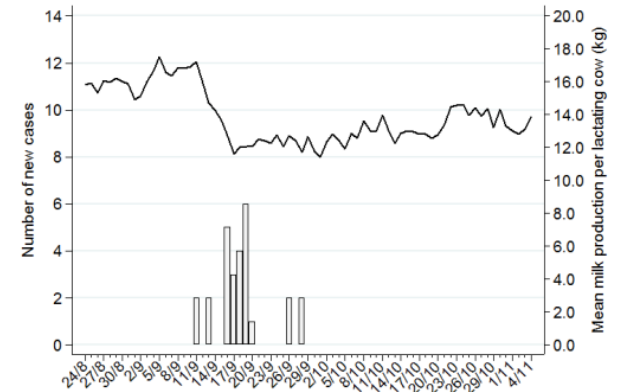
Supplementary material 6.1. Mean daily milk production per group on Farm 1 from one week prior to the farm index case (31/8/2012) to approximately five weeks after the last case (28/9/2012). Values are based on actual daily milk production rather than averaged over the week as in the main analysis in his paper.



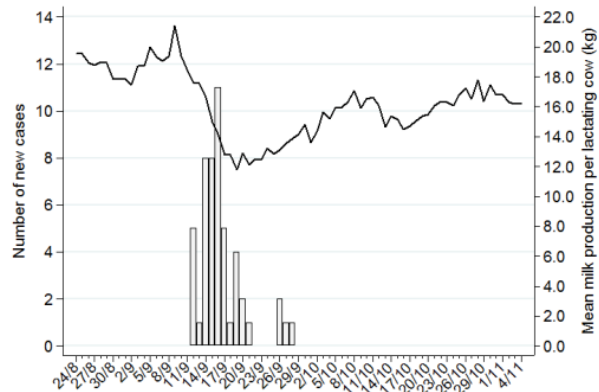
Lactating cows 1



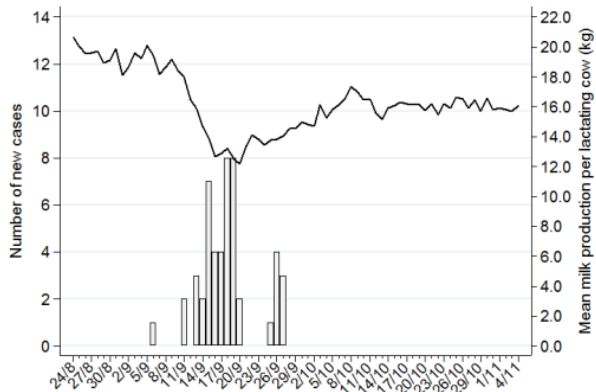
Lactating cows 2



Lactating cows 3



Lactating cows 4



Lactating cows 5

Chapter 7. Farm 2 – Outbreak description and vaccine evaluation

7.1 Introduction

During November 2013, an outbreak of FMD was attended on another large-scale dairy farm in Nakuru County. At the time of the visit, the outbreak had been ongoing for approximately three weeks but the owners had been keeping records of cases as they occurred and were willing to share data for research purposes (Appendix A). The following description and analysis follows the same format as Chapter 4 with a discussion comparing the two outbreaks in Chapter 8 and a further discussion of the implications in Chapter 11. Supplementary materials for this outbreak are at the end of this chapter (page 163). This chapter has not been submitted for publication as a research paper. Further details on data management are in Appendix D.

7.2 Materials and methods

7.2.1 Farm background

The 240 hectare farm was located in Rongai sub-county (formerly district) of Nakuru County near the administrative town of Kambi ya Moto. The farm primarily bred high genetic merit Jersey cattle for milk production and animal sales although Holstein-Friesian (HF) and various Jersey-HF cross-breeds were also present. The farm did not purchase cattle. In addition to the cattle, the farm had approximately 60 horses and numerous dogs. There were no sheep, goats or pigs. The farm was surrounded by a single electrified perimeter fence which was checked twice daily. Apart from small antelope (that may occasionally have transgressed the perimeter fence) there was no FMD susceptible wildlife present on the farm. Although there were other large-scale and smallholder farms in the area, the cattle on this farm did not graze near the perimeter minimising the possibility of direct contact with other livestock.

The farm employed approximately 40 staff of whom 20 had regular contact with the livestock. Some of these workers lived off the farm and may have owned FMD susceptible animals. Staff were assigned to work with a particular management group (e.g. calves) although they assisted with other groups as required.

7.2.2 Study population

The farm had approximately 350 cattle. Animals were managed in nine groups depending on their age, sex and production status (Table 7.1). Calving occurred in the close-up dry cow group ("C"). The dam and calf were moved to the group L1 immediately upon calving where the calf has the opportunity to consume colostrum from the dam. After four days, they were separated with the dam entering the appropriate lactating cow group (L2 if parity one, L3 if >1 parity) and the calves housed as part of group Y1. Male calves not retained for breeding were usually euthanased at this time. Calves were housed in converted stables in groups of up to three individuals for the next two weeks of life before being moved outside if considered strong enough. The movements of calves between housing and paddocks were not recorded (i.e. group Youngstock 1 [Y1] includes housed and non-housed calves) but all other intra-group stock movements were manually recorded. The exact location of groups were not recorded although dry cows were typically located in the centre of the farm with lactating cow groups in various other paddocks depending on grazing availability. Adult bulls were kept in their own individual paddocks separated by electric fencing although "nose-to-nose" contact was possible between them.

All cattle except bull calves destined for euthanasia were individually identified with unique ear tag numbers and names. Calving occurred all year around with breeding through a combination of artificial insemination or natural service with the pedigree Jersey bulls on the farm. No animals with zebu genetic background were present on the farm. Cows were milked through an automated parlour located in the centre of the farm and all lactating cows had daily milk yields recorded. Prior to the FMD outbreak, the mean daily yield was approximately 11 litres per lactating cow. Milk was collected by a tanker every other day. All farm records were paper based.

For external parasite control, all cattle were put through a spray race on the farm once a week.

7.2.3 FMD history and current outbreak

At the onset of the outbreak (21st October 2013), the owners suspected FMD and notified the local government veterinary officer. The officer later attended the outbreak but was unable to collect a sample suitable for diagnosis. The DVO later reported the outbreak to a EuFMD training course that happened to be searching for FMD demonstration cases at the time. A visit was undertaken on the 15th November 2013 at which time several animals were examined

(Appendix E) and epithelium samples from fresh lesions were taken for submission to the National FMD laboratory in Embakasi, Nairobi. Results of the antigen ELISA were positive for serotype O. At the time of writing, no sample had yet been sent to the World Reference Laboratory (WRL).

All cases of FMD were recorded in a treatment book, since all affected cows were treated with oral sodium carbonate (“Magadi soda”) and antibiotics as appropriate. The farmer identified cases on the basis of hyperptyalism with a depressed appetite. Detailed clinical signs were not recorded.

The farm vaccinated for FMD using a locally produced quadrivalent vaccine incorporating strains for the A, O, SAT1 and SAT2 serotypes. All cattle over the age of six months were vaccinated every four months, regardless of concurrent illness or proximity to calving. No second dose was given as part of the primary course. This policy had been performed for several years and all animals present on the farm had been exposed to the same dosing schedule. Vaccination was last performed prior to the outbreak on 12th July 2013. The farm records the batch numbers of all vaccines used, and stored vaccines in a fridge on arrival at the farm prior to use. Vaccines were delivered direct from the manufacturer using a courier with the vaccines packed on ice in sealed insulated containers. On arrival at the farm they were used as soon as possible, usually within a couple of days of delivery.

The farm had experienced previous outbreaks of FMD in March 2004 and December 2010. For the former, serotype SAT2 was detected by the National FMD laboratory, although for the 2010 outbreak no viral antigen could be detected. The identification of individual clinical cases was recorded from both of these outbreaks. The farmer recalled that the clinical signs were mild in the 2010 outbreak which had affected approximately 15% of the herd. The farmer also reported that those affected during the 2010 outbreak tended not to be affected during the 2013 outbreak.

In this 2013 outbreak, FMD had been present on local smallholder farms and movement of personnel was considered the most likely source of introduction.

7.2.4 Data analysis

Each animal on the farm, excluding the bull calves that were destined for euthanasia, had an individual record card that corresponds to the unique ear tag number and name. This card contained all relevant health and breeding events including abortions (Appendix D). FMD

recording was done separately on a treatment sheet according to the case definition given in the previous section (7.2.3). All relevant data were manually entered by the author into a Microsoft Excel spreadsheet prior to import into Stata 13.0 (Statcorp, Texas, USA) for analysis. A cohort study approach was utilised to describe the outbreak. The primary outcome was being recorded as a clinical case of FMD. The entry date into the study was the date of the index case. Cases exited the analysis on the date of disease onset. Non-cases were censored on the date of the last case or the date of exiting the herd for other reasons (e.g. death or being sold). To account for the correlation within groups, a mixed effects Poisson regression model was utilised incorporating group ID as a random effect to generate rate ratios.

Vaccine effectiveness was calculated by the equation $1 - \text{Risk Ratio (RR)}$. Risk ratios were estimated using a generalised linear model (GLM) with a binomial distribution and logarithmic link function. Parameters were estimated using a Fisher scoring optimisation algorithm. The lifetime number of doses received was included as the explanatory variable.

7.3 Results

7.3.1 Demography

During the 59 day outbreak period, a total of 351 live cattle were present on the farm representing a total of 9,699 cattle-days at risk (Table 7.1). Thirty cows gave birth during the outbreak producing 14 and 15 live heifers and bulls respectively (includes one set of male twins). Two heifers were born dead so that a total of 31 calves were born. Twelve other females died during the outbreak (see details below). Of the live bull calves, two were retained in the herd while the remaining were euthanased between zero and five days of age (five were euthanased on day zero, the day of birth). Four adult bulls were present on the farm and kept in individual paddocks (mean age 4.7 years, range 3.9-5.5). One 18 month old bull was kept in the “dry cow and bulling heifers (D/B)” group whilst one four month old bull calf was in the Youngstock 1 (Y1) group.

The most common breed present was purebred Jersey (236/346, 68.2%) followed by HF. A small number of crosses between these breeds were also present (Table 7.2).

Table 7.1. Descriptive data on animals present and FMD incidence by management group during the FMD outbreak on Farm 2.

Group ID	Cattle-days	N ^a	Age (mean, range) ^b	Parity (mean, range)	Lifetime vaccine doses (mean, (median; range))	Incidence rate per 100 cattle-days (95% CI)	Date of onset
Youngstock 1 (Y1)	985	30	21.0 (-49, 142)	N/A	0.0 (-)	0.81 (0.41, 1.6)	11/11/13
Youngstock 2 (Y2)	625	19	163.8 (30, 211)	N/A	0.0 (-)	1.9 (1.1, 3.4)	15/11/13
Youngstock 3 (Y3)	330	44	331.9 (154, 433)	N/A	0.9 (1; 0, 2)	12.1 (8.9, 16.5)	26/10/13
Dry cows and bulling heifers (D/B)	1329	103	3.5 (1.2, 11.7)	1.4 (0, 7)	8.4 (4; 2, 32)	5.4 (4.3, 6.8)	21/10/13
Close-up dry cows (C)	505	57	3.2 (-0.15, 11.7)	2.4 (0, 8)	8.4 (5; 0, 32)	1.2 (0.53, 2.6)	9/11/13
Fresh calvers and sick (L1)	291	49	3.0 (-0.15, 10.4)	2.6 (0, 8)	8.0 (4; 0, 28)	0 (-)	-
Lactating – first calvers (L2)	1206	42	2.8 (1.9, 3.6)	1 (-)	6.6 (6; 4, 9)	2.2 (1.5, 3.3)	2/11/13
Lactating – others (L3)	4288	111	6.6 (2.4, 11.9)	4.3 (0, 10)	17.5 (17; 5, 32)	0.98 (0.72, 1.3)	7/11/13
Bulls (B)	140	4	4.7 (3.8, 5.5)	N/A	12.3 (12.5; 10, 14)	2.1 (0.69, 6.6)	16/11/13
All groups	9699	346	1270 (-56, 4336)	1.9 (0, 10)	8.6 (6; 0, 32)	2.2 (1.9, 2.5)	21/10/13

Note: Age and parity are based on the status at the start of outbreak (21st October). All animals were kept in freely mixing groups except Youngstock 1 that are housed in groups of up to three calves for the first two weeks. The five bull calves euthanased on the day of birth are excluded from the analysis. N/A = not applicable. ^a For groups, total number of individual cattle that were in the group at some stage of the outbreak. ^b Days for youngstock and “All groups”, years for other groups. Negative values reflect animals being born during the outbreak.

7.3.2 FMD outbreak description

The outbreak commenced on the 21st October with the last case detected on 18th December. The overall incidence risk was 211/346 (60.1%). The epidemic curve for the outbreak is shown in Figure 7.1 with individual group curves shown in Supplementary material 7.1. The index case was among the dry cows and bulling heifers group, located in a central farm location (Figure 7.1). The final case was a calf born during the outbreak. This animal was 19 days old at disease onset which began after it left the indoor housing and entered the outdoor free-mixing paddock. The previous case, with onset 13 days previously, was also in the outdoor calf group. Both of these cases were in the sub-group of Y1 at pasture and no cases were seen among calves in the housed area. Clinical signs observed during the outbreak include hyperptyalism, milk drop, inappetence and oral, feet and teat vesicles (Appendix E). The farm reported that the mean daily milk yield fell as low as 8.5 litres per cow during the outbreak.

A network of direct contacts between groups during the outbreak was elucidated with help from the farm staff (Figure 7.2). Approximate group locations are provided in Figure 7.3. Three clusters of inter-group direct contacts can be seen. The outbreak was initially among the D/B and Y3 groups which were in direct contact with each other but not with the other management groups. Group Y3 became affected five days after the onset of disease in group D/B, consistent with the incubation period for FMDV. A second wave of cases (Figure 7.1) began with group L2 and spread to all other cattle groups that were interconnected through likely direct contacts (Figure 7.2). The last group affected was the bulls (B) which had no direct contacts during the outbreak. The weighted mean group incidence rate was 2.2 (95%CI 0.84-5.2) per 100 cattle-days, varying from 0 to 12.1 between groups. Although group L1 appears to have had no cases, several affected animals were present in group L1 but all had disease onset in other groups, either before entering this group or after departing.

Twelve animals exited the herd during the outbreak. Four cows were culled for reasons unrelated to FMD and went direct to slaughter. Of the remaining eight, three died suddenly with no clinical signs. These animals were aged between four and five months. No post-mortem was performed but it is possible that they died as a consequence of FMDV infection which is known to cause deaths among cattle of this age. The five remaining deaths were all ascribed to FMD according to the farmer. One was a six month old calf that died the day following disease onset. The remaining were all adult cows (ages 2.6, 3.5, 6.4 and 10.6 years) that died between seven and 19 days after FMD onset showing gradual deterioration and

failing to respond to supportive treatment. Excluding the cull cows, euthanased bull calves and stillborn, the overall mortality rate was 8/331 (2.4%). No abortions were recorded during the outbreak.

Interestingly the farmer reported two cases that developed clinical lesions and then relapsed with fresh lesions after apparent recovery. The first was a five year old cow that showed clinical signs of FMD on the 12th November which then calved on the 20th November, moved groups as per normal farm routine, before fresh lesions were observed again on the 30th November. A similar course was seen in a seven month old calf that showed fresh lesions 16 days apart although with no associated movement between groups. For the analysis, both of these were included as single cases on the date lesions were first recorded.

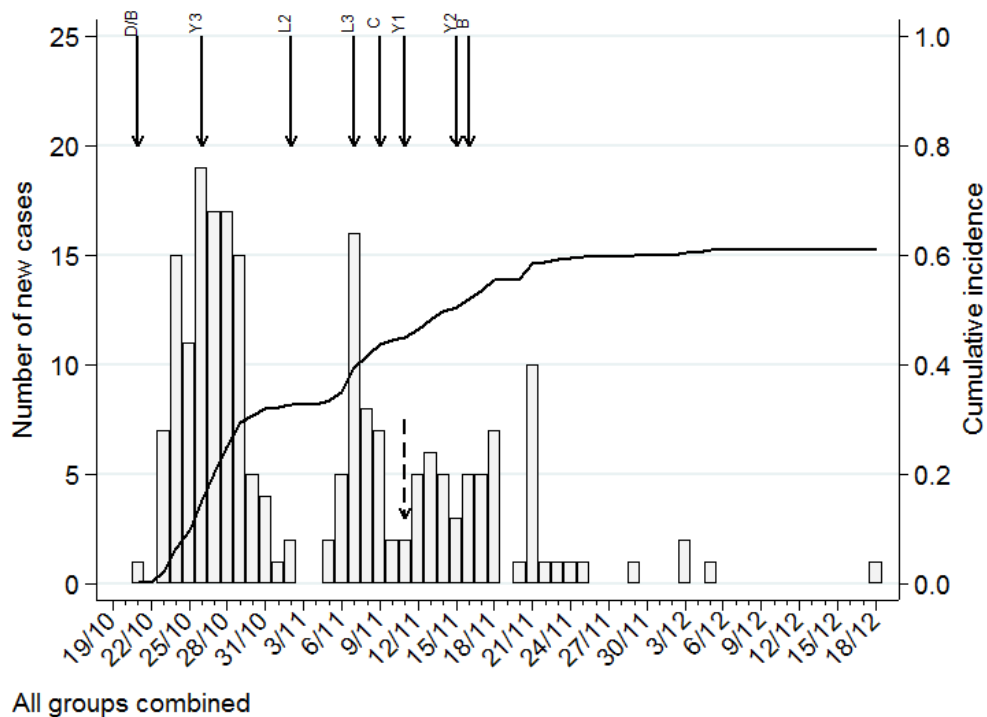
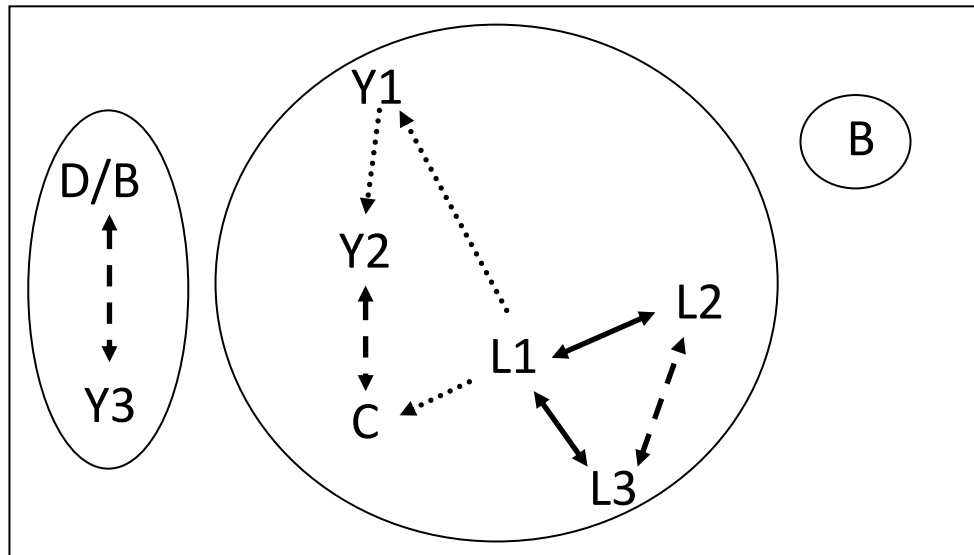


Figure 7.1. Epidemic curve and cumulative incidence of FMD for all management groups combined on Farm 2. Solid arrows indicate the dates of FMD onset within each group. Dashed arrow represents the timing of vaccination (11th November). Denominator for cumulative incidence is the mean daily number of cattle present on the farm over the outbreak period (21st October-18th December). See Supplementary material 7.1 for individual group epidemic curves.



-→ Contact due to animal movements between paddocks
- Contact from being in neighbouring paddocks
- Contact from both animal movements and being in neighbouring paddocks

Figure 7.2. Schematic outline of contacts between different management groups during the outbreak period on Farm 2 showing three clusters of direct contacts (each circled). Contact is defined by animals potentially having “nose-to-nose” interactions that are represented by arrows. Arrow direction reflects the direction contacts may occur (i.e. the direction of animal movement). L2 and L3 both had “over-the-fence” contact with L1 when taken to the parlour for milking when a nose-to-nose interaction was possible. Group B had no direct contacts. The index case was in group D/B.

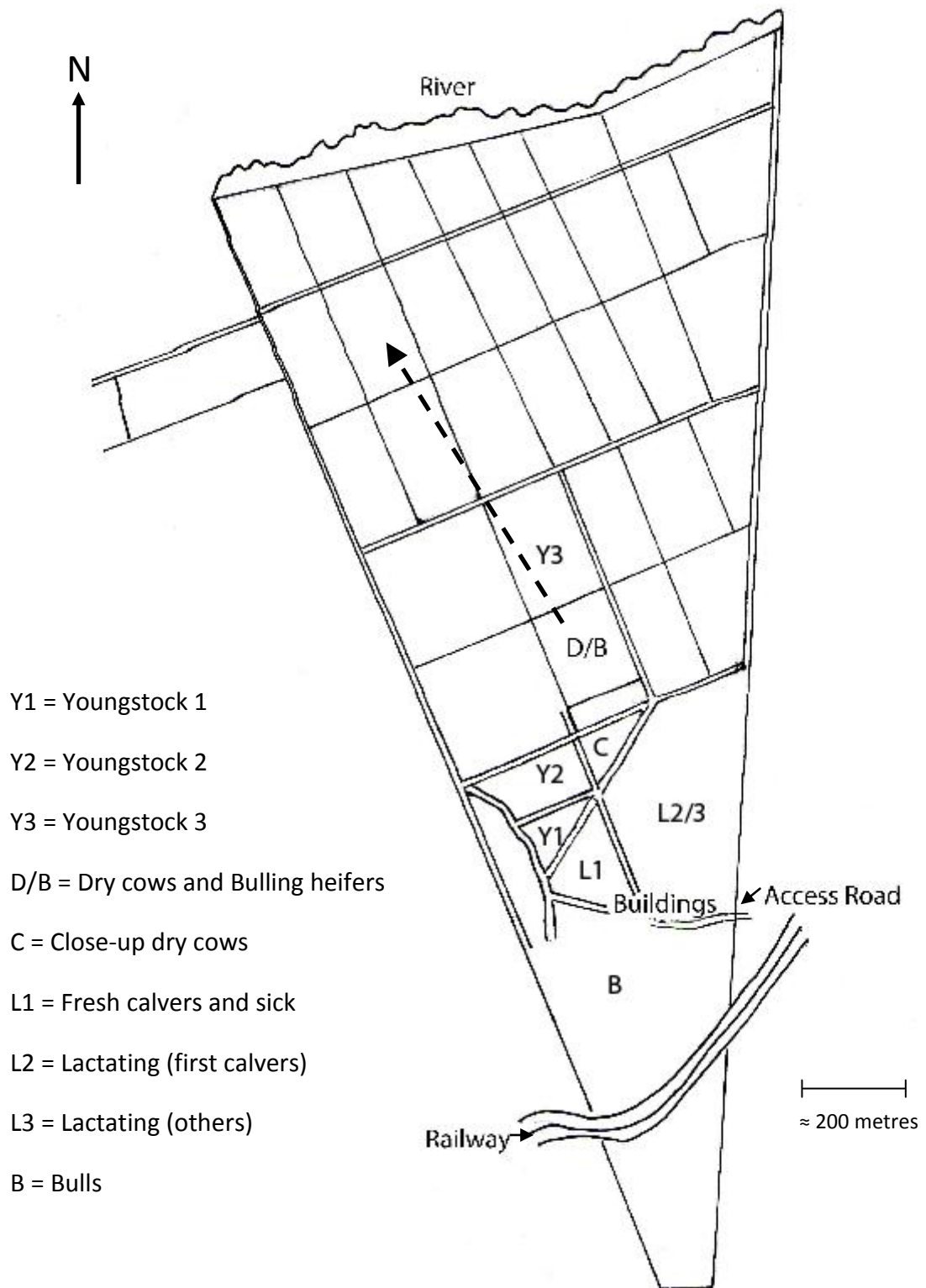


Figure 7.3. Outline of Farm 2 showing paddock and group locations during outbreak. Groups L2 and L3 were in neighbouring paddocks not marked on map. The index case was in group D/B. Dashed arrow represents movement of the D/B group at the start of the outbreak as an attempt at isolation. Single lines represent a single fence whereas roads are represented by two parallel lines. “Buildings” show where the parlour was located.

7.3.3 Farm response to FMD outbreak

The first cases were in the dry cow and bulling heifer group (D/B) which had only had contact with group Y3 but was located in a central part of the farm. On seeing the cases, the farmer attempted to isolate the affected group by moving it to an area further away from the centre of the farm (represented by the dashed arrow in Figure 7.3). Cases began in group Y3 (which were not moved) five days later and thereafter cases began in other groups. No other attempts at isolation were made. All affected animals were treated with oral sodium carbonate and topical/parenteral antibiotics as deemed appropriate by farm staff. This was performed in a crush located in the middle of the farm in close proximity to the buildings highlighted in Figure 7.3.

Vaccination was performed on the 11th November (day 22 of the outbreak), in line with the scheduled four monthly routine, not as an additional “reactive” vaccination. After discussions with other large-scale farmers, the decision was made to vaccinate all animals irrespective of age differing from their previous vaccination programme (described in section 7.2.3). The farm was intending to continue this practice in future due to a perceived lack of effectiveness of the previously used schedule.

7.3.4 Patterns by age, sex, parity, lactation, breed and previous disease status

The incidence was highest (over 90%) in animals aged between six and 18 months of age. At older ages the incidence showed a gradual decline up to four years of age when the incidence plateaus at 40-50%. The incidence among animals younger than six months of age was similar to that among animals over four years of age (Table 7.2). After adjusting for the management group, the rate ratio increased to a peak in the 1-1.5 year age group and then declined to a plateau from ≥ 4 years.

When examining the effect of parity, animals in parity two or greater had a significantly lower rate of disease compared to the baseline parity zero category. There was no significant difference between cattle in the parity zero and parity one categories (Table 7.2).

The incidence risk was higher among female animals, but this was likely to be a function of age (and/or number of lifetime vaccine doses received) since most males were over four years of age. Indeed when adjusted for management group, there was no statistical evidence for this effect (Table 7.2). There was no significant effect of lactation stage or breed on the risk or rate of disease.

Of the animals present during the current outbreak, 159 were present during the December 2010 outbreak of which 13 (8.2%) had been clinical cases. For animals present during the 2010 outbreak, those that had clinical disease during that outbreak had an overall lower incidence in the 2013 outbreak although the difference was not statistically significant (Incidence in 2010 cases = 5/13 [38.5%]; incidence in non-2010 cases = 71/146 [48.6%]; $P=0.48$). Nineteen animals present during the current outbreak had been present in March 2004 outbreak but similarly there was no association with those diseased then and being subsequently affected in the 2013 outbreak (Incidence in 2004 cases = 5/12 [41.7%]; incidence in non-2004 cases = 2/7 [28.6%]; $P=0.66$ [Fisher's exact test]).

Table 7.2. Descriptive risk factor analysis evaluating associations with being a case of FMD on Farm 2.

Variable	N	FMD cases (row%)	P-value ^a	Rate ratio (95%CI)	P-value ^b
Age category (n=351)					
Born during outbreak	29	2 (6.9)		0.17 (0.028, 0.97)	0.047
<6 months	24	12 (50.0)		0.51 (0.17, 1.5)	0.23
6m-<1y	27	25 (92.6)		1.46 (0.66, 3.3)	0.35
1-<1.5y	44	43 (97.7)		1.75 (0.94, 3.3)	0.077
1.5-<2y	27	22 (81.5)		0.90 (0.47, 1.7)	0.74
2-<3y	50	36 (72.0)		Baseline	-
3-<4y	33	23 (69.7)		0.80 (0.45, 1.4)	0.45
4-<5y	22	10 (45.5)	<0.0001	0.31 (0.14, 0.69)	0.004
5-<6y	12	3 (25.0)		0.11 (0.033, 0.39)	0.001
6-<7y	20	7 (35.0)		0.22 (0.088, 0.53)	0.001
7-<8y	27	12 (44.4)		0.26 (0.13, 0.55)	<0.0001
8-<9y	14	7 (50.0)		0.45 (0.18, 1.1)	0.079
9-<10y	12	6 (50.0)		0.37 (0.15, 0.94)	0.037
10-<11y	5	3 (60.0)		0.45 (0.13, 1.5)	0.20
11-<12y	5	0 (0.0)		-	-
Sex (n=351)					
Female	330	206 (62.4)	0.0005	Baseline	-
Male	21	5 (23.8)		1.3 (0.31, 5.4)	0.72
Parity (n=330)					
0	152	118 (77.6)		Baseline	-
1	52	36 (69.2)		0.64 (0.30, 1.3)	0.25
2	26	10 (38.5)	<0.0001	0.14 (0.065, 0.32)	<0.0001
3	16	9 (56.3)		0.25 (0.11, 0.55)	0.001
≥4	84	33 (39.3)		0.15 (0.085, 0.26)	<0.0001
Lactation stage (n=182)					
Early-lactation (<0-100d)	61	27 (44.3)		Baseline	-
Mid-lactation (101-250d)	61	32 (52.5)	0.48	1.2 (0.73, 2.1)	0.42
Late-lactation (>250d)	44	23 (52.3)		1.2 (0.68, 2.1)	0.53
Dry	16	8 (50.0)		1.1 (0.42, 2.7)	0.89
Breed (n=346)^c					
Holstein Friesian (HF)	80	44 (55.0)		0.78 (0.56, 1.1)	0.16
HF crossbreed	5	3 (60.0)		1.1 (0.35, 3.6)	0.83
HF x Jersey	15	6 (40.0)	0.27	0.41 (0.18, 0.92)	0.032
Jersey	236	151 (64.0)		Baseline	-
Jersey crossbreed	10	7 (70.0)		0.83 (0.39, 1.8)	0.64

Note: Rate ratios are generated from Poisson regression incorporating group ID as a random effect. Five bull calves euthanased at birth not included in rate ratio calculation. Animals are categorised based on the status at the beginning of the outbreak except lactation stage. Cows calving during the outbreak are included in the early-lactation category. Baseline groups are selected based on having the highest number of animals. ^a Chi-square test. Chi-square test for trend used for parity and lactation stage. ^b Wald test. ^c Not available for bull calves euthanased on day of birth

7.3.5 Patterns by lifetime vaccine doses received

Figure 7.4 shows FMD incidence by numbers of vaccine doses received. The highest incidence of FMD was among animals that had received one dose of vaccine. A gradual decline in the incidence can be seen with the number of doses received, reaching a plateau at around 11 doses. The risk of FMD was similar between animals that received no doses of vaccine (which according to the schedules used on this farm are mostly below the age of six months) and animals that received approximately nine or more doses of vaccine. The incidence among older animals could be confounded by exposure in previous outbreaks although there was no association between cows reported to have been affected and being clinically affected during the current outbreak. The protection seen in animals that had received no doses of vaccines is likely due to passive immunity from their dams.

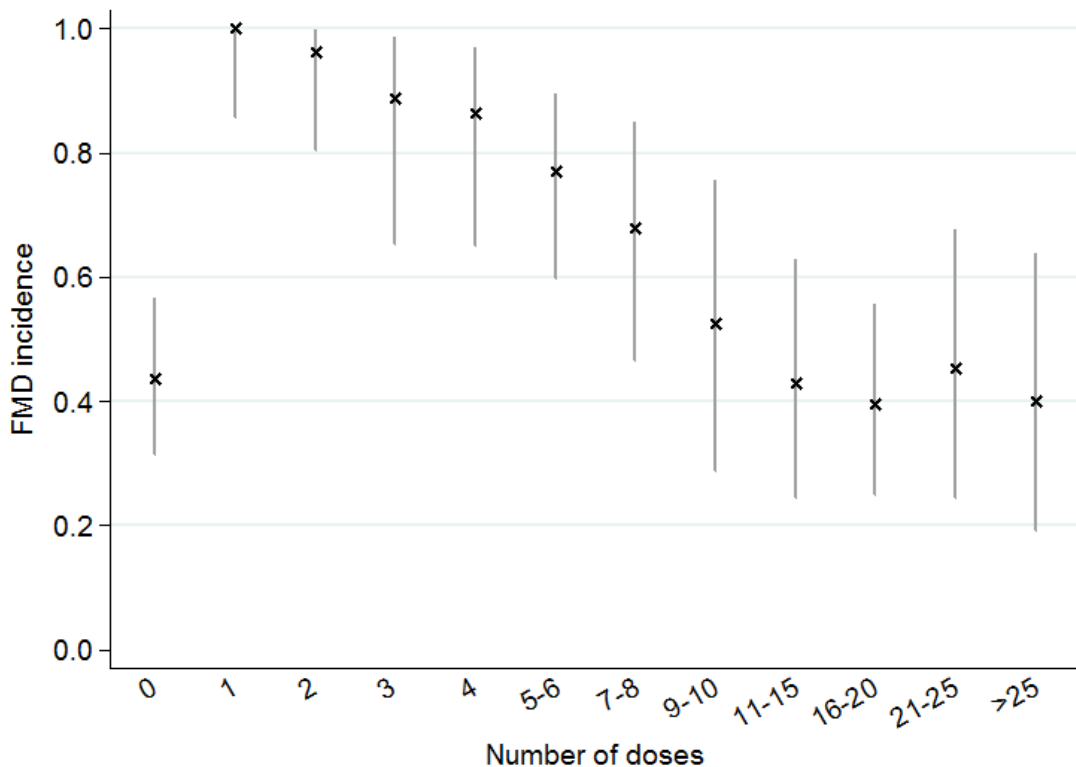


Figure 7.4. Incidence risk (with 95% confidence intervals) of FMD by the lifetime number of FMD vaccine doses given to all animals present on Farm 2 during the outbreak period (21st October-18th December 2013). Number of doses does not include the vaccination given during the outbreak.

7.3.5.1 Estimation of relative vaccine effectiveness

Animals below the age of six months were excluded due to the effect of maternal antibody which from the farm's vaccine policy removed all animals receiving zero doses of vaccine from this analysis. Due to the non-linear association between number of doses received and FMD incidence risk (Figure 7.4), the former was included as a categorical variable. The baseline group was animals receiving one or two doses of vaccine because the incidence risk in those receiving one dose was 100% prohibiting estimation of risk ratios using a GLM. Figure 7.5 shows how the relative vaccine effectiveness varies with the number of doses received. Evidence of a gradual increase in protection with an increase in the number of doses can be seen reaching a plateau at ≥ 11 doses with a relative effectiveness of around 50-60%. Due to the absolute collinearity between the number of doses received and age, the latter could not be adjusted for in the statistical model.

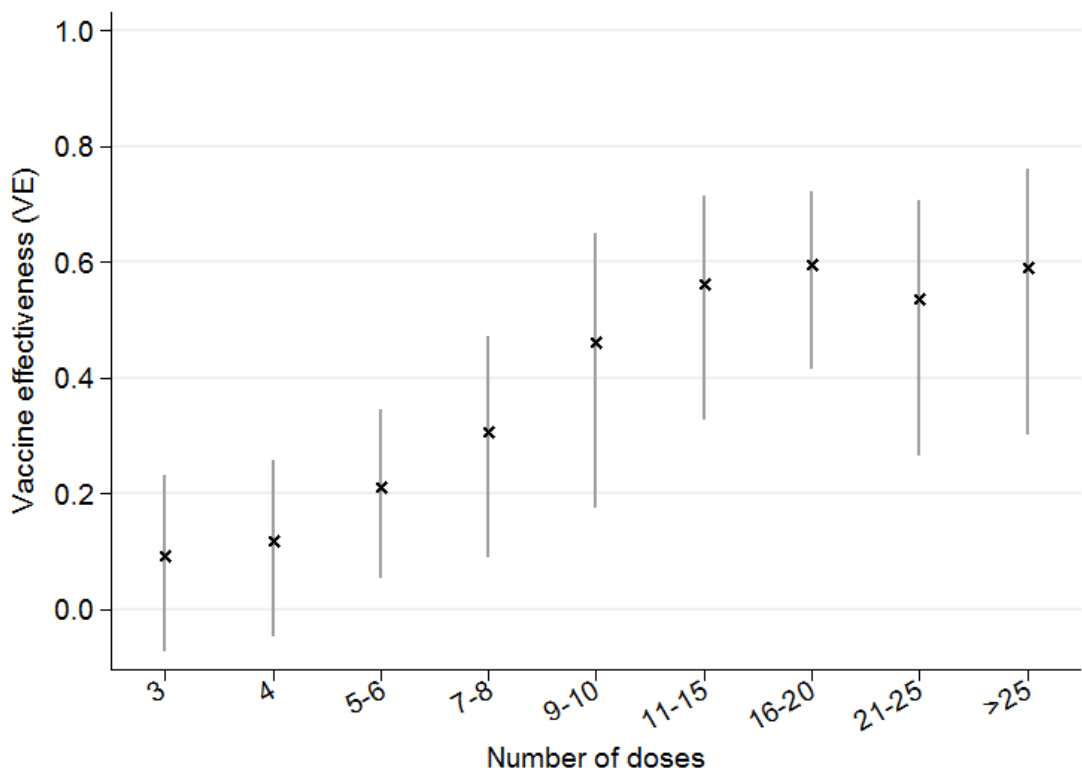


Figure 7.5. Relative vaccine effectiveness by the number of lifetime doses of vaccine received with 95% confidence intervals for Farm 2. The baseline category is animals receiving 1 or two doses of vaccine. Vaccine effectiveness is calculated from the equation $1 - \text{risk ratio}$. Risk ratios were estimated using a GLM with a log link and binomial distribution.

7.4 Discussion

The main findings from this outbreak are the high incidence among animals up to the age of four years with evidence of vaccine protection only being apparent after animals had received several doses of vaccine. Evidence was also found of a protective effect likely due to passive immunity from the dam shown by the relatively low disease among animals less than six months of age. Neither breed nor lactation stage was associated with disease incidence. Females were at higher risk than males but this is likely confounded by age and the number of doses of vaccine received.

The outbreak originated in a central farm location among the dry cows. This suggests that introduction by direct contact through the perimeter is unlikely. With no animals being purchased and a lack of contact between visiting people and vehicles with the livestock, the most likely route of introduction was indirectly through workers living off the farm. This is an issue that should be addressed by farm owners, who should consider implementing appropriate biosecurity measures for high risk workers (i.e. those that own livestock) particularly when FMD is known to be in the area (see further discussion on farm biosecurity in section 11.4.1). Once on the farm, infection initially spread between two groups that were in neighbouring paddocks (Figure 7.2). Infection then spread to the main cluster of groups that were all connected by direct contacts (Figure 7.2). This led to the second peak of cases evident in Figure 7.1. The last affected group was the bulls that had no direct contact with any other groups during the outbreak. With farm staff moving between groups and clusters without using adequate bio-security, indirect spread through personnel is likely to have been the main route of spread between the three clusters. Possible ways of limiting the spread on a farm once disease is detected is discussed in detail in section 11.4.1.

The incidence among young calves was low probably due to the presence of maternal derived antibody (MDA). The number of doses of vaccine that lactating cows had received is relatively high by virtue of their age with cows on the farm typically calving for the first time between two and three years of age (Table 7.1). The antibody levels in calves would be expected to reflect this older, more heavily vaccinated population through passive acquired MDA consumed in colostrum until the age of around six months after which time MDA has typically waned (Nicholls et al., 1984). It is therefore interesting to see that the incidence among these older cows is very similar to that among the calves below the age of six months that had received no vaccine but has recently received MDA.

The incidence peaked between ages six and 18 months and then declined, appearing to plateau from the age of four years onwards. In terms of the number of doses of vaccine and the relative vaccine effectiveness calculation, some protection was apparent in animals receiving over five doses of vaccine but the best protection was seen among animals receiving 11 or more doses. Compared to a baseline of one or two doses, the maximum vaccine effectiveness was around 50%.

Although being affected in a previous outbreak was not associated with being a case in the study outbreak, there still could be age related effects that were not accounted for in this analysis. For example, some residual immunity cannot be ruled out. However the serotype in 2004 was SAT2 and there is not thought to be any cross protection between serotypes with FMDV (OIE, 2009). The serotype from the 2010 outbreak is unknown but it should be borne in mind that animals in previous outbreaks are also older and this would confound the association through having a higher number of doses of vaccine. This correlation means that the relative vaccine effectiveness estimates are limited as they cannot be adjusted for age. As highlighted in section 4.5, the inability to adjust for the possible confounding effect of age in the model is a major limitation of the approach and highlights the difficulties in evaluating vaccine effectiveness on herds using a routine vaccine policy applied to all animals at the same time.

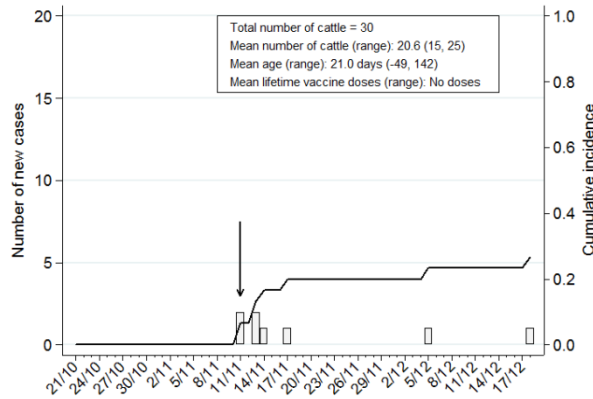
The timing of vaccination during the outbreak was in keeping with the routine vaccination schedule the farm administers every four months rather than as part a reactive vaccination to limit the impact of the outbreak. As can be seen in Figure 7.1, by the time the vaccine was administered, six groups had already reported cases and the two remaining groups (Y2 and B) developed disease four and five days later respectively. This is unlikely to be enough time to allow the vaccination to work particularly when virus exposure is likely to have occurred so soon after administration. This combined with the small number of animals in these groups and being the first dose of vaccine received by group Y2 (and so has no appropriate comparison group) evaluation of the effectiveness of this strategy was not possible from this outbreak.

Also of note in this outbreak is the mortality among adults. Usually FMD is associated with mortality in younger animals connected to myocarditis. In this study outbreak, three sudden deaths were seen in youngstock and one further young animal died after developing FMD lesions. Four further deaths were seen in adults that the farmer ascribed to FMD. Rather than an acute mortality as seen with myocarditis, these were related to prolonged anorexia.

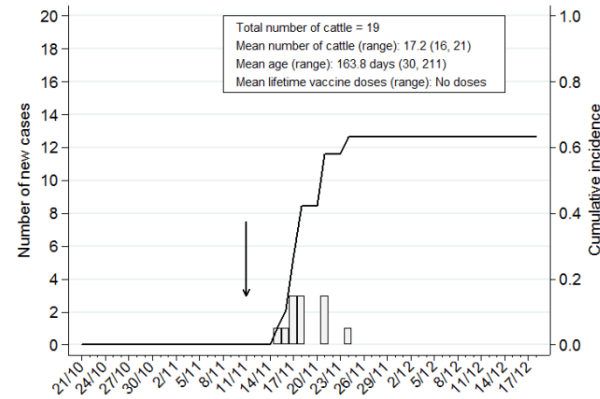
Although unconfirmed, this report suggests that the impact of FMD is more severe among adults than usually thought although the overall mortality was consistent with other reports (for further discussion, see section 8.3).

Further discussion of this outbreak and a comparison with the observations on Farm 1 are presented in Chapter 8 and Chapter 11.

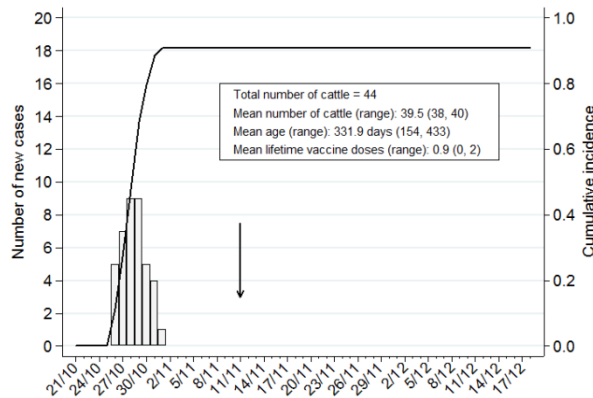
Supplementary material 7.1. Epidemic curves for each group on Farm 2. Note there is no curve presented for group L1 because no cases began in this group although diseased animals did pass through after disease onset. Arrow represents the date of vaccination during the outbreak. Continued on next page.



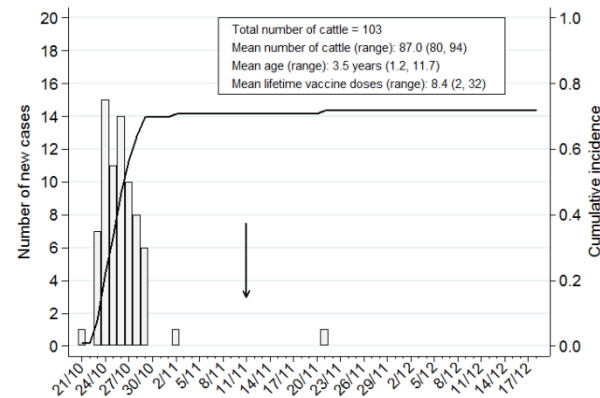
Youngstock 1



Youngstock 2

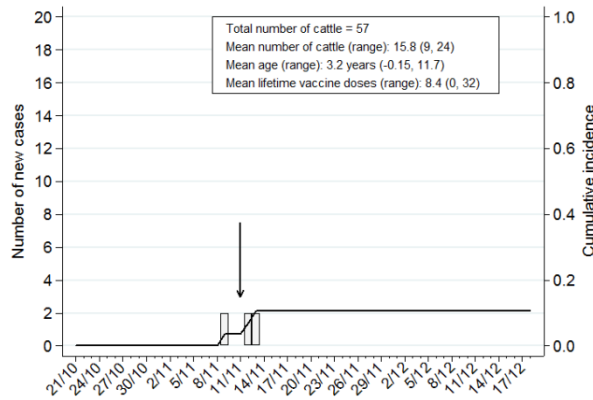


Youngstock 3

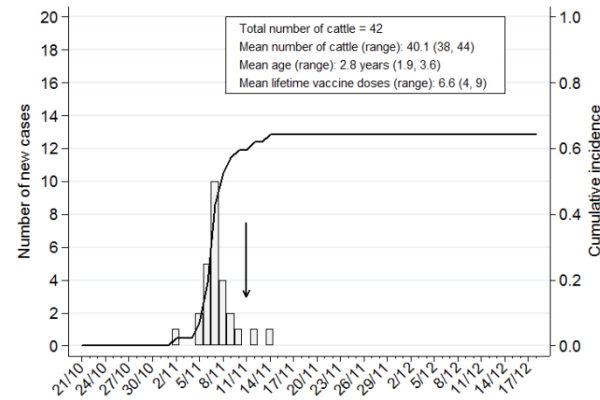


Dry cows and bulling heifers

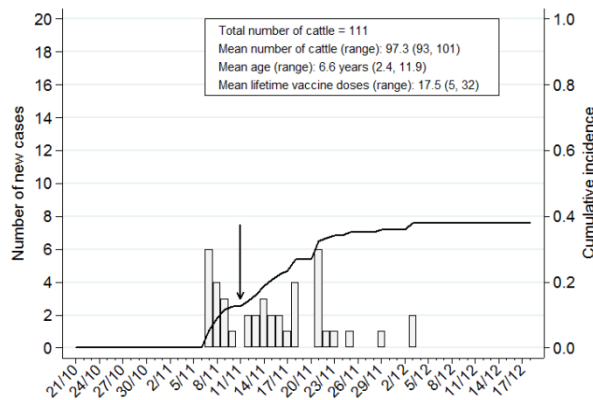
Supplementary material 7.1. Continued from previous page.



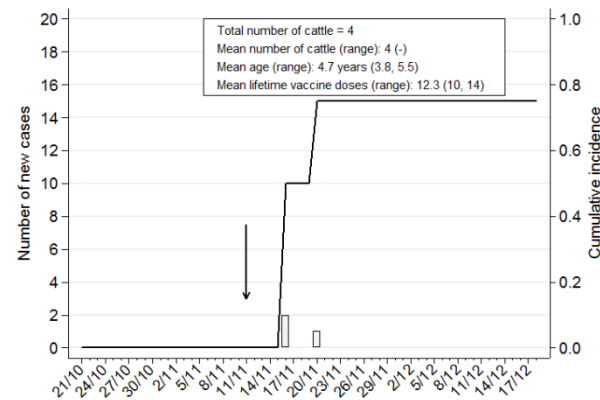
Close-up dry cows



Lactating - first calvers



Lactating - others



Bulls

Chapter 8. Discussion of the FMD outbreaks on Farm 1 and Farm 2

Two outbreaks of FMD on large-scale dairy farms that use prophylactic vaccination have been described in detail in Chapter 4 and Chapter 7. There are several key similarities and differences between these outbreaks that give insights into FMD epidemiology and aspects of vaccine performance. The following is a comparison of these outbreaks and a discussion of the implications of the findings. Both farms used locally produced vaccine manufactured by the same company. Major features of the farms, the animals and the FMD outbreaks are summarised in Table 8.1.

8.1 Farm backgrounds

Both farms are located in Rongai Sub-county of Nakuru County and are approximately 14km apart (or 20km by road). Both are large-scale dairy farms producing milk that is sold to a dairy company. Both breed pedigree cattle with Farm 1 (Chapter 4, from here referred to as Farm1/SAT2) in particular focussing on receiving income through the sale of in-calf heifers and first lactation animals. Compared to Farm 2 (Chapter 7, from here referred to Farm 2/O), Farm 1/SAT2 has more animals (including small ruminants), employs more people and also receives significant additional income through the sale of crops. Both calve all year around and use artificial insemination for breeding, although Farm 2/O also uses natural service through home-bred bulls.

8.2 Farm demographics

Due to different breeding and sales policies, the age distributions differ between the herds as shown in their respective population pyramids (Figure 8.1). The number of males is low in both herds as they are both dairy farms that usually remove males at an early age or use sexed semen as with Farm 1/SAT2. There are obvious differences in the age distributions, with Farm 1/SAT2 being a younger herd and most animals tending to be between the ages of 1 and 4 years. This reflects their policy of selling heifers or first lactation animals as an important source of income and tending not to retain older animals. In contrast Farm 2/O has a much broader age distribution. This reflects their policy of retaining cows for repeated breeding and milk production.

There were some differences in the breeds on the farms, with Farm 1/SAT2 incorporating limited zebu genetics, but the large majority of animals were of European varieties.

Table 8.1. Summary of Farm 1 and Farm 2 outbreak characteristics.

	Farm 1 (Chapter 4)	Farm 2 (Chapter 7)
Farm		
Number of susceptible cattle	644	346
Farm size (ha)	1600	240
Number of livestock workers	25	20
FMD vaccine schedule	All animals every 4-6 months	All animals over 6 months old every 4 months
Animals		
Mean yield pre-outbreak (per lactating cow per day)	17.0kg	11.0 litres
Age at start of outbreak (days; mean, median, range)	850.5 (746.5; -28, 3543)	1270 (919.5; -56, 4336)
Number of management groups	18	9
Predominant breed	Holstein-Friesian crossbred (248/644, 38.5%)	Jersey (236/346, 68.2%)
Small ruminants on farm	Yes	No
Outbreak		
Dates	31 st August – 28 th September 2012	21 st October -18 th December 2013
Length of outbreak (days)	29	59
Serotype	SAT2	O
Overall incidence	400/644 (62.1%)	211/346 (60.1%)
Mortality rate	3/644 (0.47%)	8/331 (2.4%)
Vaccine doses (mean, median, range)	5.0 (5; 0-13)	8.6 (6; 0, 32)
Days between last vaccination and index case	99	99

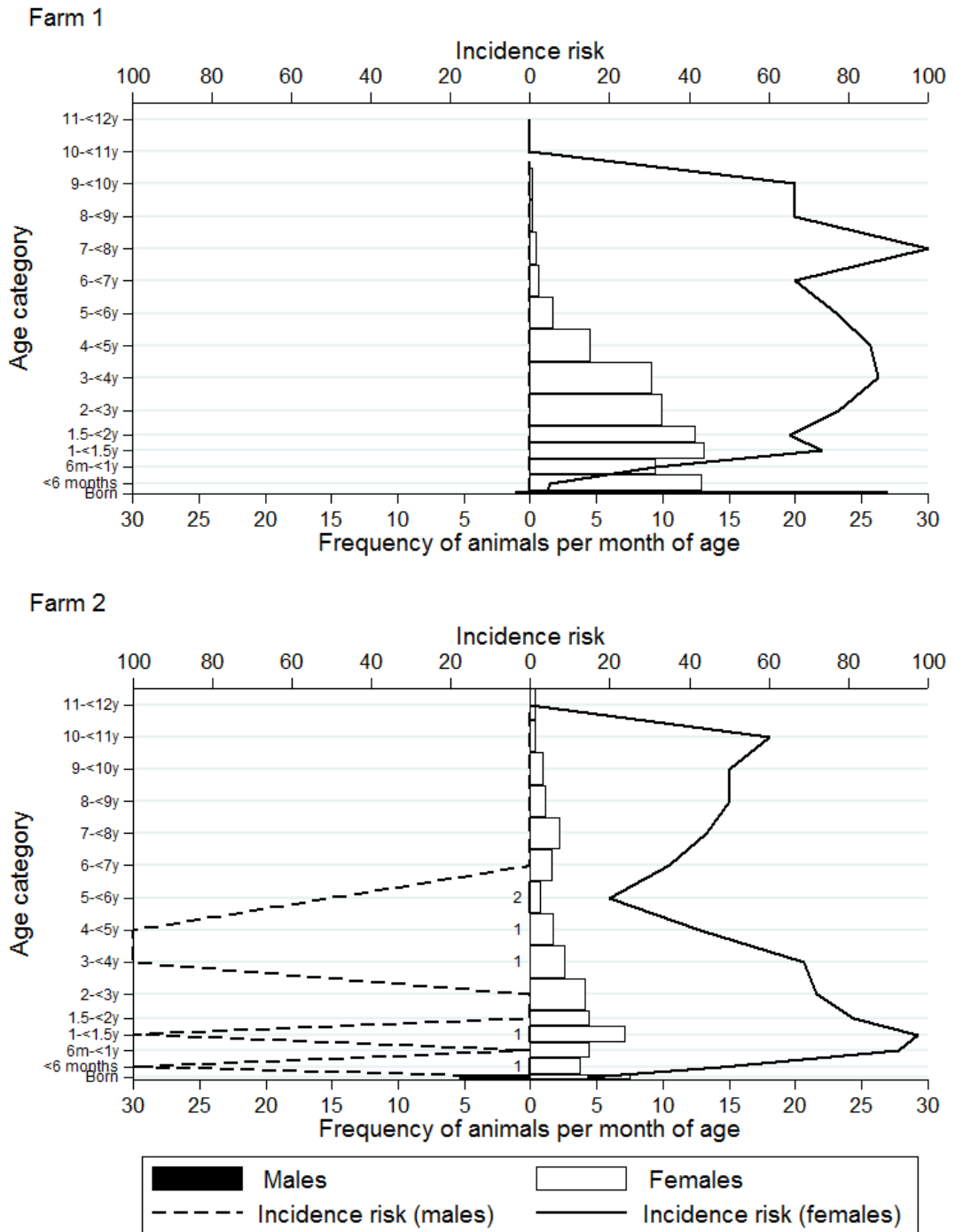


Figure 8.1. Population pyramid and age specific incidence for Farm 1/SAT2 and Farm 2/O. Top graph = Farm 1/SAT2, Bottom graph = Farm 2/O. Males are represented on the left whilst females are on the right. The age used was the age at the beginning of the outbreak period. Age category “Born” means the animals were born during the outbreak. So that each age category is appropriately adjusted the bottom x-axis is the frequency of animals in the age category divided by the number of months the category contains. In farm 2 where there were small numbers of males that cannot be easily visualised so numbers have been inserted to represent the number of males in that age category. Farm 1 only has males in the “Born” category. On Farm 2, the five male calves that were euthanased on the day of birth are not included.

8.3 Outbreak descriptions

The two outbreaks were caused by different serotypes: SAT2 and O for Farms 1 and 2 respectively. The epidemic curves are similar with two main peaks of cases (Figure 8.2) comparable to that seen in another outbreak on a Kenyan commercial dairy farm (Figure 1.6, Gakuya et al. (2011)). This coincides with the onset of cases in different groups reflecting the spread of infection around the farms. The intervals between peaks on the farms are 10 and 12 days for Farms 1 and 2 respectively.

In both outbreaks, the source is likely to have involved farm workers bringing virus onto the farm, perhaps on clothing or other contaminated items. The reasons for this speculation are partly by exclusion of other possible transmission routes and particular circumstances surrounding the outbreaks. Both farms have a secure perimeter fence; both restrict animal grazing near the farm boundary; both have a policy of not buying in animals; and both allow limited access of visitors to the animals. In both instances, the outbreak began in a central farm location (i.e. not near the farm boundary) and there are a large number of livestock workers some of whom live off the farm and own their own FMD susceptible livestock. On both farms, outbreaks were also present in the surrounding area on smallholder farms with the same serotype detected in samples submitted to the National FMD laboratory.

The overall *incidence* of FMD for both outbreaks is comparable at around 60% (Table 8.1). This is similar to the 95/166 (57.2%) and 123/207 (59.4%) reported on other commercial dairy farms in Kenya due to serotypes SAT1 and O respectively in which the routine vaccination also appeared ineffective (Gakuya et al., 2011; Mulei et al., 2001). The incidence was much lower than that reported by Kimani et al. (2005), who described an overall morbidity rate of 2211/2430 (91%) on four large-scale dairy farms in Kenya which had not been vaccinated for the responsible serotype (SAT1) in the previous five years. Closer examination of the age-incidence patterns on the farms in this study shows some differences (Figure 8.3). The incidence risk on Farm 1/SAT2 *increased* with age up to the 1-1.5 year age category then plateaued around 70-90% thereafter, with all the confidence intervals overlapping. In contrast on Farm 2/O, the incidence peaked in animals between the ages of six months and 1.5 years, being nearly 100% in the 1-1.5 year age group, and then gradually *declined* to a plateau of around 40-50%. Examination of the confidence intervals shows that the six month to 1.5 year old age categories were at significantly higher risk than those of four years and above. A similar incidence between younger (<six months old) and older animals (Figure 8.3) is consistent with a protective effect of maternal derived antibody (MDA) for calves less than six

months of age. Age-incidence patterns were not presented in the other outbreaks reported on Kenyan large-scale farms with the objectives of those studies being FMD economics or treatment effectiveness (Gakuya et al., 2011; Kimani et al., 2005; Mulei et al., 2001).

A different pattern was seen on Farm 1/SAT2 with a comparatively low incidence in younger animals compared to a high incidence in their respective dams. As discussed in Chapter 4, one reason for this pattern on Farm 1/SAT2 could be that the particular strain of virus only caused mild clinical signs among younger animals in comparison to the strain on Farm 2/O that caused relatively severe clinical signs. Alternatively, case ascertainment among calves may have been poorer on Farm 1/SAT2 although a similar case definition was used by both farms. It is also possible that MDA could have had a protective effect on Farm 1/SAT2 if the level of antibody in the colostrum and thus the calf was at a higher level than their dams and therefore were more protected (i.e. immunity wanes quicker for the dam after vaccination than it does for calves that source their immunity through colostrum). With the last vaccination being over three months prior to the start of the outbreak, this scenario seems unlikely.

The overall **mortality rates** were similar at 3/644 (0.47%, 95% CI 0.16-1.4) and 8/331 (2.4%, 95% CI 1.2-4.7) for Farm 1/SAT2 and Farm 2/O respectively. Although deaths in adults were reported on both farms, the overall mortality was similar or lower than other reports in the literature from endemic settings. In Ethiopia, Negussie et al. (2011) reported an overall mortality rate of 0.3% (19/7,127, 95% CI 0.17-0.42) among cattle from eight investigated outbreaks. Chowdbury et al. (1993) reported a 50.9% mortality rate among calves in Bangladesh, although the definition of a calf and the rate among older animals was not presented. Among Kenyan pastoralists, an overall mortality rate of 3% has been estimated (Onono et al., 2013). A study of four Kenyan large-scale dairy farms reported an overall mortality rate of 200/2430 (8.2%, 95%CI 7.2-9.4) (Kimani et al., 2005). These outbreaks had been due to serotype SAT1 for which no recent vaccination for the SAT1 serotype had been performed. Another report from Kenya revealed a mortality of 9/207 (4.3%, 95%CI 2.3-8.1) on a vaccinated herd affected with serotype O that had been vaccinated for this serotype 104 days prior to the index case (Mulei et al., 2001). Based on the 95% confidence intervals, all three Kenyan large-scale farms that had an outbreak despite vaccination appear to be significantly lower than the outbreak that had an outbreak with no history of recent vaccination. This implies that the vaccine may have had some protective effect against death. However, these estimates are unadjusted and don't account for baseline mortality rate on these farms so caution should be taken when comparing them.

Management systems for calves varied between Farm 1/SAT2 and Farm 2/O which may have affected the rate of virus transmission and levels of immunity. On Farm 1/SAT2, calves were immediately placed into individual hutches in a paddock up until the age of eight weeks (group Y1) when they were moved into free mixing groups (Y2). Exposure by direct contact in the hutches was unlikely with indirect routes such as through shared equipment being more likely. On Farm 2/O, they were housed in a converted stable until the age of two weeks in groups of up to three individuals per stable. Thereafter they were placed into free mixing groups (all included in group Y1). Therefore one would expect more effective contacts in this setting compared to Farm 1/SAT2.

All calves on Farm 1/SAT2 were fed four litres of pooled colostrum by farm workers. This is more likely to have led to more consistent levels of immunity among the calves compared to Farm 2/O that were left to suckle from their dams. Holstein-Friesians have been associated with producing lower quality colostrum (i.e. with lower IgG1 concentrations) compared to other dairy breeds (Godden, 2008). Although Farm 1/SAT2 had a greater proportion of Holstein-Friesian pedigree than Farm 2/O, this is unlikely to have made a significant difference to the colostrum quality as these cows were lower yielding than those under the European or North American system in which poor quality colostrum is usually associated. Moreover, Farm 2/O had a significant Holstein-Friesian presence on the farm (Table 7.2).

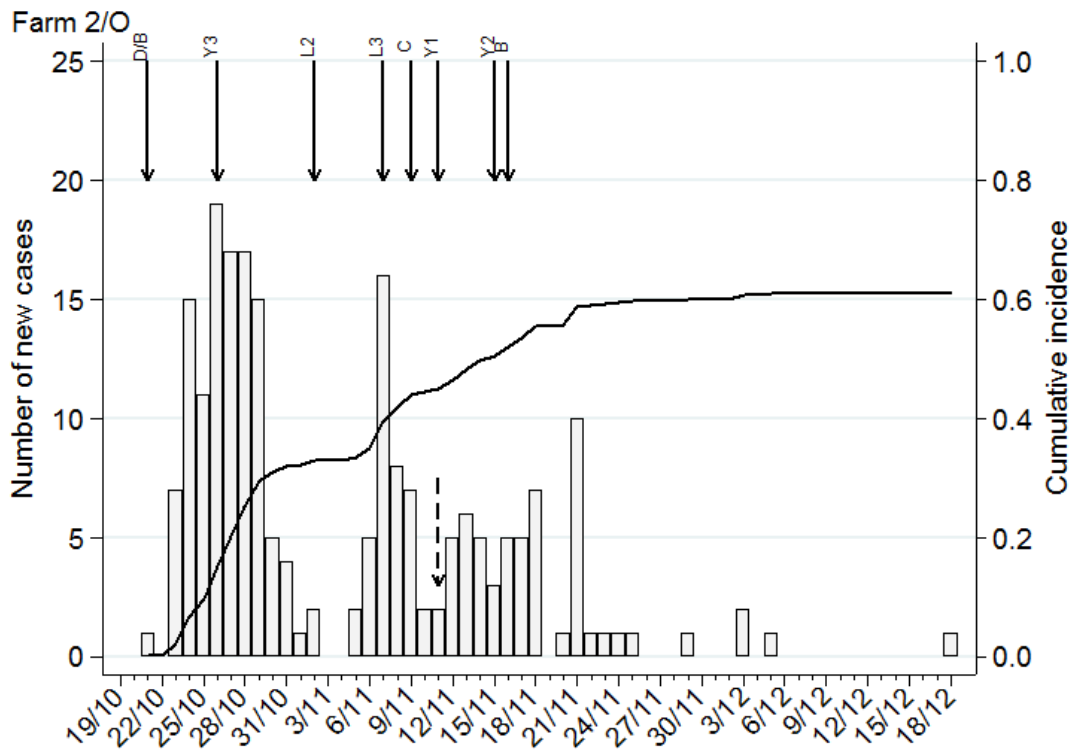
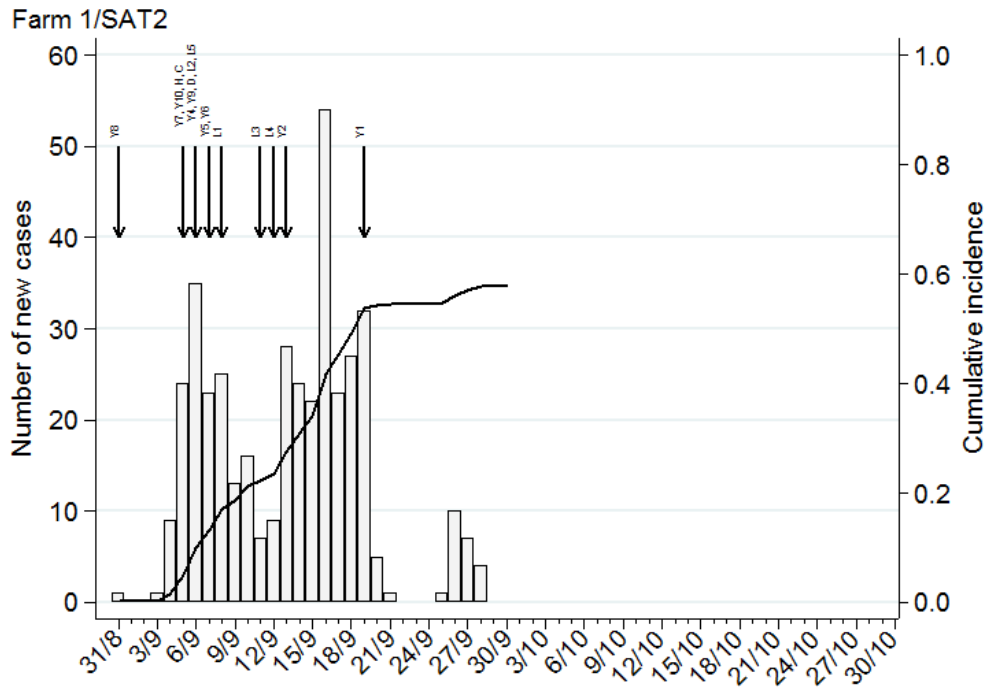


Figure 8.2. Epidemic curves for Farm 1/SAT2 and Farm 2/O with solid arrows showing the onset of cases in each affected group. Dashed arrow for Farm 2/O represents the vaccination date. To ease comparison, the time scale has been modified for Farm 1/SAT2 compared to that shown in Figure 4.2.

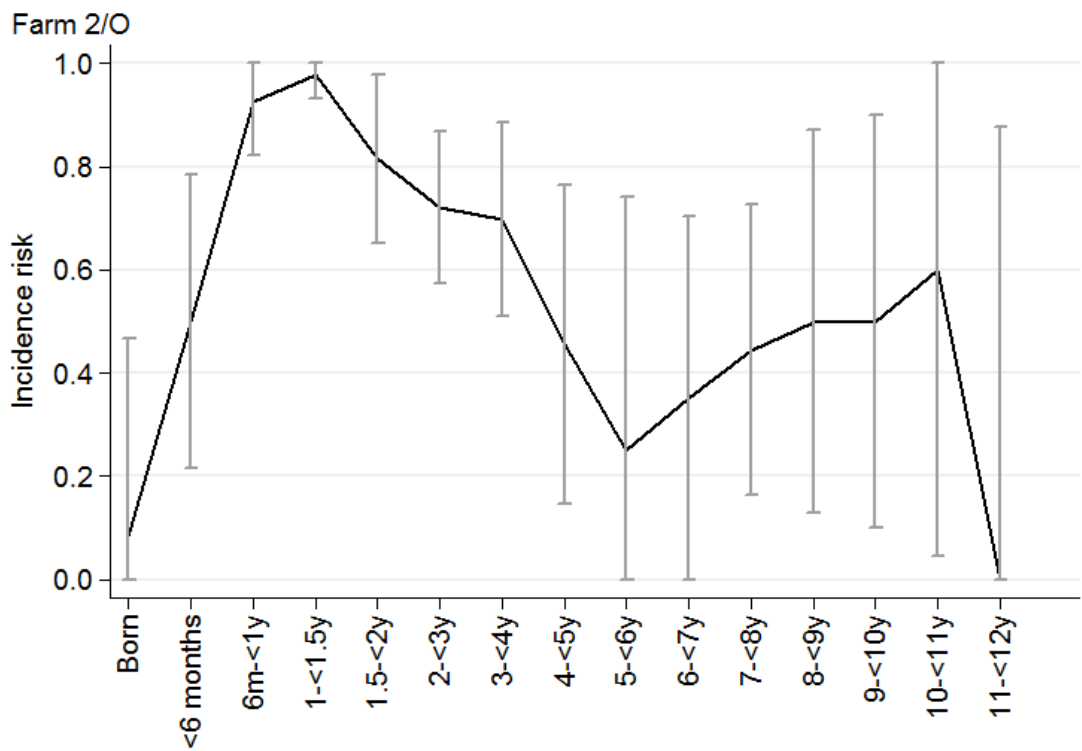
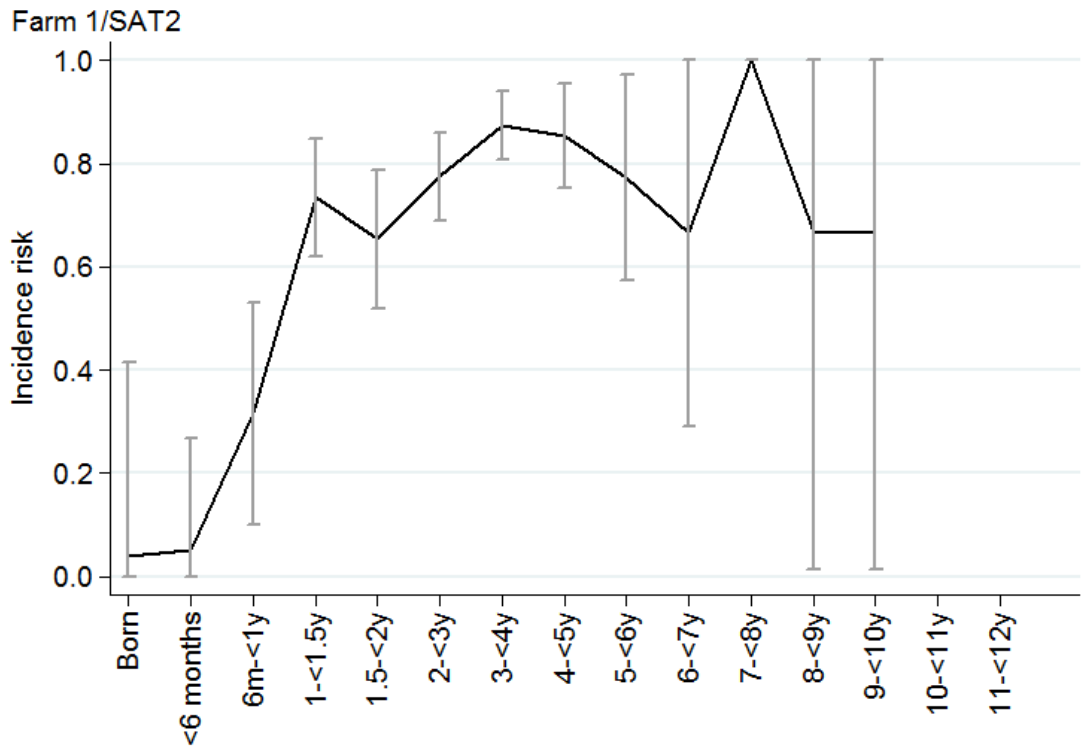


Figure 8.3. Incidence of FMD by age category for Farm 1/SAT2 and Farm 2/O with 95% confidence intervals.

8.4 Vaccine use, effectiveness and schedule modification

Both farms used the same locally available killed, aqueous-adjuvanted, non-NSP purified quadrivalent vaccine containing serotypes A, O, SAT1 and SAT2. The manufacturer's recommendation was to vaccinate all animals on the farm at least every 6 months but preferably every 4 months³. A second or third dose as part of a primary course was not recommended. The two farms described here employed differing vaccine schedules prior to the outbreaks. Farm 1/SAT2 vaccinates all animals every 4-6 months irrespective of age. Farm 2/O vaccinates every four months but only animals over the age of six months. Although the former vaccinates at a younger age, the slightly less frequent vaccination and younger age structure on Farm 1/SAT2 means Farm 2/O is a much more heavily vaccinated population (Figure 8.4) reflected in the average number of vaccine doses received being 8.6 compared to 5.0 on Farm 1/SAT2 (Table 8.1).

Both outbreaks occurred within four months of the most recent vaccination. Estimation of the absolute vaccine effectiveness was not possible on either farm due to all animals being vaccinated at the same time and the high collinearity between the number of doses and age meaning there were no appropriate comparison groups. However, the incidence patterns by the number of doses of vaccine received provide insights into possible reasons underlying the poor effectiveness. On Farm 2/O, unadjusted *relative* vaccine effectiveness estimates were possible. Such an analysis was not appropriate on Farm 1/SAT2 due to the much lower incidence risk among younger animals meaning any risk ratios would have been over 1, with subsequent vaccine effectiveness estimates all being negative.

On Farm 1/SAT2 there appears to have been no or very limited protection offered by the vaccine evidenced by the high incidence even among multiply vaccinated animals (Figure 8.5). The low incidence among animals receiving relatively low number of doses may be an age effect with younger animals showing less obvious clinical signs as described in Chapter 4. In contrast, on Farm 2/O there is some evidence of cumulative protection with the number of doses received with this protective effect plateauing from 11 doses. Examination of the confidence intervals shows animals receiving five or more doses were significantly more protected than those receiving one or two doses (Figure 7.5). The higher incidence among animals receiving 1-2 doses is likely due to an "immunity gap", a term used to describe the situation in which protection from maternal antibodies has waned but insufficient doses of

³ http://www.kevevapi.org/index.php?option=com_content&view=article&id=127&Itemid=123. Accessed 01/07/2014.

vaccine have been received to confer protection like that seen in animals receiving five or more doses. Although Farm 2/O was a more heavily vaccinated population, there were still a high number of animals receiving five or more doses of vaccine on Farm 1/SAT2 (Figure 8.4) yet the incidence was high among those animals. Indirect protection “herd immunity” effects on Farm 2/O from the population being more heavily vaccinated are unlikely to be so strong as to be responsible for the differences in effectiveness seen between the two farms. Due to the age and number of doses being highly collinear (Figure 8.4), age cannot be adjusted for in these estimates and so may be confounding this association.

In both outbreaks, problems with the **cold chain** are considered unlikely due to the high number of vaccine doses received on multiple occasions. Farm 1/SAT2 sources vaccine through the local government veterinary office. Vaccine is delivered to the office directly through a courier when requested by the farmer. On arrival, the farmer is notified and the vaccine is collected, taken back to the farm and immediately refrigerated in a temperature monitored refrigerator up until the point of use. There are refrigerators at the local government office although the farmer cannot be completely sure that the cold chain has been satisfactorily maintained from leaving the manufacturer and arriving on the farm. Therefore on Farm 1/SAT2, a break in the cold chain cannot be completely ruled out as contributing to the ineffectiveness seen although is still considered unlikely. However since the outbreak, the farmer now elects to collect vaccine direct from the manufacturer. In contrast, Farm 2/O receives vaccine directly to the farm from the manufacturer using a courier. They report that the vaccine is reliably delivered and is always chilled on arrival and packed with non-melted ice when received. Both farms order vaccine according to need, use as soon as possible after being delivered to the farm, and use cool boxes packed with ice when vaccinating the herd. After a vial is breached it is used immediately and not kept for the following vaccination dates. Both farms also ensure that the vaccine has not passed the expiry date prior to use.

Conversations with the vaccine producer in August 2014 as a consequence of the poor performance seen in Farm 1/SAT2 revealed that this farm hadn't consistently order enough vaccine to vaccinate all animals in the herd prior to the outbreak. The farmer claims this is because they often had vaccine left-over from previous vaccination rounds and insists all cattle are routinely vaccinated at the same time. In addition, they have a refrigerator on the farm with a temperature monitor to ensure the temperature is kept between 2 and 8°C while stored. Additionally they discard breached vials after use and never use vaccine that is beyond the expiry date. However, the SAT2 antigen is known to be less stable than other serotypes

(Parida, 2009) and this could mean it has a relatively shorter shelf life even under refrigerated conditions. This aspect of SAT2 vaccines should be investigated (see section 11.4.5 on further research priorities).

In the case of Farm 1/SAT2, it seems unlikely that changes to the vaccine schedule would have made any difference to the vaccine effectiveness as the incidence was so high in multiply vaccinated animals. The reasons behind the lack of effectiveness could be due to poor vaccine potency or a high disparity between the field and vaccine strains. It is possible that more frequent vaccination could have improved effectiveness through reducing the time between vaccination and exposure but this is unlikely to be acceptable to the farmer.

In contrast, on Farm 2/O, more aggressive vaccination among younger animals may reduce the time between MDA waning and being able to mount a full response to the vaccine. Additional doses in the primary course or more frequent vaccinations up to a certain age (for example every 3 months) decreasing in frequency when older (for example biannually after the age of 3 years) may improve effectiveness. Increasing number of doses among youngstock would also likely have indirect effects through reducing the level of exposure among older animals. Interestingly after this 2013 outbreak, Farm 2/O decided to change the vaccine schedule based upon a conversation held with another local large-scale farmer who had not had an FMD outbreak in recent years using a modified schedule. As a consequence, all animals on the farm were to be subsequently vaccinated, rather than just those over six months of age, and youngstock were to be given a two dose primary course, one month apart. However with an incidence risk still around 30-40% in multiply vaccinated animals there are likely to be other variables limiting the vaccine's effectiveness. At the time of writing, no vaccine matching tests had been performed comparing the reactivity of sera from vaccinated animals to the field and virus strains. Indeed, there has been no vaccine matching performed in recent times for any Kenyan field strains using the current KEVEVAPI vaccine. Therefore it is likely that a combination of suboptimal vaccine schedule and field/vaccine strain mismatch is contributing to the lack of effectiveness on this farm.

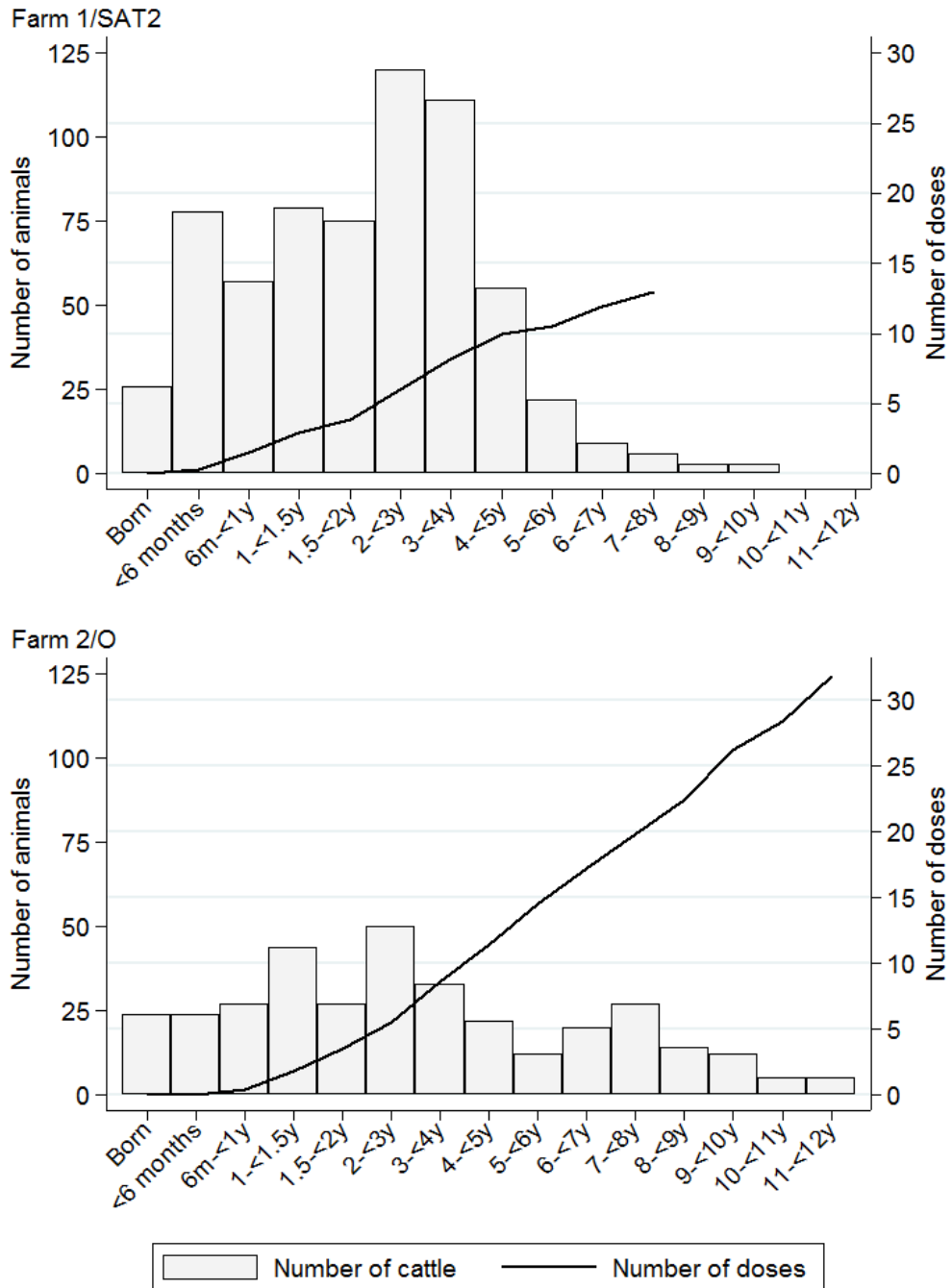


Figure 8.4. Mean number of doses of vaccine received by age category of Farm 1/SAT2 and Farm 2/O with different vaccine schedules. For Farm 1/SAT2, the total number of doses received was not available for cattle over 8 years of age (n=9).

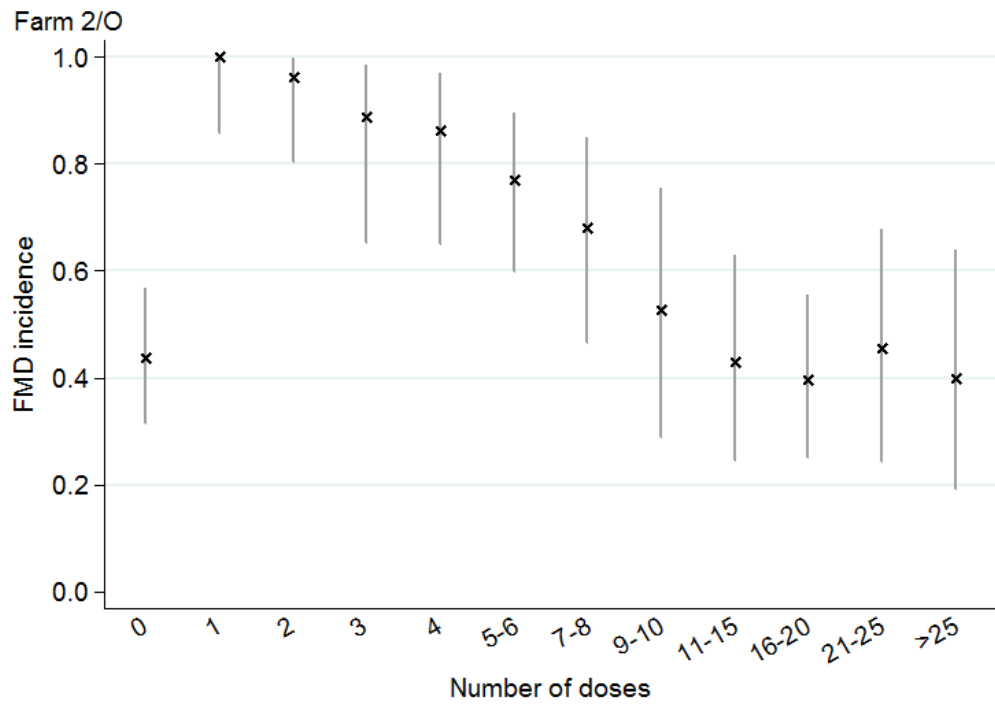
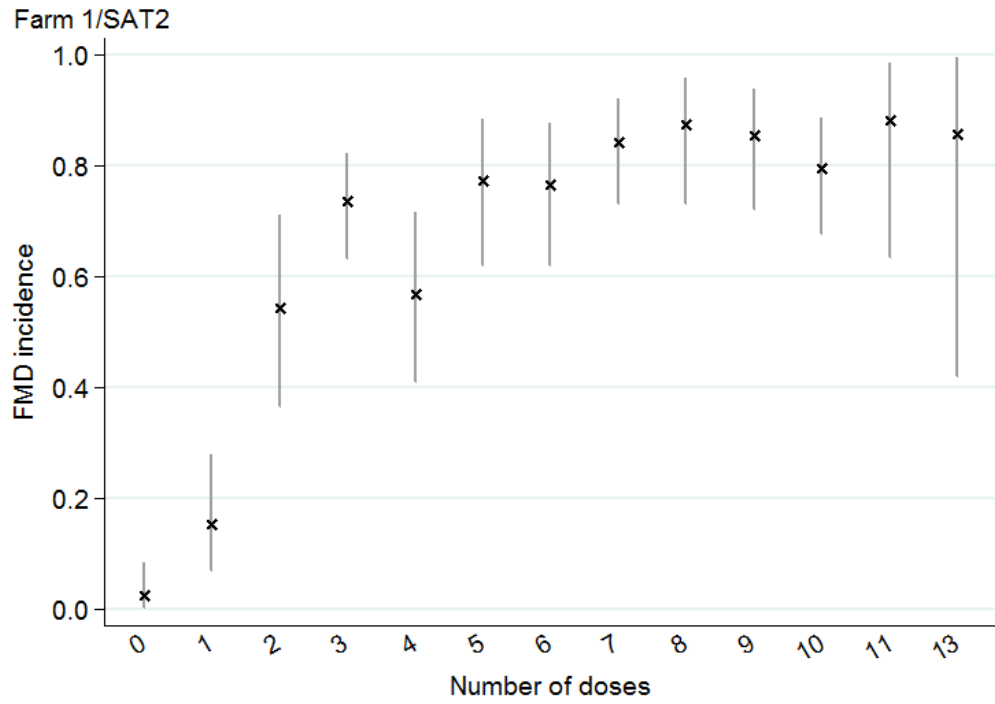


Figure 8.5. Incidence of FMD by number of doses of vaccine received for Farms 1/SAT2 and Farm 2/O with 95% confidence intervals.

Part B – Serology

Chapter 9. Literature review 2 - Decay of maternally derived antibody in the calf and the influence on response to FMDV vaccination

As demonstrated in Chapter 7 and discussed in Chapter 8, there is evidence that the vaccine or vaccine schedule among youngstock may need modification, and that maternal immunity may be responsible for some of the protection seen in animals below six months of age. A comprehensive literature review was thus undertaken in order to establish how maternal immunity may interfere with vaccination.

The specific objectives of this comprehensive literature review are:

- 1) Determine the normal kinetics of declining FMD-specific maternally derived antibody (MDA) in calves born to vaccinated dams
- 2) Appraise the evidence for the influence of MDA on the antibody response to FMD vaccination using commercially available vaccines in calves

This review informs the analysis of data provided by the Kenyan Veterinary Vaccine Production Institute (KEVEVAPI) who produce the only available FMD vaccine in Kenya and whose product was used in both of the large-scale farms whose data were analysed in Part A of this thesis. The aim is to use the information from the literature review and analysis (Chapter 10) to inform vaccine schedules in youngstock among large-scale dairy farms in Kenya.

9.1 Decline of maternally derived antibody for FMD in calves

The newborn calf is born without antibody due to the *in utero* separation of the maternal and foetal blood preventing transplacental transfer (Godden, 2008). This is seen in all ungulate species and is in contrast to the situation in primates in which the haemochorial placental structure allows transfer of IgG antibody through the placenta (Cervenak and Kacskovics, 2009). Therefore the newborn calf is completely reliant upon colostrum as a source of antibody until endogenous production begins.

The main antibody present in colostrum is immunoglobulin G1 (IgG1) although other antibodies are present in low amounts in addition to other macromolecules, such as immune cytokines, growth factors, and maternal leukocytes. Selective accumulation of IgG1 in colostrum is thought to be through FcRn receptors on the mammary epithelial cells resulting in colostrum IgG being 10-40 times the concentration of plasma, sufficient to lower the level of

maternal serum IgG1 in the last month of pregnancy (Cervenak and Kacskovics, 2009). Colostral molecules, including immunoglobulin, are able to pass through the neonatal small intestine through non-specific pinocytosis that occurs mainly in the first 24 hours of life before a process known as “gut closure” prevents further transfer. Maternal leukocytes also pass to the calf and traffic to neonatal lymphoid tissue. The influence these have on the neonatal immune system is unclear but may enhance the neonatal response to antigens to which the dam has previously responded (Donovan et al., 2007).

The amount of antibody that is transferred to the calf and enters its circulation depends on several factors including the concentration of antibody in colostrum (often referred to as colostrum quality); the volume fed; timing and method of feeding; and the metabolic status of the calf (Godden, 2008). For some cattle vaccines, in particular to those for rotavirus, coronavirus and *Escherichia coli*, it has been shown that vaccination of the dam 3-6 weeks prior to calving can increase the quality of the colostrum and protect calves from clinical disease including under field conditions (Castrucci et al., 1987; Crouch et al., 2001; Waltner-Toews et al., 1985).

The half-life of IgG is considerably longer than that of other isotypes due to the presence of the FcRn on endothelial cells. These allow the continual uptake of IgG from serum into endosomes which then either transport IgG back to the serum or into the interstitial fluid. In cattle there is some variation in IgG half-life ranging from 10-22 days and being slightly longer for IgG2 compared to the other IgG isotypes (Cervenak and Kacskovics, 2009).

For FMD specifically, several studies have looked at the decline of maternal antibodies in calves receiving colostrum from dams that have been vaccinated for FMD. An early study by Graves (1963) looked at the issue in an experimental setting. Eleven heifers were vaccinated with two doses of a formaldehyde inactivated serotype A (119) vaccine 30 days apart and then proceeded to give birth between 30 and 117 days after the second vaccination. Calves were left with the dams to ingest colostrum and sera were tested for FMD antibodies with a virus neutralising test (VNT) using a virus strain homologous to the vaccine. A single calf was blood sampled hourly for five hours after ingestion of colostrum which showed a rapid rise in neonatal antibody levels reaching a plateau five hours post vaccination. Three calves were sampled at “appropriate intervals” up to 90 days of age. For two calves, the half-life was 15 days whilst in the third calf it was 19 days.

In a review of the subject, van Bekkum (1966) stated that according to their experiments the half-life varies from between 12 and 24 days and that "...antibody could usually be detected up to an age of 60 to 160 days in calves born from cows that had been vaccinated repeatedly under field conditions."

Figueroa et al (1973) looked at VNT titres for serotype O in 16 calves of different ages born from dams repeatedly vaccinated with a commercial, inactivated polyvalent FMDV vaccine. They found declining titres with age and detectable antibodies up to seven months of age. No regression coefficient or half-life was stated in the paper.

Researchers in India took weekly blood samples from two calves beginning from one day of age to six weeks (Shankar and Uppal, 1981). These calves were born to dams that had received multiple doses of quadrivalent (A, O, C, Asia-1) vaccine. VNT titres (measured as the logarithm [base 10] of the reciprocal of the last dilution where antibodies were detected) to the serotype A and C strains revealed a mean decline from 2.75 to 1.6 and 3.0 to 1.25 respectively. Regression coefficients were -0.0315 and -0.0382 respectively. No half-life estimates were provided, but from the regression coefficients are equal to 9.6 and 7.9 days respectively.

Nicholls et al (1984) determined the decay in VNT titres in 11 calves for strains of serotype O, A and C. Dams were vaccinated 4-6 months prior to the calves being born and the latter were blood sampled five times over a four month period (age range 7-142 days while sampled). A statistically significant decline in antibody levels was seen with linear regression for each strain ($P < 0.01$ for each). The mean half-life for all the serotypes was 22.2 days. By 103 days of age, antibodies had declined to the lowest measurable levels. Two were excluded from the analysis as there were no detectable antibodies at the first sampling.

As part of a study performed by Sadir et al (1988) in Argentina, three calves had samples taken periodically on three, five and six occasions to examine the decay in VNT titres. Sampling began at 1-2 weeks of age and lasted up to around six months. These calves were born between 60 and 70 days after dam vaccination. The average regression coefficient and half-life was estimated at -0.014 and 21.5 days respectively. Also in Argentina, Späth et al (1995) blood sampled 14 calves at five time points starting from between 42 and 66 days old up to around 180 days of age. Liquid phase blocking ELISA (LPBE) were performed for four different strains of FMD virus, two of which were serotype A with the remaining being O and C. Based on 62 observations, the half-lives ranged from 19.1 to 25 days with an overall average of 21 days (Table 9.1).

Table 9.1. Regression and correlation coefficients and associated half-lives for different strains of FMD virus (Adapted from Spath et al, 1995).

FMDV Strain	Regression coefficient	Correlation coefficient	Half life
A79	0.014	0.78	20.8
AB7	0.012	0.70	25
CBS	0.016	0.80	19.1
O1C	0.014	0.81	21.1

Note: Estimates were based upon sampling calves at five time points between the ages of 42 and 180 days.

Dekker et al (2014) were also able to calculate the rate of MDA decay in control groups used in a vaccine safety trial and found an average half-life of 21 days (95% confidence interval 19-24 days) for strains A Turkey 14/98, O Manisa and Asia-1 Shamir. These calves were born from dams that had been vaccinated three times with a double oil emulsion vaccine in each trimester of pregnancy. Similarly Bucafusco et al (2014) were able to use data from control groups to estimate half lives in calves having received pooled colostrum from dams receiving at least three vaccine doses, the last dose given within six months of calving. The vaccine used was an oil-adjuvanted commercially available type including strains O1/Campos, A24/Cruzeiro, A/Arg/01 and C3/Indaial. The estimated decay half-life ranged from 17.1 to 18.4 days.

9.1.1 Discussion

Table 9.2 summarises the results of the available studies examining the decline of FMD specific maternal antibodies in calves receiving colostrum from vaccinated dams.. The range in half-life estimates is 8-25 days. The predominant method of measuring the titres is through VNT, although one study compared serology methods (LPBE, VNT, IgG1 and IgG2 isotype ELISA) and the half lives ranged from 17.7 to 18.4 indicating little difference between the serology method used (Bucafusco et al., 2014). Although the decline of MDA to the SAT serotypes has never been examined, there is no reason for it to be different from MDAs to the other serotypes. In support of this, Dekker et al. (2014) showed that there was no statistically significant difference between serotypes in their study of MDAs to serotypes A, O and Asia-1.

This range in half-life estimates is wider than quoted in a recent general review on the subject of passive immunity in cattle which gave a range of 10-22 days (Cervenak and Kacskovics, 2009). However, the studies that included estimates outside of this range were based on very small number of animals. A single calf was used to predict a half-life of 25 days (Späth et al., 1995), and similarly single calves were used to estimate half-life values of 7.9 and 9.6 days (Shankar and Uppal, 1981). Most studies do not provide confidence intervals around estimates, or present appropriate data such that a combined estimate can be calculated.

Table 9.2. Summary of studies examining the decline of maternal antibodies in calves receiving colostrum from dams vaccinated for FMD.

Study/country	Number of animals	Serotype(s)	Dam schedule (and timing of last dose relative to calving)	Serology test(s)	Half life estimates (days)
Graves (1963)/USA	3	A	2 doses 30 days apart (30-117 days)	VNT	16.3 (range 15-19)
Figueroa et al. (1973)/Chile	16	O	Repeatedly (not given)	VNT	Not given
Shankar and Uppal (1981) India	2	A, C	Several (not given)	VNT	Not given (calculated as 9.6 and 7.9 for serotypes A and C respectively.
Nicholls et al. (1984)/Uruguay,Brazil	11	A, C, O	Not given (4-6 months)	VNT	22.2
Sadir et al. (1988)/Argentina	3	A	Not given (60-70 days)	VNT	21.5
Späth et al. (1995)/Argentina	14	A, C, O	> 6 doses (5 months)	LPBE	21 (range 19.1-25.0)
Dekker et al. (2014)/Netherlands	5	A, O, Asia-1	3 doses (last trimester)	VNT	21 (95%CI 19-24)
Bucafusco et al. (2014)/Argentina	7	O	Minimum 3 doses (within 6 months)	VNT, LPBE, IgG/IgG2 ELISA	17.1, 17.8, 18.4, 17.6 ^a

Note: All studies were longitudinal, taking repeated samples from the same animals except Figueroa et al. (1973) who correlated age and titres based on single samples. ^a Antibody half-life for LPBE, VNT, IgG1 and IgG2 ELISA respectively.

9.2 Influence of maternally derived antibody on the response to commercially available FMD vaccines in calves

Although the transfer of maternal antibody to the neonatal calf is important to ensure protection from disease, it is acknowledged that this passive immunity may interfere with the calf's ability to respond to vaccines through preventing access of the immune system to antigen. Therefore, an understanding of its impact on vaccination is important for informing vaccine schedules, in particular the timing of the first dose. However, in a relatively recent review on the subject related to all vaccines used in calves, Chase et al (2008) stated "In assembling this article, it is clear that many "vaccine recommendations" have been made but little research is available to indicate the true effectiveness of vaccine timing or ideal protocols for use in young calves." This literature review summarises the published studies on the subject in relation to FMD, several of which are extensions to the studies examining the decline of MDA cited above. These studies are summarised in Table 9.3, and are presented in the context of the vaccine formulation, serological assay used, and study hypothesis.

There is a lack of consistent terminology between studies on the use of the terms "primary", "booster" and "re-" vaccination (or dose) that needs clarification. Generally a "primary course" or "primary series" constitutes one or more doses that allow the "development of immunity" (Kroger et al., 2013). Periodic vaccination to maintain this immunity is called a "booster". An example from the human vaccinology field is with Diphtheria, Pertussis and Tetanus (DPT) schedule, according to which infants are given the "primary course" at 2, 3 and 4 months of age. A booster dose is given at 3-4 years old. In this review the responses under consideration are in young animals and refer to the interference of MDA. Therefore it is the primary course that is being considered and so for consistency when comparing studies, doses shall be referred to simply as first, second or third doses.

9.2.1 Early studies with formalin inactivated vaccines

Early FMD vaccines were inactivated with formalin and used an aqueous adjuvant, either aluminium hydroxide alone or in combination with saponin. In the study by Graves (1963), one calf that had MDA was given a single dose of serotype A vaccine at 21 days of age which was repeated when the calf was 120 days old. No response was seen in neutralising titres to the first dose but an increase from 1.0 to 2.0 log₁₀ reciprocal titre was seen with the second dose,

a response similar to that seen when vaccinating an immunologically naive adult. The exact timing of the blood sampling is not clear from the methodology presented in this study.

Further experiments by Figueroa et al (1973) in Chile used six calves with MDA that were vaccinated with serotype O vaccine. Blood sampling occurred at vaccination and weekly for four weeks thereafter. A progressive week-on-week increase in VNT titre was detected. The ages at vaccination and range in MDA titres of those vaccinated was not stated, but the geometric mean at vaccination (1/32) and the regression line of decay in MDA suggests vaccination may have been at about 3.5 months.

Uppal et al (1975) vaccinated six randomly selected 5-21 day old calves with either a full or half dose of a quadrivalent (A, O, C, Asia-1) vaccine born to multiply vaccinated dams. VNT titres at vaccination were variable ranging from 1.0 to 3.5 \log_{10} reciprocal titres. A rise in antibody post-vaccination was seen irrespective of the dose and whether maternal antibodies were present or not. A higher serological response was seen if the maternal antibody titre was lower at vaccination. Further studies in India by the same research group compared responses in 1-90 day old calves born to dams in herds using regular vaccination (n=10) to those born in unvaccinated herds (n=8) (Shankar and Uppal, 1982). Neither herd had recorded an FMD outbreak in the previous 6-8 years. Calves were either given one "full" dose of a serotype A vaccine or two half doses, 21-days apart. VNT titres to the serotype A strain were measured. Significant increases in titres were observed in both groups compared to unvaccinated controls (P=0.05) after the first dose irrespective of MDA status although no significant difference was seen between the two groups. A further increase was seen after the second half dose inducing significantly higher titres than the single dose group, measured 50 days after the first vaccination. The authors stated that lower MDA titres in calves were associated with a higher response after vaccination although data were not presented demonstrating this effect. They concluded that MDA might interfere with the vaccine response but that it does not block it completely and recommended a two dose schedule among calves.

9.2.2 Aqueous versus oil adjuvanted vaccines

Due to reports of incomplete inactivation with formalin and vaccine associated disease outbreaks, inactivation was later performed with the more reliable binary ethyleneimine (BEI) (Bahnmann, 1975) which is still commonly used for the production of FMD vaccines. Around the same time, oil adjuvants also started to be used and today both aqueous and oil adjuvanted vaccines are commercially available.

Using BEI inactivated vaccines, several studies investigated the influence of adjuvant type on the response to vaccination, under the hypothesis that oil-based vaccines were able to circumvent the effect of MDA. A study in Brazil compared the response among calves with varying levels of MDA to oil (n=28) and aluminium hydroxide-saponin (n=31) adjuvanted trivalent (A, C, O) vaccines (Gomes, 1984). All dams had been vaccinated every six months with oil based vaccine. Calves were aged between 8 and 169 days at first vaccination and were blood sampled at first dose, second dose (105 days later) and seven days later. Antibody levels for all three serotypes were measured though the mouse protection index (MPI) ⁴ for which a cut-off of 2.0 had been shown by the same research group to be associated with clinical protection in cattle when intradermolingually challenged (Gomes and Astudillo, 1975). Increasing titres were seen with both vaccine types when given the first and second doses, regardless of age and serotype. However, if the MPI was greater than 2.0 a poor response was seen regardless of vaccine adjuvant and with both first and second doses. No statistical tests were performed with these data.

Also in South America, Sadir et al (1988) made similar comparisons, following their observations of a poor vaccine response in calves less than 30 days old with no MDA contrary to reports elsewhere. Dams had received multiple doses of the oil-adjuvanted vaccine with the last dose given around 70 days prior to calving. When using the aqueous vaccine, the authors reported no increase in VNT titre even if given a second dose 60 days later and that titres were lower than what would be expected from usual MDA decay. A plot was presented demonstrating the decline of titres over time although no statistical tests were presented and undefined ranges around points on this graph overlap with the theoretical half life curve bringing this evidence into question. Additionally, the number of calves on which this aqueous-adjuvant study was based is unclear from the description. For evaluating the oil vaccine, 26 calves aged 1-90 days with MDA (n=15) and without MDA (n=11) were used. For calves with MDA, those given a first dose at 1-21 days old showed no increase in VNT titres. Although some increase was seen when a second dose was given 60 days later, the ranges around points on the graphs don't support a significant increase. Conversely, if calves were over 30 days old at first vaccination, a greater response was seen together with a greater response to the second dose. For calves born with no MDA, a 30-60 day lag in response was seen after first vaccination when given at three and seven days of age although a more rapid response was

⁴ The mouse protection test (MPT) is where comparisons are made between mice "treated" or "non-treated" with immune serum and challenged with different titres of homologous FMD virus. The mouse protection index (MPI) is the log difference between the virus titres causing disease in the serum treated and non-treated mice.

seen in those vaccinated over 21 days of age. All of these calves showed a response to a second dose, although there were a maximum of two animals in each age category and only one received a second dose. No statistics are provided alongside these findings making their interpretation problematic. Additionally, it is difficult to compare these results with that of the aqueous vaccine when the neutralising antibody level appears lower with those receiving oil-adjuvanted vaccine at first vaccination according to the figure presented in the paper.

A group in India, compared oil and aqueous adjuvanted quadrivalent (O, A, C, Asia-1) vaccine (Patil et al., 2014). Calves (n=18) were aged between two and three weeks and born to vaccinated dams. Ten were given oil adjuvanted vaccine whilst eight were given aqueous vaccine. Animals were blood sampled at 0, 15, 30 and 60 days post vaccination. Similar MDA levels were seen in both groups at vaccination. Using the oil adjuvanted vaccine, an increase in VNT titre was seen peaking at the 15 day sampling for serotype O and 30 day sampling for the other serotypes. With the aqueous-adjuvanted vaccine, a decline of VNT titres was seen after vaccination. The differences in post-vaccination antibody levels between the two adjuvants were statistically significant throughout the study period ($P < 0.0001$). The authors recommended first vaccination of animals at 8 weeks of age with the oil-adjuvanted vaccine. No data were presented from unvaccinated animals.

9.2.3 Evaluation using non-VNT serology

All the studies so far have focussed on titres as measured using VNT. Several studies have considered the effect on antibodies measured using alternative assays.

Späth et al (1995) performed two experiments using a commercially available oil-adjuvanted vaccine for strains O1C, A79, A87, C85, by measuring liquid phase blocking ELISA (LPBE) titres. Dams had all received greater than six doses of the same vaccine. In the first experiment, three groups of 20, 30 and 40 day old calves (18 per group) were all given a single dose of vaccine and blood sampled at 0, 15, 30, 60, 94 and 124 days post vaccination. A control group of 14 calves were given no vaccine, sampled 15, 30, 60, 94 and 124 days after the commencement of the trials (range 25-49 days old at commencement). Similar responses were seen in the different age groups, although the difference seen was only statistically significant at 90 and 120 days post vaccination when comparing it to non-vaccinated calves. The authors refer to the "Expected Percentage of Protection" (EPP) measure (covered previously in Chapter 2, section 2.1) and on this basis claimed around 40-50% of calves had protective titres compared to 0% with the non-vaccinated calves at 120 days post vaccination. Higher initial MDA antibody

titres were associated with a lower response to vaccination demonstrated through linear regression. The slope was greater for serotype O, although no statistics supporting these observations were presented.

In the second experiment by Späth et al. (1995), four groups of 14-16 animals were used and comparisons made between calves vaccinated at three months and four months of age. One three month and one four month old group received two doses (day 0, day 120) of vaccine. The remaining two groups received three doses (day 0, day 60, day 120). Similar titres were achieved with all four groups at 180 and 240 days post first vaccination (and nearly 100% protection based on EPP). A statistically significant increase in titre was apparent after the second dose, with a greater increase seen for the serotype O strain (to which responses were generally higher). The increases in titre post vaccination were also inversely related to the MDA titre although these data were not presented.

On the basis of their two experiments, Späth et al. (1995) concluded that vaccinating calves as young as 20 days generated a protective titre even in the presence of high MDA titres which lasted up to four months, contrary to that seen in non-vaccinated calves, and that a second dose given 60-days after the first ensures high antibody titres. For calves receiving vaccine at 3-4 months old, at the “end of the colostral protection period”, protection lasted for four months irrespective of receiving an additional dose.

Another assessment was performed by Bucafusco et al (2014) in Argentina. Several serological tests were utilised to further understand the effect of MDA on vaccination response. Commercial oil-based vaccines with strains O1/Campos, A24/Cruzeiro, A/Arg/01 and C3/Indaial were used in two experiments. Dams had received at least three vaccine doses with the last dose within six months of calving and calves received pooled colostrum from these dams. The first experiment involved three groups of four calves being vaccinated at one month, three months or five months of age alongside a non-vaccinated three animal control group. Blood sampling took place approximately every 1-2 weeks and serological tests included: LPBE for O1 Campos; VNT for O1 Campos; single dilution avidity ELISA; IgG1/IgG2/IgM isotype ELISA. When animals were vaccinated at one month of age, levels declined in all the serological tests although were significantly higher than the non-vaccinated group by 165 days post vaccination for LPBE ($P < 0.05$). In contrast, statistically significant increases were seen in all tests when receiving the first dose at five months of age ($P < 0.05$). Those receiving the first dose at three months had significantly increased titres in all tests

except the IgG1 ELISA. In this age group, responses were slower to develop and lower than the five month group.

In the second experiment, two groups were selected on the basis of low (<1.3, n=13) and high (>2.9, n=12) MDA titres to O1 Campos measured by LPBE. Ages differed between the groups at 165-215 days and 14-42 days respectively. Both groups were vaccinated twice, 35 days apart, and blood sampled at 0, 7, 21, 35, 55 and 120 days post primary dose. When MDA levels were low, one dose of vaccine raised titres in all tests and was sufficient to induce protective levels of antibody 21 days later (based on the EPP). Although IgG1 levels decayed from 55 to 120 days post first vaccination, IgG2 levels were maintained over time. For the high MDA group, after the second dose titres were lower than the low MDA group and were significantly different for both LPBE and VNT ($P<0.05$). LPBE titres were significantly higher than expected from normal maternal decay ($P<0.05$) although VNT titres were not significantly different. The authors concluded that the presence of MDA as measured by VNT tended to interfere with the response to vaccination rather than the MDA measured by LPBE which measured total antibody levels.

9.2.4 Evidence from the field

Nicholls et al (1984) conducted a series of FMD vaccine studies in experimental and field settings. Thirty-eight calves in Uruguay born to cows vaccinated ten days to four months prior to calving were vaccinated with an aqueous (aluminium hydroxide and saponin) adjuvanted vaccine at one week of age with blood samples taken at vaccination and 21 days later. These were compared with six calves born in the UK that were vaccinated with the same vaccine batch but had no MDA. A higher VNT response to vaccine was seen in the UK based calves with no MDA. Analysed through linear regression, a significantly lower response was seen when the MDA titres were higher ($P<0.01$) with similar slopes seen for each serotype. The antibody level was observed to decrease post vaccination in the Uruguayan calves for all three serotypes which the authors noted was beyond the decrease expected from normal decay. They speculated that the vaccination could have a depressive effect on antibody levels.

A separate study described in the same paper, involved 59 vaccinated calves in Brazil divided into one, two, three and four month age groups. Blood collection occurred at vaccination and at 30 and 60 days later. Some animals received a second dose at the 30 day sampling point. They found that responses to the first and second doses both varied with the MDA levels as in their Uruguayan study ($P<0.01$), and that the maximum titres were seen after the second dose

although there was “considerable animal to animal variation in this response”. The authors claim that low levels of MDA that were “scarcely demonstrable” appeared capable of interfering with responses.

The same authors went on to conduct a field study on four large farms in Brazil where FMD was endemic. This study was previously described in Chapter 2 (section 2.4) of this thesis but is included again here due to its repeated relevance. The study compared the incidence of FMD among calves given two different schedules. The traditional vaccine schedule (dosed every four months irrespective of age) was used on half the calves on three farms and on all the animals on the remaining farm (n=9,056 calves). A revised schedule was used on all remaining animals (n=7,951 calves), delaying the first dose to 5-6 months old with a second dose given one month later. The clinical incidence was monitored for a 12 month period after implementing the change. Among calves, the incidence on the farms using the traditional schedule was 11.0% compared to 0.09% with the revised schedule, a difference unlikely due to chance ($\chi^2= 741, P<0.01$). Combined with the other findings in the paper, they recommended that vaccination should be delayed in calves until 5-6 months of age. Although it is encouraging to see field data provided to support a modification to the vaccine schedule, there are several limitations to this study including: a) “calves” and “adults” not being clearly defined; b) no explanation on the likelihood of exposure of all the groups (the authors assume “the possibility of an FMD outbreak was equal in all vaccination groups”); c) no comparison groups on the one farm which just received the traditional schedule d) numbers of cases and animals were not provided at the individual farm level. No vaccine effectiveness estimate was attempted by the authors in this study and given these methodological problems it cannot be calculated from the data presented.

9.2.5 Alternative strains

Dekker et al (2014) tested the hypothesis that if calves with MDA are vaccinated with a strain which is different to that given to their dams, a greater increase in neutralising titre will be seen after vaccination. Five groups of five calves each were used in the study, with three having MDA. Dams had been vaccinated as part of a vaccine safety trial with a trivalent (A Turkey 14/98, O Manisa, Asia-1 Shamir) double oil emulsion vaccine given once in each trimester of pregnancy. Two groups of calves with MDA were vaccinated at 2-3 weeks old with either A Turkey 14/98 (i.e. homologous to the MDA) or A22 Iraq (i.e. heterologous to the MDA). As controls, two groups without MDA were similarly vaccinated and one group with MDA was unvaccinated. VNTs to the A Turkey 14/98 and A22 Iraq strains were performed at

weekly intervals for 6 weeks after vaccination and analysed with a linear mixed effects model accounting for repeated sampling. A significantly greater increase in titre was seen when calves were vaccinated with a heterologous vaccine strain compared to using a homologous strain ($P=0.002$) which led to a decline in titres in line with the normal MDA decay half-life.

9.2.6 Reviews and unpublished data

A series of reviews have been written on the influence of MDA on FMD vaccination including by those working in the pharmaceutical industry. Based at the Institute for Animal Health (now the Pirbright Institute, Surrey, UK), Kitching and Salt (1995) speculated on the mechanism by which MDA leads to poor responses with vaccination including: "Antigen blockage" from high MDA preventing an appropriate immune response; MDA inducing negative signals in B-cells as part of a negative feedback mechanism; suppression of T-helper cells; and upregulation of T suppressor cells. The authors described their experiences on large-scale dairy farms in the Middle East. They reported that animals between six and 18 months old frequently get FMD on these farms. Animals five to six months of age had received at least two doses of vaccines but appeared susceptible to clinical disease presumably due to maternal immunity interfering with the vaccine response. They speculated that environmental shedding from these animals was occasionally sufficient to overcome the immunity in older animals leading to wider outbreaks of disease. Studies they performed on these farms found vaccination at one day or one month old was ineffective at inducing an antibody response even with second dose given 3-4 weeks later. However, they did not see suppression of antibody levels as reported by Nicholls et al. (1984). When these same animals received a third dose at 4-5 months old, there was no evidence of a priming effect with animals giving a similar response to unvaccinated animals of similar age from the same herd. Their conclusion from these data was that "the most effective vaccination program for calves of well vaccinated dams, was vaccination of all calves at 4, 5, and 6 months of age, to ensure that all calves had good levels of protection". Details of the studies and the actual data were not published so the logic behind these recommendations cannot be appreciated.

In a review by Doel (1999) based at Merial Animal Health Ltd, a major global producer of FMD vaccine, it was stated that "as maternal antibody wanes, there is an increasing opportunity for the vaccine to induce a significant titre of neutralising antibody and, in general, the population will respond well to vaccination after 2.5 months of age". Additionally, "it is also the case that maternally derived antibodies do not always interfere with development of vaccine induced immunity for reasons which are not clear". Work done in the author's laboratory suggested

that the anamnestic response depends on the interval between the first and second dose with a two week period not inducing a strong response and suggested 3-4 weeks as an appropriate boosting interval. Data supporting these recommendations were not presented and it was not specified if this work was done in the presence of MDA.

In a later review by the same author (Doel, 2003), the possibility was acknowledged that MDA might influence or prevent the development of immunological memory but no supporting data were provided. A pragmatic approach to vaccinating youngstock was proposed, acknowledging the impracticalities of withholding vaccination from calves until MDA has completely waned due to the prolonged “window of susceptibility” that will result. A recommendation was made that with vaccines containing either adjuvant type, calves from vaccinated dams should receive their first dose at 2.5 months of age “unless the epidemiological circumstances are very severe in which case 2.0 months is prescribed”. These “epidemiological circumstances” are not defined, but imply circumstances consistent with high risk of exposure.

All three reviews comment on the effects of oil-based vaccines. Kitching and Salt (1995) speculated that the success seen with oil-adjuvant may be related to improved immunogenicity compared to aqueous vaccines. Doel (1999) remarked that there are several reports that don’t support the claim of oil-adjuvanted vaccines overcoming passive immunity, reiterated in the later review (Doel, 2003).

9.2.7 Discussion

9.2.7.1 Response to the first dose

The results of the studies looking at the impact of MDA on response to vaccination are summarised in Table 9.3. It is apparent from these studies that MDA can interfere with the response to vaccination although this effect appears to vary between studies. Among calves with MDA, studies have reported an increase in titres after first vaccination to both oil and aqueous adjuvanted vaccines with ages ranging from a few days to five months old. Other studies that have reported no change or a decline in titres after first vaccination have tended to be in younger calves less than 3 months of age with the notable exception being the study by Nicholls et al. (1984) who saw decreasing titres in 1-4 month old calves given their first dose of an aqueous-adjuvanted vaccine.

It has been claimed that having an oil-adjuvant can circumvent the problem of MDA, particular from the study by Sadir et al. (1988) who directly compared their work to that of Nicholls et al (1984) in claiming the benefits of oil vaccines. They ascribe this benefit to the slow release of antigen from oil emulsified vaccines and therefore more persistent stimulation which has been reported elsewhere as a feature of vaccines containing this adjuvant (Aucouturier et al., 2001). This persisting effect may be responsible for the delayed response reported by Späth et al. (1995) who vaccinated calves less than 40 days old and only saw differences with the unvaccinated group at 90 and 120 days post vaccination. Based on the literature reviewed here, the response with oil and aqueous-adjuvanted vaccines is inconsistent as titre increases have been reported with both adjuvant types in the presence of MDA. Comparing the studies is not easy due to the different vaccine types used; lack of information on vaccine potency and antigen dose given; and varying MDA levels when vaccine is given. Only three studies made direct comparisons between these adjuvants in equivalent study populations. Gomes (1984) showed no difference, Sadir et al. (1988) showed a response to oil if calves were over 30 days of age which was not seen with an aqueous adjuvant, and Patil et al. (2014) found increased responses with oil adjuvants. On the evidence presented, it is not clear that oil based vaccines are superior to aqueous when given in the presence of detectable MDA titres. This conclusion is in agreement with reviews on the subject (Doel, 2003, 1999; Kitching and Salt, 1995).

9.2.7.2 Response to repeated doses

Several studies looked at the effects of a second dose, which was given between 21 and 120 days after the first. In three studies both aqueous and oil adjuvanted vaccines increased titres with a second dose after the first failed to elicit any increased response (Graves, 1963; Nicholls et al., 1984; Sadir et al., 1988, Table 9.3). In three other studies, the second dose was ineffective at increasing the titre after an ineffective first dose, again demonstrated for both oil and aqueous adjuvanted vaccines (Uppal et al., 1975; Sadir et al., 1988; Bucafusco et al., 2014, Table 9.3).

The second dose tended to be more effective when given at an older age, probably related to further MDA decay. Although the study by Sadir et al. (1988) saw no response to a second dose with an aqueous-adjuvanted vaccine but did with an oil-adjuvanted equivalent in animals of similar ages, the number of animals receiving the aqueous booster is not made clear in the paper and so must be treated with caution.

9.2.8 Conclusion

On the basis of this literature review, it is not possible to make general recommendations for the age of first dose, the necessity or possible timing of a second dose, or the relative benefits of using oil versus aqueous-adjuvanted vaccine. The majority of the studies are based in experimental settings using relatively small numbers of animals with no consideration to sample size. Only one study presented field evidence (Nicholls et al., 1984), but this was a poor study design that was inappropriately analysed so cannot be used as convincing evidence of a benefit of giving a second dose. Many of the studies are pre-1995 and there have likely been improvements in vaccine quality and potency in subsequent years. Few studies quoted the antigen dose used and little consideration was given to this aspect. There is also a notable absence of any SAT serotypes in any of the studies and none of studies are based in an African population. The variability in the study designs, vaccines and serological assays precludes combining the results, for example as a meta-analysis. There is a need for standardised protocols to ensure comparability of these studies.

None of the studies considered natural immunity and the effect these colostral titres have on the response to vaccination, although this is likely to have been relevant in the one study presenting field data from an endemic area (Nicholls et al., 1984). A number of studies comment on the variability of titres seen in calves receiving their first dose at an equivalent age, and these titres will depend not only on the type of vaccine used in the dam but also with the FMD epidemiology and possibly the animal genetics that predominate in certain areas. Based on the study by Dekker et al. (2014), natural immunity is likely to have some impact on the response to vaccination presuming a different strain is circulating compared to the vaccine. These observations combined with the clear variability in results seen with vaccine types in different areas suggest vaccine schedule recommendations are likely to vary with the vaccine used and the particular FMD epidemiology though this is poorly documented in endemic areas. There is a need for further studies in the field in a variety of different settings using relevant (i.e. locally available) vaccines so that one can correctly inform optimal use in endemic countries.

There are limitations to this review that should be acknowledged including the presence of studies in foreign journals that were either not available or not in English. Additionally it is acknowledged that there are many vaccine producers in the world for FMD vaccines (over 56). It is very likely that some, if not all, of these companies have their own data that are not publically available. The experiences relayed in the reviews by Doel (1999, 2003) are valuable

in this regard but this only represents one producer and data are not presented so are closed to academic rigour and informed discussion. Appraisal of the schedule recommendations of several large-scale producers of FMD vaccines in endemic settings (Table 9.4), it is apparent that there is some variation with oil based vaccines tending to not have a second dose in the primary course soon after the first dose. This is contrary to recommendations with the aqueous vaccine where a second dose 2-4 weeks is advised with the only exception being the Kenyan manufacturer where a second dose is not currently recommended. It is also noteworthy that two producers have different age recommendations depending on the presence of MDA (Merial/BVI and MSD Animal Health) and only two recommend vaccinating all ages (Brazil and Kenya).

Table 9.3. Summary of studies examining the antibody titre response to the first and second vaccine dose according to the age and adjuvant type used. Continued on next page.

Study/Country	N	Age	Serotype(s)	Adjuvant	Titre response – first dose	Titre response – second dose (dpv ^a)
Early studies with formalin inactivated vaccines						
Graves (1963)/USA	1	21 days	A	Aqueous	None	Increase (120)
Figuerola et al. (1973)/Chile	6	3.5 months (approx.)	O	Not specified	Increase	N/A ^b
Uppal et al. (1975)/India	6	5-21 days	O	Aqueous	Increase	None (21)
Shankar and Uppal (1982)/India	18	1-90 days	A	Aqueous	Increase	Increase (21) ^c
Oil versus aqueous adjuvants						
Gomes (1984)/Brazil	31	6 to 169 days	A, C, O	Aqueous	Increase if MPT <2.0	Increase if MPT <2.0 (105)
	28	8 to 167 days		Oil	Increase if MPT <2.0	Increase if MPT <2.0 (105)
Sadir et al. (1988)/Argentina	-	1-90 days	A	Aqueous	None	None (30)
	26	1-90 days		Oil	None if 1-21 days old. Increase if >30 days old	Increase for both age categories but greater increase if >30 days for primary dose (30)

Note: Increase or decrease in titre is defined relative to the titre when the dose was given. All titres measured with VNT except by Gomes (1984) (MPT) and Späth et al. (1995) (LPBE). Bucafusco et al. (2014) used LPBE and VNT. N/A = not applicable. "N" represents the total number of animals used in the study.

^a dpv = days post primary vaccination ^b N/A = Not applicable ^c Booster regime was half dose twice 21 days apart. Increase titre seen with both full and half dose. ^d For serotype O, higher increase than other strains with 60 day dose but decrease seen with 120 day dose. ^e Tests include LPBE, VNT, IgG1, IgG2, IgM, avidity ELISA

Table 9.3. Continued from previous page.

Study/Country	N	Age	Serotype(s)	Adjuvant	Titre response – first dose	Titre response – second dose (dpv ^a)
<i>Oil versus aqueous adjuvants (continued)</i>						
Patil et al. (2014)/India	8	2-3 weeks	A, C, O, Asia-1	Aqueous	Decrease	N/A
	10	2-3 weeks		Oil	Increase	N/A
<i>Evaluation using non-VNT methods</i>						
Späth et al. (1995)/Argentina	68	20d, 30d, 40d groups	A, C, O	Oil	Decrease but higher than non-vaccinated controls at 90 and 120 dpv. No difference with age groups.	N/A
	62	3 months and 4 months		Oil	Increase	Increase (60, 120 ^d)
Bucafusco et al. (2014) ^e /Argentina	15	1, 3, 5 months	O	Oil	Only increase with 3 and 5 month group	N/A
	13	14-42 days		Oil	Decrease	Decline but LPBE, IgG1, IgG2 and avidity assay levels were higher than expected than normal decay (35)
	12	165-215 days		Oil	Increase	Increase (35)

Note: Increase or decrease in titre is defined relative to the titre when the dose was given. All titres measured with VNT except by Gomes (1984) (MPT) and Späth et al. (1995) (LPBE). Bucafusco et al. (2014) used LPBE and VNT. N/A = not applicable. “N” represents the total number of animals used in the study.

^a dpv = days post primary vaccination ^b N/A = Not applicable ^c Booster regime was half dose twice 21 days apart. Increase titre seen with both full and half dose. ^d For serotype O, higher increase than other strains with 60 day dose but decrease seen with 120 day dose. ^e Tests include LPBE, VNT, IgG1, IgG2, IgM, avidity ELISA

Table 9.3. Continued from previous page.

Study/Country	N	Age	Serotype(s)	Adjuvant	Titre response- – first dose	Titre response – second dose (dpv ^a)
<i>Evidence from the field</i>						
Nicholls et al. (1984)/Uruguay,Brazil	44	1 week	A, C, O	Aqueous	Decrease	N/A
	59	1, 2, 3, 4 months		Aqueous	Decrease	Increase (30)
<i>Alternative strains</i>						
Dekker et al. (2014)/Netherlands	25	2 weeks	A	Oil	Increase to heterologous strain. Decrease with homologous strain.	N/A

Note: Increase or decrease in titre is defined relative to the titre when the dose was given. All titres measured with VNT except by Gomes (1984) (MPT) and Späth et al. (1995) (LPBE). Bucafusco et al. (2014) used LPBE and VNT. N/A = not applicable. “N” represents the total number of animals used in the study.

^a dpv = days post primary vaccination ^b N/A = Not applicable ^c Booster regime was half dose twice 21 days apart. Increase titre seen with both full and half dose. ^d For serotype O, higher increase than other strains with 60 day dose but decrease seen with 120 day dose. ^e Tests include LPBE, VNT, IgG1, IgG2, IgM, avidity ELISA

Table 9.4. Summary of a selection of commercially available FMD vaccines and suggested schedules in cattle. MDA = maternally derived antibodies. Continued on next page. All aqueous vaccines are adjuvanted with aluminium hydroxide and saponin.

Manufacturer	Product	Adjuvant	Age first dose	Schedule	Source
Merial	Aftovaxpur DOE	Oil (double oil emulsion, DOE)	2 months	Primary course: 1 dose Booster: Every 6 months	European medicine agency website ^a
	Aftovax	Aqueous	2 weeks (unvaccinated dams) 2.5 months (vaccinated dams)	Primary course: 2 doses, 3-4 weeks apart Booster: Every 4-6 months	Botswana vaccine institute website ^b
Indian Immunologicals	Raksha	Aqueous	4 months	Primary course: 2 doses, 2-4 weeks apart Booster: Every 6 months	Indian Immunologicals website ^c
	Raksha-Ovac	Oil (double oil emulsion)	4 months	Primary course: 1 dose Booster: 9 months, then every 12 months	

^ahttp://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/002292/WC500147985.pdf (accessed 05/11/2014)

^b<http://www.bvi.co.bw/products/AFTOVAX.html> (accessed 05/11/2014)

^c<http://www.indimmune.com/livestock-vaccines.pdf> (accessed 05/11/2014)

Table 9.4. Continued from previous page.

Manufacturer	Product	Adjuvant	Age first dose	Schedule	Source
Bayer	Bayovac® Oleosa	Oil	All ages	Primary course: 2 doses, 90 days apart Booster: Every 6 months	Bayer Brasil website ^d
Centro Diagnóstico Veterinaria	Aftosa	Oil	3 months	Primary course: 2 doses, 6 months apart Booster: Every 12 months	CDV website (Argentina) ^e
MSD Animal Health	Foot-and-mouth disease vaccine	Oil	3 months	Primary course: 3 doses at 4-6 weeks and 24 weeks (6 months) Booster: Every 44-48 weeks (10-11 months)	MSD Animal Health website ^f
	Decivac	Oil (DOE)	2 weeks (no MDA) 4 months (with MDA)	Primary course: Single dose Booster: Every 6 months	MSD Animal Health Website - Philippines ^h
Kenya Veterinary Vaccines Production Institute	Fotivax	Aqueous	All ages	Booster: Every 4-6 months	KEVEVAPI website ^g

^dhttp://200.182.3.36/ContentAH/animproducao.nsf/Bayovac_oleosa?OpenPage (accessed 05/11/2014)

^e<http://www.cdvs.com.ar/vademecum-vacuna-anti-viral-aftosa-ficha.php> (accessed 05/11/2014)

^fhttp://www.msd-animal-health.co.in/Products/Foot_and_Mouth_Disease_Vaccine/020_Product_Details.aspx (accessed 05/11/2014)

^ghttp://www.kevevapi.org/index.php?option=com_content&view=article&id=127&Itemid=123, accessed 01/07/2014)

^hhttp://www.msd-animal-health.ph/products/131_118551/ProductDetails_131_118625.aspx

Chapter 10. Decline of maternal antibodies and the impact on vaccination response

10.1 Introduction

The Kenyan Veterinary Vaccine Production Institute (KEVEVAPI) is a state-owned FMD vaccine production company that is the sole provider of FMD vaccines in Kenya and the largest producer of FMD vaccine in Africa. Their vaccine (Fotivax™) is an inactivated aqueous based product with aluminium hydroxide gel and saponin adjuvants. Each batch of vaccine undergoes potency tests conforming to OIE standards (OIE, 2009) performed by the National FMD laboratory which is based on the same site as the vaccine institute but is a separate government institution. The manufacturer's recommended schedule is to administer vaccine to animals of all ages via subcutaneous injection at least every six months but four months for "better protection"⁵. It is licensed for cattle, pigs, sheep and goats.

As part of the quality assurance (QA) at KEVEVAPI, four dairy cattle farms in different regions of Kenya are vaccinated every four months with free quadrivalent (O, A, SAT1, SAT2) vaccine in exchange for KEVEVAPI's being able to take serum samples at vaccination and 21 days later. These samples are tested for FMD antibody by virus neutralisation tests (VNT). Vaccination, sampling and laboratory tests are all performed by the QA department at KEVEVAPI with appropriate measures undertaken to maintain the cold chain up until the point of vaccination. The primary analysis is to assess the proportion of animals that are over the 1.36 titre threshold which the manufacturer considers to correlate with clinical protection. The results of the sampling are fed back to each participating farm. At sampling, animals are classified as calves (<9 months old), yearlings (9-18 months old) and adults (>18 months old).

Contact was made with one of the QA farms which was conveniently located, easily accessed, had individual animal records and agreed to allow access to relevant data to allow analysis of the VNT data. KEVEVAPI had been using this farm for QA purposes for more than 20 years with some data being available electronically as far back as 2006. In light of the findings on the large-scale farms alongside the conclusions of the preceding literature review, the objective of this analysis is to evaluate the effect of maternally derived antibody (MDA) on the response to FMD vaccine using historical data from this farm. It is hoped that the combination of this

⁵ http://www.kevevapi.org/index.php?option=com_content&view=article&id=127&Itemid=123, Accessed 01/07/2014.

analysis with relevant epidemiological data will inform policy for vaccination in Kenya and other countries in East Africa.

10.2 Methods

10.2.1 Farm background

The study farm was located to the west of Nairobi and had approximately 200 cattle of Ayrshire, Friesian, Guernsey and Jersey breeds along with various crosses between them. Calving took place all year around and no cattle were purchased. Each animal was individually identified with a unique ear tag number and name and had a unique identification card. Basic information was kept on the card such as the date of birth and sire/dam identification along with all information related to disease events and fertility (e.g. calving dates, offspring names, services). Electronic records were not routinely kept.

For colostrum, calves were left with their dams for a seven day period before being transferred to group pens for further rearing. An outbreak of FMD was recorded on the farm in September/October 2009 from which SAT1 serotype antigen was detected at the National FMD laboratory although no virus could be detected when sent to the World Reference Laboratory (WRL), Pirbright, UK. No other FMD outbreaks have been recorded on the farm since the early 1990s.

10.2.2 Vaccination and sampling

All cattle over the age of three months were vaccinated at four month intervals, although animals could be excluded if calving is expected within two to three days of vaccination. Approximately 75% of animals vaccinated were blood sampled, with sampling generally not being performed on cows during high yielding periods due to a concern that this might compromise milk production and cows in late gestation so as to minimise the risk of abortion. The virus strains included in the vaccine (serotypes O [K77/78]; A [K5/80]; SAT1 [T155/71]; SAT2 [K52/84]) were the same for each vaccination although the batches varied over time (Table 10.1).

10.2.3 Laboratory tests

All VNTs were performed by KEVEVAPI using homologous virus strains. Two-fold dilutions of serum beginning with a 1/8 dilution were tested by their ability to neutralise the cytopathic

effect of FMD virus on Bovine Hamster Kidney (BHK) cells. End point titres were determined by a pH driven colour change that is prevented when neutralising antibodies are diluted to levels insufficient to prevent a viral-mediated cytopathic effect. Titres are recorded as the logarithm (base 10) of the reciprocal of the last dilution where a colour change was detected. If no colour change occurs at the lowest dilution of serum the result is referred to as $<1/8$ (or <0.9 as the reciprocal logarithm to base 10 equivalent).

10.2.4 Data analysis

Due to an outbreak of FMD which occurred on the farm in late September 2009, only results prior to this date were selected. VNT data were made available either electronically through KEVEVAPI or in hardcopy from the farm and manually entered. Data were entered into Microsoft Excel and imported into Stata 13.0 (Statacorp, Texas, USA) for analysis. For analysing the decline of MDA by age, tobit regression was used to account for left censored VNT titres below the threshold detection limit of $1/8$ (or 0.9). For the analysis of response to first vaccination, calves were defined as “Responders” if there was any increase in VNT titre 21 days post-vaccination. If there was no change or titre decrease, the calf was classified as a “Non-responder”. MDA levels were categorised as <0.9 , $0.9-1.19$, $1.2-1.49$, $1.5-1.79$, $1.80-2.09$ and >2.1 . Animals were defined as seroconverting if there was a four-fold increase in titre post vaccination, a cut-off that has been used previously for FMD when considering sub-clinical infection (Cox et al., 2010). For repeated vaccination, geometric mean titres at each dose and 21 days later were considered and all titres <0.9 were recorded as zero and a “protective” threshold of 1.36 was used.

10.3 Results

VNT results were retrieved for all vaccination dates going back to August 2006, this being the first date for which electronic records were available, covering ten vaccinations (Table 10.1). The last of the vaccinations occurred just prior to the aforementioned outbreak and the 21-day follow-up testing was not performed in this instance. Date of birth records were found for 60 calves which formed the dataset for analysis. Nine different vaccine batches were tested, two of which were used on two occasions. On one vaccination date two different batches were used although it was not recorded which animals received which batch on this occasion (Table 10.1).

Table 10.1. Dates of vaccination, batch number(s) of vaccine used and the number of animals sampled that were being vaccinated for the first time for which farm records were available

Vaccination date	Batch number	Number of animals sampled when receiving first dose
29/08/2006	K01099	2
28/12/2006	K01105	2
26/04/2007	K01119	8
06/09/2007	K01119	9
17/01/2008	K01135	4
22/05/2008	K01137	8
11/09/2008	K01144/K01146	8
15/01/2009	K01150	5
14/05/2009	K01150	11
10/09/2009 ^a	K01165	3

^a 21-day follow up sampling not available.

Frequency distributions of titres of all the samples show a right skewed distribution with most animals being in the 0.9-1.19 category (Figure 10.1). The numbers of animals at first vaccination above the 1.36 titre which the laboratory considers consistent with protection are 5/60 (8.3%) 3/60 (5.0%), 10/50 (20.0%) and 11/49 (22.4%) for serotypes A, O, SAT1 and SAT2 respectively. Associations between the age at first sampling and the level of neutralising titres give an indication of the decay of MDA over time (Figure 10.2). For all serotypes except SAT1 there is a decreasing trend of titre with age, although this is only statistically significant for serotypes O and SAT2. The half-lives for serotypes O and SAT2 are 141 (95%CI 78-687) and 158 (95%CI 87-911) days respectively. Regression analysis indicated there was no significant association between the titres for the different serotypes indicating limited cross protection.

VNT titres in respective dams were available for 22 calves from this study (Figure 10.3). The median titre was highest for serotype O. The distributions are broadest for serotype A, with the highest inter-quartile range, and SAT1 which has two outlier values (one dam had a titre of 0.9 and one was <0.9). The mean time difference between the dam and calf being sampled was 69 days (standard deviation 33.7, range 8-125).

Analysis of the response to the first dose compared to the neutralising titre of MDA showed that an increasing level of MDA was associated with fewer responders to first vaccination for serotypes A and SAT1 but there was no statistical evidence of a trend for O and SAT2 (Table 10.2). Although for serotype O all animals with titres <0.9 responded to vaccination in contrast to animals with higher titres, for serotype SAT2 there was no indication at all that lower titres

was associated with a response. For SAT2 there even appeared to be an upward trend although there was no statistical evidence to support this observation (Table 10.2). No animals with titres above 1.5 responded to vaccination for any serotype. This analysis was supported by assessment of seroconversion rates, defined as a fourfold increase in VNT titres 21 days after vaccination (Figure 10.4). For all serotypes, very high seroconversion rates approaching 100% can be seen if the MDA titres are less than 0.9 except for SAT2 which is much lower at around 20%. Based on chi-square tests for trend, for serotypes A, O and SAT1 there was very strong evidence of a linear trend (all $P < 0.0001$) whereas there was no significant evidence for SAT2 ($P = 0.11$).

In order to assess the effect of repeated doses, data were collected for up to five doses of vaccine (Table 10.3). For serotypes A and O, after receiving three doses of vaccine the mean titre is above the protective cut-off at the time the fourth dose of vaccine is given. This is not the case for the SAT serotypes for which the mean titre dropped below the protective level after each of the five doses indicating poor persistence of titres for these serotypes (Figure 10.5). For serotypes A and O, after each dose of vaccine, the proportion still protected by the time the next four-monthly dose is given appears to be increasing with successive doses indicating probable increasing persistence of titres for these serotypes. This effect is less pronounced with the SAT serotypes which are still far below 50% protected even after the fifth dose of vaccine compared to 50% or greater for the A and O serotypes (Figure 10.6).

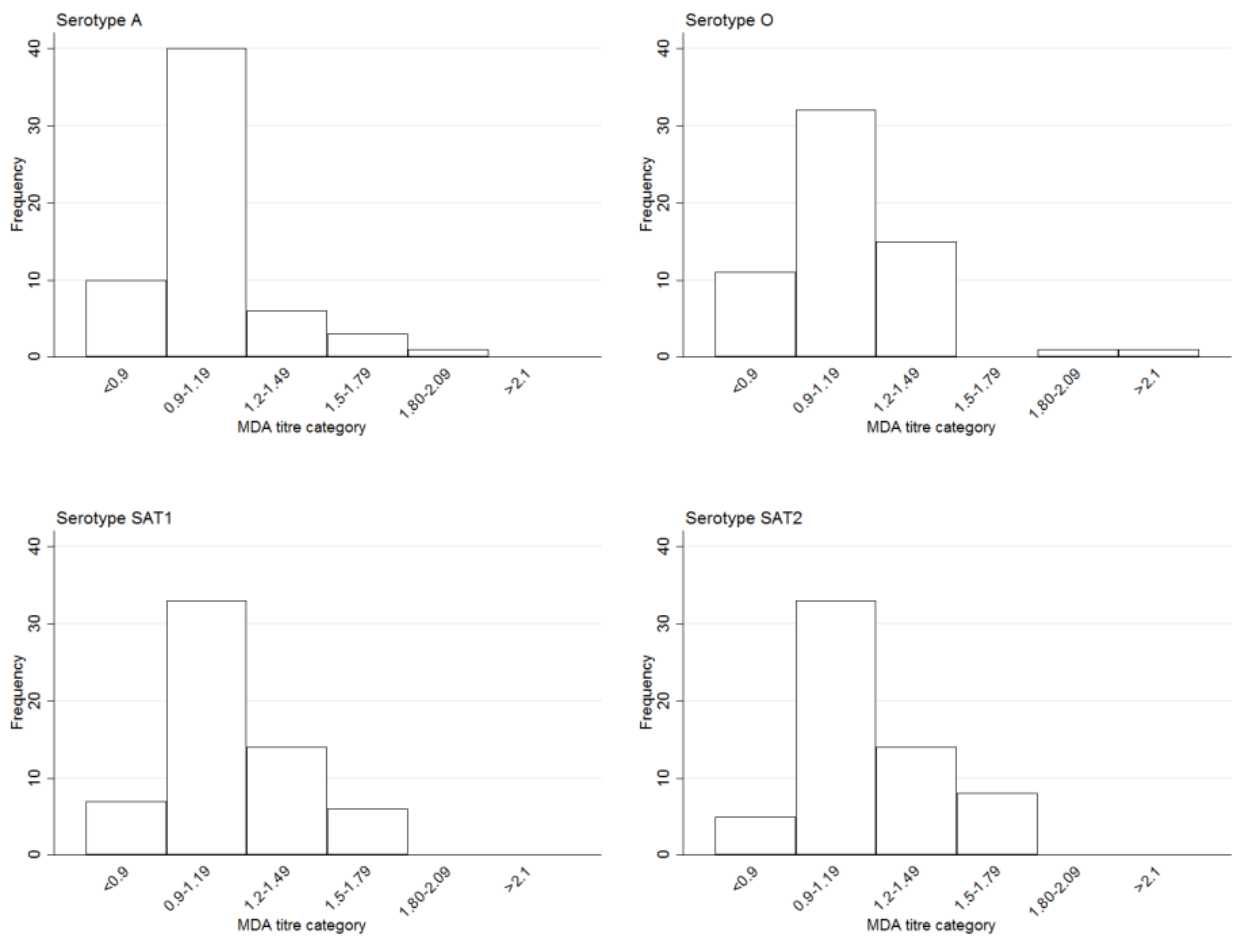


Figure 10.1. Frequency distribution of neutralising antibody titres for different FMDV serotypes at the time of first vaccination. Percentage of animals with “protective” titres: Serotypes A 5/60 (8.3%); O 3/60 (5.0%); SAT1 10/50 (16.7%); SAT2 11/49 (18.3%). MDA = Maternally derived antibody.

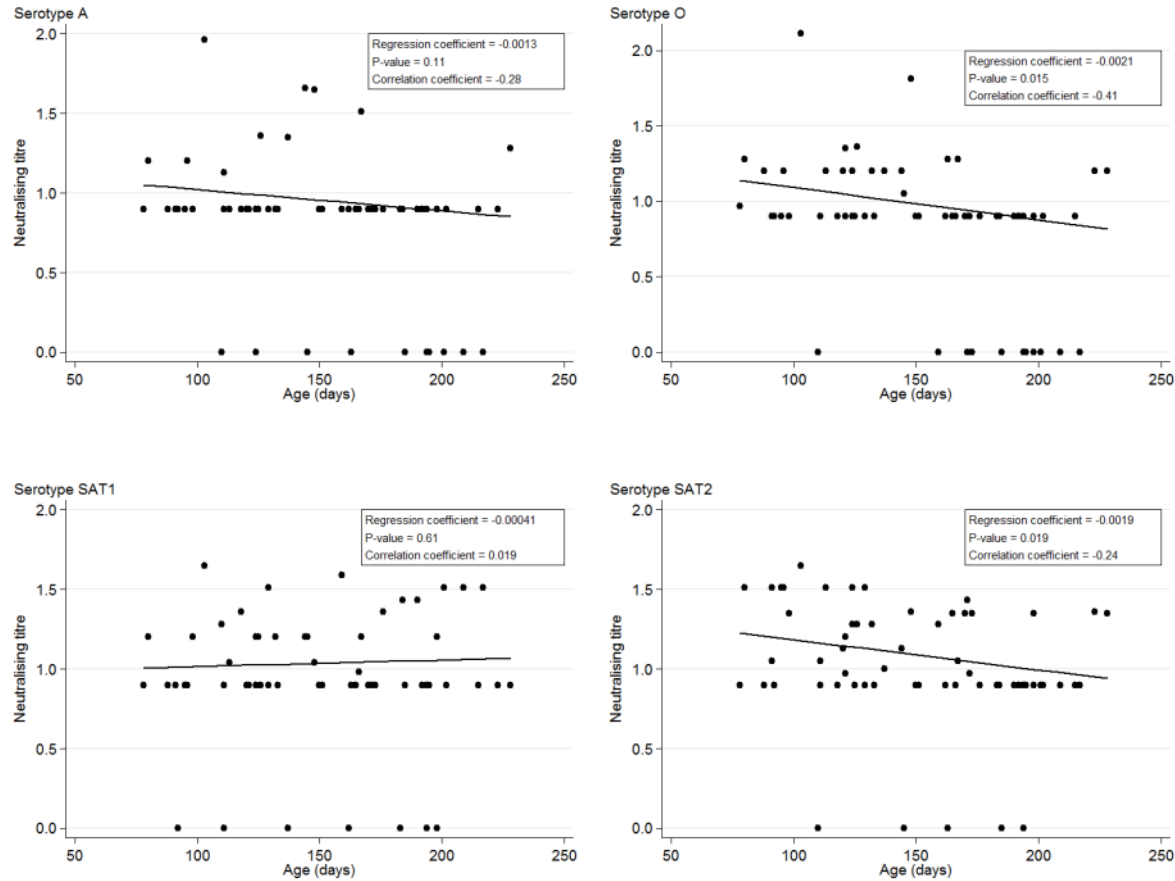


Figure 10.2. Scatter plot of neutralising titres of calves blood sampled at the time of first vaccination versus age with regression line predicted using the output of a tobit regression model to account for left censoring of values below the serological threshold detection limit. Titres are measured with VNT. Animals are sampled (vaccinated for first time) only when over the age of three months. Regression coefficient with associated P-value and the correlation coefficient are shown in boxes within each graph.

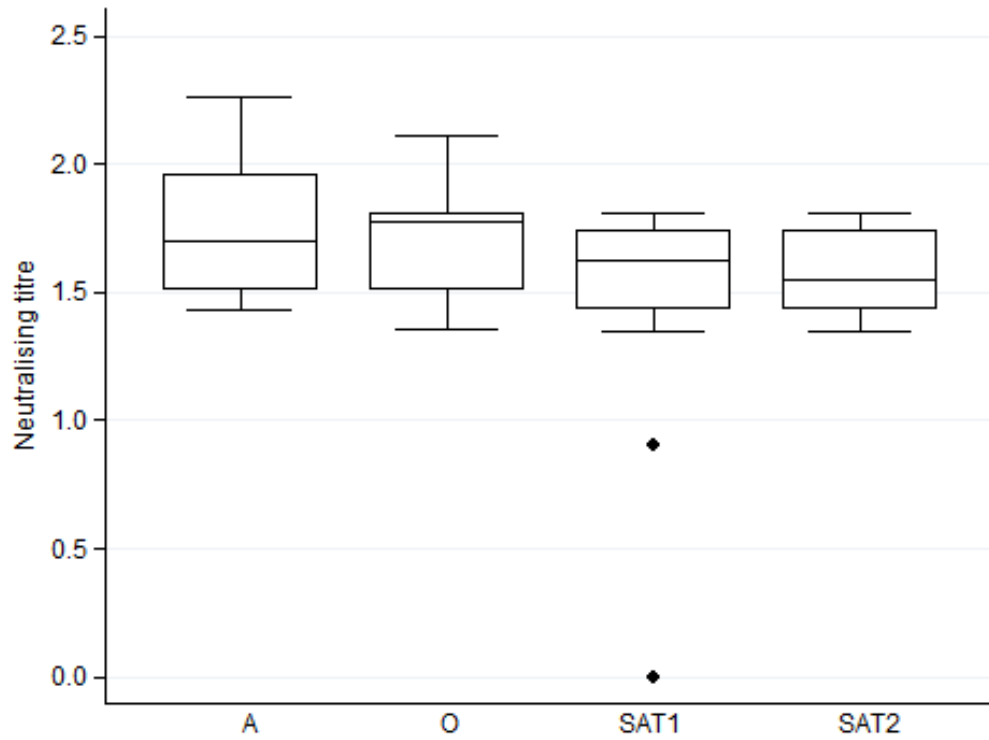


Figure 10.3. Box plot representing the distribution of neutralising titres for 22 dams of calves in the study population for each serotype.

Table 10.2. Number of animals responding to first vaccination by the level of maternally derived antibody (MDA) for each serotype.

Serotype	VNT titre at vaccination (MDA level)												P-value ^c
	<0.9			0.9-1.19			1.2-1.49			1.5-1.79			
	N ^a	R ^b	%	N	R	%	N	R	%	N	R	%	
A	10	9	90.0	36	23	63.9	5	3	60.0	2	0	0.0	0.020
O	11	11	100.0	30	9	30.0	12	9	75.0	0	-	-	0.29
SAT1	6	6	100.0	28	20	71.4	14	9	64.3	5	0	0.0	0.0014
SAT2	5	1	20.0	30	17	56.7	13	11	84.6	5	0	0.0	0.96

^a N = number. ^b R = Responder, defined by an increase in titre 21 days after vaccination. ^c Chi-square test for trend.

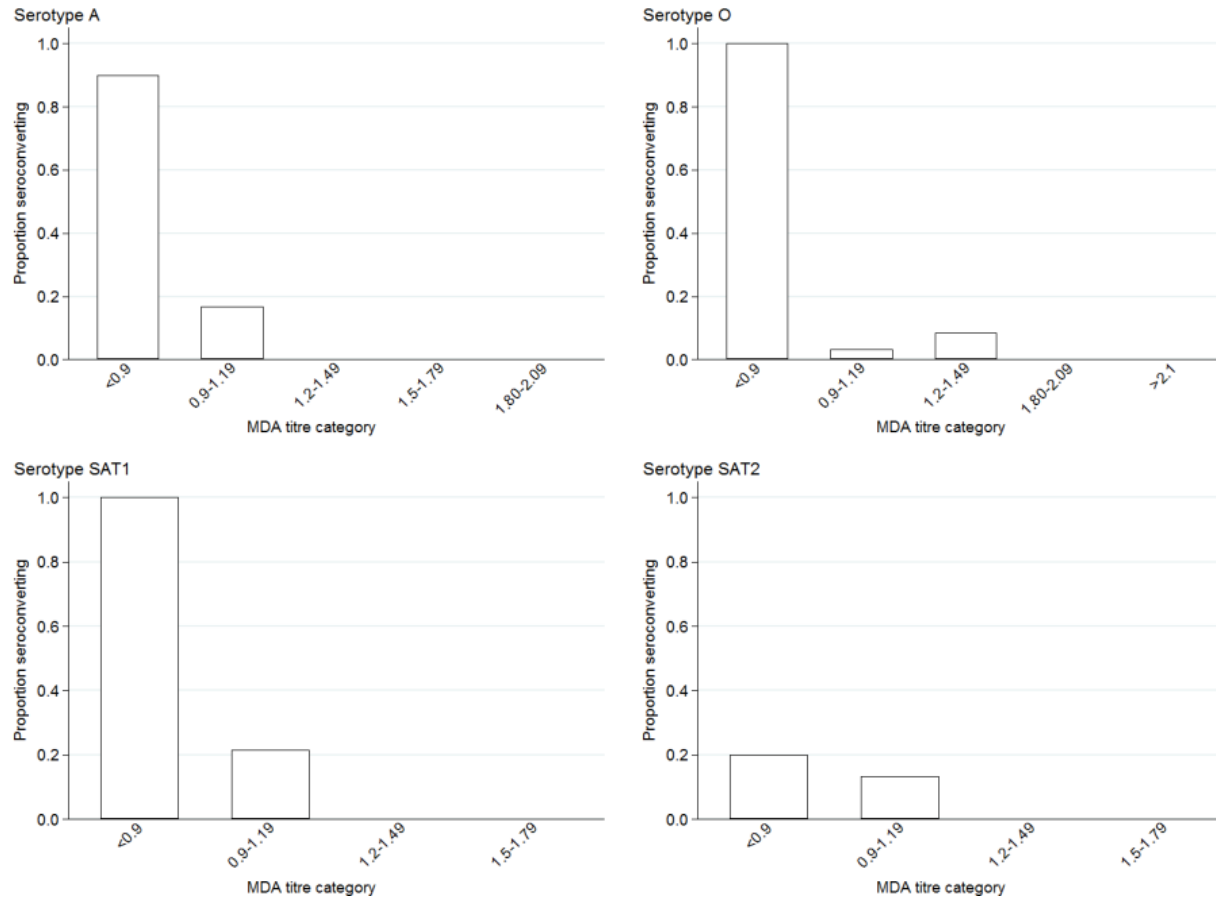


Figure 10.4. Proportion of animals seroconverting to the first dose of vaccine by the level of MDA present at vaccination as measured by virus neutralisation test. Seroconversion is defined by a fourfold increase in titre measured 21 days after vaccination. For serotypes A, O and SAT1 there was very strong evidence of a linear trend based on chi-square tests for trend (all $P < 0.0001$). For SAT2 there was no significant trend ($P = 0.11$). MDA = Maternally derived antibody.

Table 10.3. Repeated doses of vaccine – titres at vaccination and 21 days later.

Vaccine dose	Number of animals	Geometric mean titres (standard deviations)			
		A	O	SAT1	SAT2
D1	60	0.84 (0.44)	0.86 (0.47)	0.95 (0.41)	1.0 (0.39)
D1+21d	53	1.1 (0.39)	1.1 (0.27)	1.2 (0.29)	1.1 (0.43)
D2	64 ^a	0.99 (0.37)	0.99 (0.36)	1.1 (0.22)	0.98 (0.34)
D2+21d	50	1.4 (0.27)	1.6 (0.29)	1.4 (0.24)	1.3 (0.40)
D3	52	1.2 (0.25)	1.2 (0.29)	1.1 (0.30)	1.1 (0.34)
D3+21d	45	1.6 (0.13)	1.8 (0.30)	1.5 (0.25)	1.5 (0.38)
D4	46	1.4 (0.32)	1.4 (0.32)	1.2 (0.36)	1.2 (0.33)
D4+21d	37	1.6 (0.16)	1.8 (0.23)	1.5 (0.20)	1.6 (0.19)
D5	42	1.4 (0.27)	1.4 (0.29)	1.2 (0.37)	1.3 (0.35)
D5+21d	31	1.7 (0.18)	1.8 (0.22)	1.6 (0.18)	1.5 (0.27)

Note: Animals are vaccinated approximately every four months. D1 represents the first dose of vaccine the animal receives. Samples with no detectable antibodies (i.e. <0.9) were recorded as zero. Data are represented graphically in Figure 10.5.

^a For some animals the first dose titre was not recorded but the second dose was hence more animals included at D2 than D1

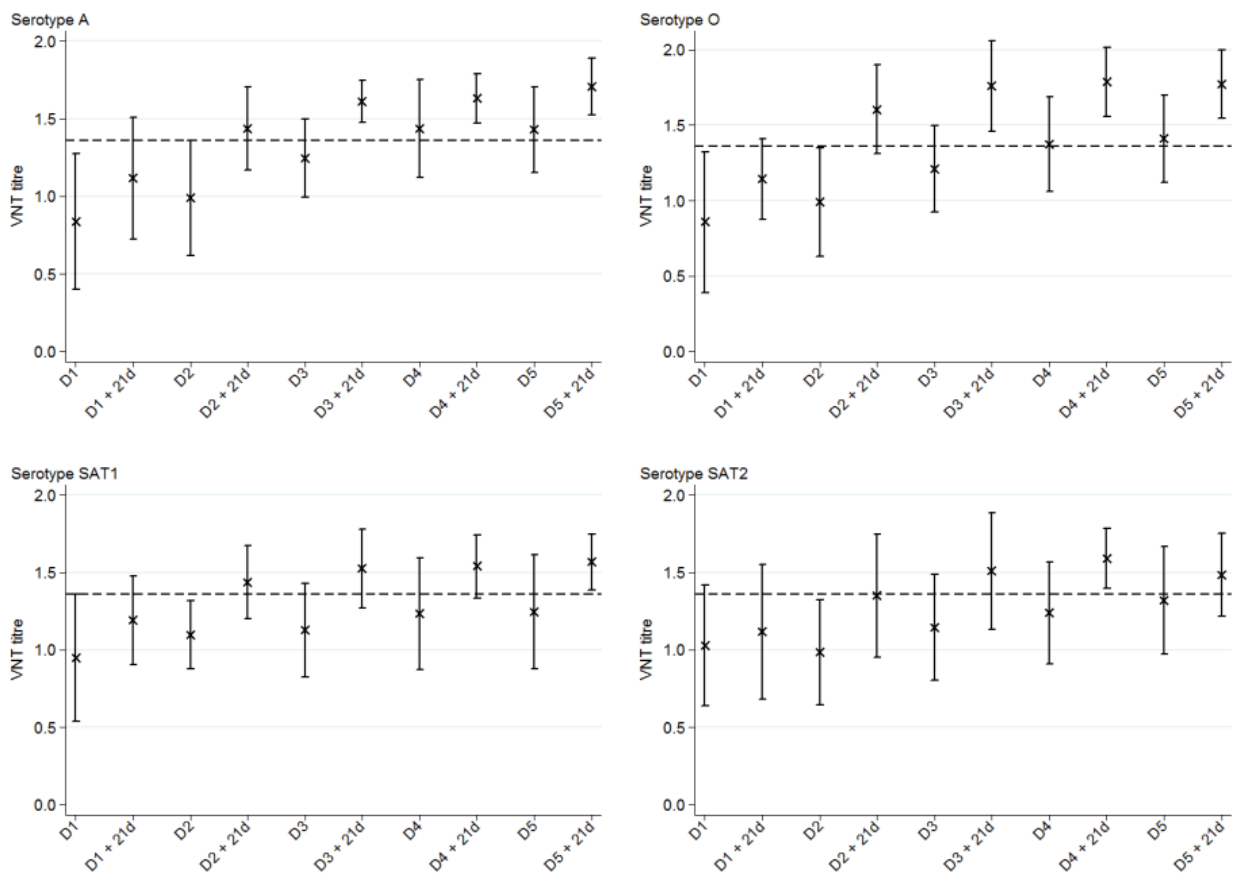


Figure 10.5. Response to the first five doses of quadrivalent vaccine reflected by an increase in neutralising titre to the homologous virus strain. D1-5 represents the (mean) titre at each vaccination with the D+21 representing the (mean) titre 21 days post vaccination. The horizontal line represents the titre considered to be protective by the manufacturer (1.36 for each). Samples with no detectable antibodies (i.e. <0.9) were recorded as zero. Data are tabulated in Table 10.3.

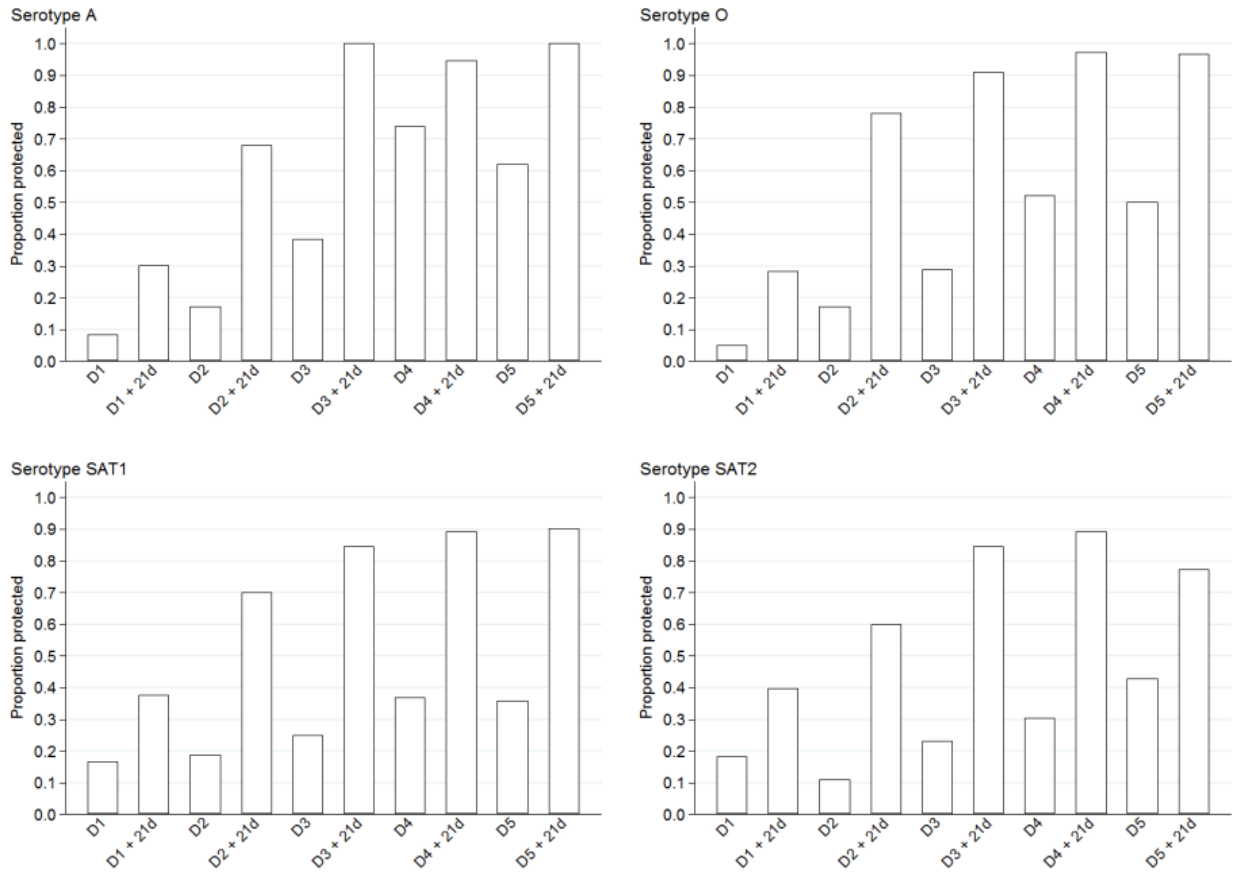


Figure 10.6. Proportion of animals with a “protective titre” (greater than 1.36) by the number of doses of vaccine. D1-5 represents the proportion “protected” at each vaccination with the D+21 representing the proportion 21 days post vaccination.

10.4 Discussion

10.4.1 Maternally derived antibody

The level of maternally derived antibody (MDA) was shown to decrease with age as expected due to waning levels over time associated with IgG catabolism (Cervenak and Kacskovics, 2009) although statistical evidence was apparent only for O and SAT2 serotypes with half-lives calculated as 141 (95%CI 78-687) and 158 (95%CI 87-911) days, respectively. These estimates are far greater than those reported in the literature and therefore cast doubt on these data. The total lack of an association between age and VNT level for A and SAT1 is surprising. For serotype A, there was a declining trend but the regression coefficient was not significantly different from zero (regression coefficient -0.0013; P=0.11). For SAT1, there was a slight upward trend and several animals had high levels of antibody (≥ 1.5) despite being over 150 days of age, more than is apparent for the other serotypes, and also indicated by the higher proportion of animals having protective levels of antibody at the first dose (Figure 10.6). A similar effect is seen for SAT2 but to a lesser extent and may indicate some cross-reactivity of the assay although regression analysis did not indicate an association between the titres of the different serotypes. By 150 days of age, one would have expected the antibody levels to have waned to very low levels. These findings may indicate virus exposure, either of the dams leading to high colostrum titres that are transferred to their offspring, or more likely exposure of the calves leading to endogenous antibody production. The farm did not report any outbreaks of disease that would explain this finding although in a vaccinated population it is possible that clinical signs may be much reduced and transmission may be at such a level as to not be obvious to farm staff, particularly if older multiply vaccinated lactating cows were not clinically affected.

There are likely to be several other reasons beyond possible exposure leading to inaccurate estimates. The absence of data in calves below the age of three months likely compromises the quality of the dataset and analytical capability. There are also many factors that can affect the quality of the colostrum, and there is likely to be variation in the initial calf titres and that will affect the half-life calculation. Unfortunately, maternal antibody levels were only available for 22 dams and few of these were close to the time of calving meaning dam levels of neutralising antibodies could not be usefully analysed alongside the calf data. However, titres in these dams were variable particularly for serotypes A and SAT1 (Figure 10.3). This variation may contribute to the lack of association between age and titres in their offspring for these

serotypes and the inaccurate half-life estimates for serotypes O and SAT2. A more reliable way of calculating the half-life would be to follow a cohort of individual calves with sampling commencing shortly after birth.

10.4.2 Response to first dose of vaccine

The increasing titre response to first vaccination was strongly related to the VNT levels, consistent with other reports in the literature, although this effect was statistically significant only for serotype A and SAT1. There was some indication that low MDA titres were associated with a greater response for serotype O but much less with SAT2, which may reflect an overall poor serological response to the SAT2 vaccine as seen in Figure 10.5. When considering seroconversion, evidence was stronger of an association with MDA. Very high seroconversion rates were seen for serotypes A, O and SAT1 if the MDA titre was less than 0.9 decreasing with higher levels. However, such evidence was not apparent from SAT2 further indicating problems with the SAT2 response. Although using a definition like this is likely to be an underestimate of seroconversion in response to vaccines when applied to settings with a high background immunity (Chandramohan et al., 2007), the strength of this association and the differences seen between SAT2 and the other serotypes is indicative of a genuine effect.

The inconsistent association of VNT levels with age means an optimal age at first vaccination cannot be confidently suggested from the results of this analysis. No increase was seen for any serotypes when titres were over 1.5. Disregarding SAT1 results that may be unreliable due to possible virus exposure, the mean and median ages of calves that had titres over 1.5 at first vaccination were 117 and 108 days respectively. This is in contrast to a number of studies that showed responses to aqueous-adjuvanted vaccines in calves younger than this age (Figueroa et al., 1973; Shankar and Uppal, 1982; Uppal et al., 1975) but in agreement with other more recent studies (Nicholls et al., 1984; Patil et al., 2014; Sadir et al., 1988). Directly relating these findings to other studies is complicated by the use of different vaccines, different study populations, and variable MDA levels with age. This study was also done in a non-experimental setting, and utilised a quadrivalent vaccine that included SAT1 and SAT2 serotypes which has not been reported previously.

10.4.3 Titre trends with repeated vaccination

Sampling with each vaccination provided the opportunity to assess how the titres fall between doses, and whether protective titres are maintained. With each dose of vaccine there is a clear

increase in titre 21-days later which then decreased by the time the next dose is given four months later for all serotypes. This decrease becomes less pronounced with each dose for serotypes A and O but this effect is not seen to the same extent with the SAT serotypes indicating poor persistence of antibody even after four doses of vaccine. Indeed, the responses look more like a “succession of primary responses rather than a continuously maturing immune response” as previously observed by Doel (1996). The overall proportion of animals “protected” (i.e. titres ≥ 1.36) 21 days after vaccination reaches very high levels for serotype A and O being over 90% after three doses of vaccine. For the SAT serotypes, levels don’t reach 90% even after five doses of vaccine. This indicates that although there was a response to the SAT serotypes at vaccination, it was less than for the other serotypes and the levels were not maintained. Higher antigen payload, an oil based-adjuvant, or higher frequency of doses may be required for the SAT serotypes although the latter will increase cost and labour and therefore unlikely be acceptable to the consumer given the high frequency of vaccination already performed. The need for a higher antigen payload for SAT2 was previously suggested on the basis of experience with potency tests (Parida, 2009), although similar suggestions for SAT1 have not been made. There is evidence that the use of an oil-based adjuvant may increase the duration of immunity (Gomes et al., 1980) including for SAT serotypes in field conditions in South Africa using equivalent antigen doses (Cloete et al., 2008). Additionally for SAT serotypes, higher titres have also been demonstrated post-vaccination for double oil emulsion vaccines compared to aqueous aluminium hydroxide/saponin equivalents (Hunter, 1996).

The overall levels of induced titres were generally disappointing with mean titres for each serotype ranging from 1.5 to 1.8 after five doses. Relating this to other studies in the literature, Pay and Hingley (1987) used an aqueous based vaccine for serotypes O, A and C and analysed how different antigen doses affected the serological response measured through serum neutralisation titres. They typically found titres over 2.0 with a single dose of vaccine at a 1:2 dose dilution. Although their study was in immunologically naïve calves, similar levels should be attained after five doses after which time maternal antibody would have long disappeared. Although these antibody levels induced by the KEVEVAPI vaccine may be sufficient for clinical protection at 21 days post vaccination, evidenced by each batch passing a potency test, the lack of antibody persistence may not provide sufficient protection and higher initial titres may therefore be required. The antigenic dose for the KEVEVAPI vaccine is not available but clearly this is an aspect of vaccine production that should be addressed.

The results of these analyses indicate a need to address the schedules of vaccines given to youngstock. For serotype A and O, at least three doses are required in order to achieve satisfactory persistence between doses. In this study, the vaccines are given to all cattle every four months. Among youngstock, a second dose given at one month after the primary dose may allow animals to reach a higher level of protection quicker minimising the time period between protective MDA being present and sufficient antibody being induced by the vaccine – the so called “immunity gap”. Thereafter the calves may fit into the herd vaccine schedule receiving a booster up to four months later. The optimal age at first vaccination in repeatedly vaccinated herds also needs to be addressed and the results of this analysis are not able to suggest the optimum time. Although there are various recommendations for this age from other settings (Table 9.4), differences between vaccine manufacturers and the population being immunized mean that population-specific studies should be performed. These studies should consider the practical difficulties in smaller herds of ensuring calves are vaccinated at the correct time which is limited by the vial size and extra labour required when vaccinating outside of the herd schedule.

There are uncertainties over the results presented particularly with reference to the decline in maternal antibodies indicating some exposure may have occurred. It also would be worthwhile getting external validation of the VNT results for example through the National FMD laboratory in Kenya or the World Reference Laboratory in Pirbright, UK. The protective titre assumed in this study is 1.36 for each strain which is the level used by the manufacturer. According to OIE guidelines “Cut-off titres for evaluating immunological protection afforded by vaccination have to be established from experience of potency test results with the relevant vaccine and target species”. No evidence from the manufacture was available publicly, but with all batches of vaccines undergoing potency tests, supplementary VNT information could be easily collected.

The data used in this analysis were from vaccinations performed under “ideal” conditions with the vaccine manufacturer performing the vaccination and therefore are a “best-case scenario” for sero-conversion in the field. Under more realistic field conditions, the vaccine is not likely to perform as well with a suboptimal cold-chain, missed or inaccurate doses and deterioration of antigen post-manufacture. Therefore post-vaccination serological monitoring in areas of vaccination, performed by independent monitors or government subsidised vaccination campaigns would add valuable information.

In conclusion, the results of these analyses indicate opportunities to modify the vaccine schedules to achieve greater levels of protection quicker in an animal's life. In addition, the antigen payload or vaccine adjuvant for the SAT serotypes needs to be addressed. Further validation of these results is required and the evidence for a protective titre needs to be clearly elucidated.

Chapter 11. Discussion

This thesis has presented data relating to the epidemiology, vaccine effectiveness and disease impact on two large-scale farms in Kenya (Part A). The vaccine evaluations are supported by the analysis of serological data provided by the vaccine manufacturer (Part B). All three research paper publications (Chapter 4, Chapter 5 and Chapter 6) include separate discussions relating to their specific objectives and findings. For consistency in structure, the non-published chapters also include separate discussion sections, including Chapter 8 comparing the epidemiological aspects of the two outbreaks. This final chapter provides a general discussion of the implications, limitations and subsequent recommendations based on the work as a whole. This is complemented by general observations from the field during the study period involving discussions with Kenyan farmers, veterinary surgeons and animal health assistants.

11.1 Key findings

Two instances of poor vaccine effectiveness on large-scale, extensively grazed dairy farms in Nakuru County, Kenya were investigated. Both had received the last vaccine dose approximately three months before the index case occurred and used a periodic vaccination policy whereby all eligible animals were vaccinated at the same time.

The exact reasons for observed low effectiveness were not confirmed in this work. However, based on the patterns of disease and complementary analysis of the VNT data provided from the vaccine manufacturer, evidence was provided for three important factors:-

1. Poor match between the field and vaccine strain ("Vaccine match", [VM])
2. Waning antibody titres between vaccination rounds
3. Sub-optimal vaccine schedules in youngstock

Of particular concern is the vaccine for the SAT2 serotype given the high incidence of disease seen despite multiple doses of vaccine together with poor seroconversion rates and rapidly waning titres seen in the VNT analysis.

On the farm that had the outbreak due to serotype SAT2 (Farm1/SAT2), where the vaccine appeared ineffective, an analysis of disease impact was performed looking at the effect of the outbreak on milk yield, clinical mastitis and culling. A clear impact of the disease on milk production was seen at a farm level decreasing from an average of 20 to around 13 kg per cow

per day. It took around 2 months to show some recovery before a further decline was seen. Despite this, no difference was found in milk yield between reported cases and non-cases. This may have been due to subclinical infections, poor sensitivity of the case definition or underreporting. Compared to predicted yields, higher impact was seen among older animals in peak-lactation with the greatest effect seen in cows of parity four or greater that were between 101 and 200 days in milk at the beginning of the outbreak. These animals produced a 305 day milk yield which was around 550 litres less than predicted. Survival analysis showed recorded FMD cases were at an increased risk of clinical mastitis in the month after the outbreak began (HR=2.9, 95% confidence intervals [CI] 0.97-8.9, P=0.057) and weak evidence that they were associated with an increased culling over the 12 months after the outbreak began (HR=1.7, 95%CI 0.90-3.4, P=0.10).

11.2 Implications

11.2.1 FMD outbreaks on large-scale dairy farms in Kenya

These studies provide useful insights into outbreaks in endemic settings that may inform control measures on affected farms. Both outbreaks occurred over 1-2 month periods with two major peaks in cases consistent with movement of virus to clusters of inter-connected groups. Both farms attempted isolation of clinical cases, but neither was successful in preventing the outbreak affecting most of the animal groups. Neither attempted any other forms of biosecurity. On Farm 1/SAT2, the early movement of animals in an attempt to protect the dairy herd is likely to be the reason the outbreak became widespread so quickly. This information is useful as it indicates that several measures are likely to be needed to prevent intra-farm spread once a farm is affected and that isolation of affected animals alone is likely to be insufficient.

FMDV is well known by Kenyan farmers for its transmissibility. The author's experience in the field revealed it was common for farmers to say that they would prefer an outbreak to affect all groups as soon as possible so as to minimise the duration of inconvenience the outbreak brings. This is a similar approach to the "aphthisation" described by Suttmoller et al. (2003) which was "common practice" globally until the 1950s. The deliberate infection of livestock meant farms avoided a period of uncertainty and long outbreak duration. From the author's experience of visiting several large-scale farms in Kenya, it appears that most farmers are resigned to fate and only make half-hearted efforts at disease control once their farm is

affected and no sustained efforts at virus containment. When farms use vaccination it is likely that vaccine induced immunity is overcome by relatively high levels of exposure to virus. Although the risk of spread cannot be eliminated, efforts to minimise it may have some effect if performed appropriately. It is apparent that farms should have contingency plans designed to minimise spread and impact in the event of an outbreak (discussed further in section 11.4.1).

In particular on Farm 1/SAT2, there was a low incidence of FMD among younger animals (section 4.4.4). This finding is reminiscent of the disease pattern seen with a serotype O strain outbreak reported in the Netherlands (Bouma et al., 2004). This suggests that more proactive surveillance among young animals may be required to detect the presence of infection. If a farm is at high risk of an outbreak, individual calves may need routine close examinations to detect FMD. It is important to identify affected groups early so that control measures may be rapidly implemented. Undetected disease in youngstock may facilitate onward transmission to other groups on the farm, particularly if intra-group biosecurity measures have not have been introduced in the absence of overt disease on the farm.

11.2.2 FMD vaccination

These are the first studies to investigate the field effectiveness of FMD vaccination in East Africa and the first studies to evaluate vaccines on farms using a routine vaccination strategy. Field investigations are limited worldwide due to a longstanding tradition among FMD researchers and vaccine manufacturers towards experimental studies for evaluating vaccines. Such experimental studies cannot account for circumstances in the field when it comes to such factors as exposure, coverage, and cold chain policies. This is apparent in this study, as the vaccines were apparently tested and approved according to the recommended PD_{50} test in the OIE guidelines (OIE, 2009), yet did not work effectively. The poor vaccine performance was similar to that observed in outbreaks in Israel reported by Elnekave et al. (2013) and in Thailand reported by Gleeson et al. (1995) each of which occurred despite a good vaccine match to the field strain. This demonstrates that even a vaccine that has supposedly passed standardised quality control procedures may not perform as expected in the field, suggesting that adjustments are needed to our evaluation approach if we are to optimise effectiveness. As it is only through a field-based epidemiological approach that such findings are revealed, it is important that such studies be encouraged worldwide in similar and different settings.

The vaccine used in these outbreaks was a locally produced aqueous quadrivalent vaccine containing single strains of serotype A, O, SAT1 and SAT2 viruses. The manufacturer-recommended **schedule** is a dose at least every six months but preferably every four months to animals of all ages (KEVEVAPI, 2014). Based on the results presented here, in particular the outbreak on Farm 2/O and the VNT analysis (Chapter 7 and Chapter 10), there is evidence that a two or three dose primary course may be needed in line with recommendations by other vaccine producers (Table 9.4). The high incidence among youngstock, which decreased with the number of doses received, suggests that more doses of vaccine need to be administered earlier in life to ensure sufficient levels of immunity among younger animals. Different schedules should be investigated by the manufacturer (see “Further research priorities”, section 11.4.5).

A history of disease in a previous outbreak was not associated with protection on Farm 2/O suggesting that the greater protection among multiply vaccinated animals was due to vaccination rather than **previous exposure** which might have occurred in older animals. However, previous disease or infection cannot be ruled out entirely. Mild, unrecorded disease may have occurred during these known outbreak periods and it is possible that virus incursions could have occurred unknown to the farmer particularly as disease is endemic in the region. Additionally there could be other reasons that immunological protection increases with age. The inability to adjust for age as a confounder between the lifetime number of doses received and the risk of FMD is a major limitation of the analysis.

Although there is evidence that additional doses are needed to increase protection in youngstock, the unacceptably high incidence among multiply vaccinated animals implies that schedule modification alone is unlikely to have made a substantial difference to the outcome on Farm 2/O (Section 7.4). Therefore schedule modification needs to be considered as part of a combination of measures aimed at improving effectiveness. Further investigations are required to establish the reason(s) behind such outbreaks.

Although these vaccines apparently passed the OIE recommended potency tests, the rapid waning of titres may indicate poor vaccine **quality**. Figure 11.1 allows comparison between the incidence by the number of doses for each farm (Figure 8.5) and the neutralising titres with repeated doses for the respective serotype (Figure 10.5). The outbreak on both farms occurred coincidentally 99 days after the previous dose (Table 8.1). For SAT2, lower titre increases post vaccination, poor persistence of titres between doses and the limited cumulative titre increase with each dose may explain the high incidence in multiply vaccinated animals on Farm 1/SAT2

at this time-point since the previous dose. For serotype O, higher post vaccination titres and greater cumulative increase with repeated doses could explain why this vaccine was more effective at this equivalent time-point since the previous dose although more vaccine had been used on this farm mainly due to the different age distribution. Titre kinetics for animals receiving further doses, preferably similar in number to what animals had received on these farms, would add to this interpretation.

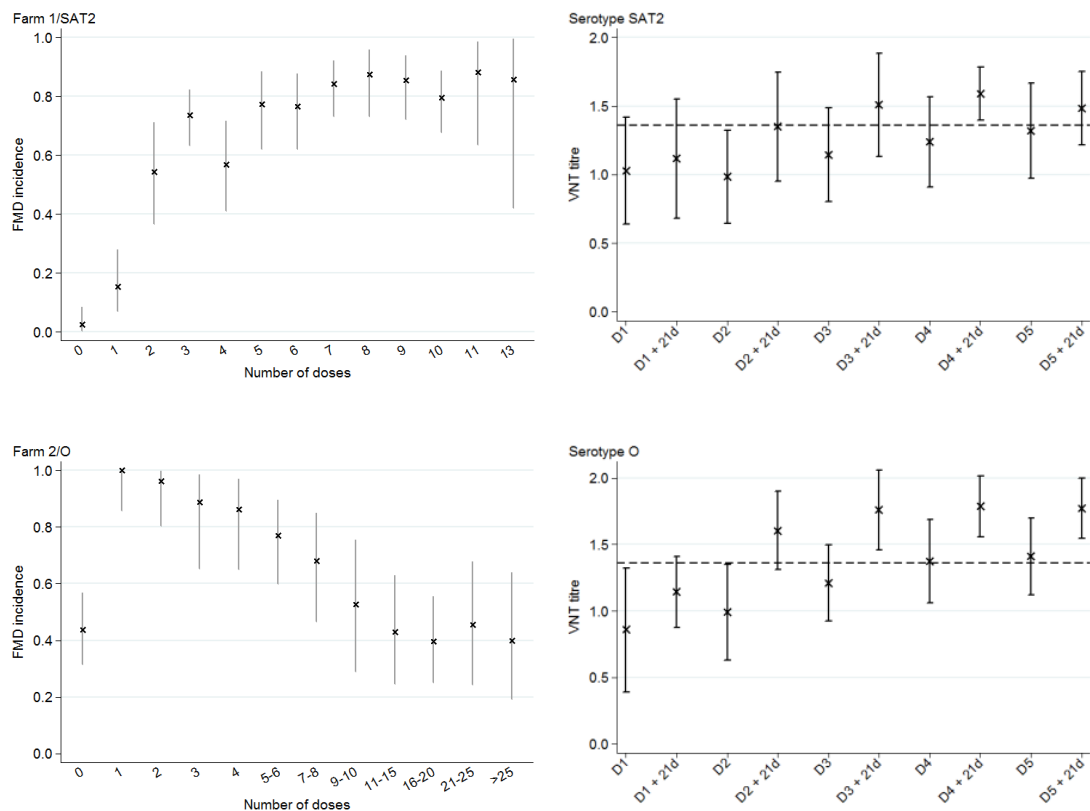


Figure 11.1. Comparison of the incidence by the number of doses for Farm 1/SAT2 and Farm 2/O and the pattern of neutralising titres with repeated doses of vaccine for respective serotype. For the VNT titres (right) the response to the first five doses of vaccine is shown (see Figure 10.5). D1-5 represents the (mean) titre at each vaccination with the D+21 representing the (mean) titre 21 days post vaccination. The horizontal line represents the titre considered to be protective by the manufacturer (1.36 for each).

The Kenyan vaccine manufacturer is currently developing a new vaccine for FMD that will be oil-adjuvanted and NSP purified. **Oil-based vaccines** have been extensively used in South America and experience there suggests that titres induced by such vaccines tend to be more persistent in cattle compared to aqueous equivalents (Sutmoller et al., 2003). More persistent

antibody responses with oil adjuvants have also been demonstrated under field conditions in Africa with SAT serotypes (Cloete et al., 2008). Advantages of oil-based vaccines have also been claimed in terms of improved potency as demonstrated in a study by Vianna Filho et al. (1993). They compared aqueous (hydroxide-saponin) and oil-based vaccines in adult (18-24 months old) cattle by IDL challenging them with serotype O virus at 21-28 dpv and 85 dpv after a single dose of vaccine. At the former time point 246/271 (91%) and 300/320 (94%) were clinically protected with the aqueous and oil adjuvant type respectively. In contrast, at 85 dpv the protection was 7/15 (47%) and 14/15 (93%) respectively. The authors conclude from these results that the oil adjuvanted vaccines perform better than the aqueous equivalent. However, benefits of oil adjuvants are controversial as highlighted in Chapter 9 with reference to the interference with MDA consistent with published reviews on the subject (Doel, 2003; Kitching and Salt, 1995). In a review by Parida (2009) no clear benefit of oil based vaccines has been apparent from an immunological perspective since both aqueous and oil-based tend to favour humoral immunity and neither induces sufficient cell-mediated response which is also required for protection. These studies imply that oil-adjuvanted vaccines may have some advantages over aqueous, although field based vaccine effectiveness studies should still be a key aspect of their evaluation.

11.2.3 FMD economics

As outlined in the literature reviews to the papers in Chapter 5 and Chapter 6, there is a lack of field evidence on FMD impact particularly in endemic settings. This lack holds back the control of FMD, as governments are unable to allocate resources appropriately in national control strategies given that resources are scarce and competing with other areas. The importance of this aspect of FMD control is set out in the guidelines of the joint FAO/OIE Progressive Control Pathway (PCP) document, which stipulates a minimum requirement for inclusion in Stage 1 as having some understanding of the socio-economic impact of FMD in the country for different stakeholders and husbandry systems (FAO, 2011).

Estimating the economic impact of disease is a highly complex subject due to the many direct and indirect effects that may occur. This combined with the lack of field data means economic impact assessments are often limited in their approach by either considering only direct effects, being based on assumptions, or using poorer quality data such as those provided by expert opinion (Knight-Jones and Rushton, 2013; Şentürk and Yalçın, 2008, 2005).

The results of the studies presented in this thesis provide objective estimates on milk yield (Chapter 6), clinical mastitis and culling (Chapter 5). These elements are poorly characterised for FMD, particularly for the latter two components that are rarely considered in economic impact assessments. Conducting a full cost analysis of the outbreak was not the objective of this thesis, and would require the development of an economic framework based on this Kenyan setting. However, the outputs of the studies reported here could be used to parameterise such economic models to estimate the overall cost of an outbreak, which could then be used to inform policy at the individual farm, national or regional levels.

11.3 Strengths and limitations

11.3.1 External validity of study results

Great caution must be taken when extrapolating an analysis based on just two farms to the general population since this study population is unlikely to be representative of all farms in the region. This applies to the analyses of epidemiology, vaccine effectiveness and economic impact. Most of the dairy production in Kenya is from smallholder dairy farms, unlike the farms described here.

The way in which an outbreak spreads on a farm is likely to vary depending on various management factors that will differ in type and extent between farms. The recognition of high risk exposure events on these farms may help to guide generic recommendations for these types of farms that may in turn be generalised to other large-scale dairy farms in Kenya and elsewhere in the world (see “Recommendations”, section 11.4.1).

The FMD vaccine investigated here would probably have behaved in a similar way on other dairy farms in Kenya under similar exposure conditions, although the inability to find hard evidence for the failures limits this generalisation (e.g. other farms may have used different schedules or different cold chain management). A survey of vaccine management and vaccination policies among these different farm types would therefore be useful. Among **smallholders** in Kenya, routine vaccination is less commonly used, and farmers usually receive vaccines only through government-subsidised “ring” (or “reactive”) vaccination campaigns (described in section 1.4.2). In such circumstances, the lifetime number of vaccine doses is likely to be much lower than that on the large-scale farms. However, since farms are being “ring vaccinated” during a period of high-exposure risk, exposure may be closer in time to when the vaccine was given. With the problems of VNT titre persistence demonstrated in this

thesis (Chapter 10), it is possible that clinical protection may be greater if the time between vaccination and exposure is shorter. In this context, it should be stated again that the VNT data used in this analysis is a “best-case scenario”, as they are from quality assurance (QA) farms where vaccination is performed by the manufacturer under ideal conditions. Lower titres may be expected during field vaccination campaigns. Additionally, as smallholder populations are at risk of exposure to virus from farms tending to use high risk management practices (e.g. shared grazing, using a common dipping facility for parasite control) a higher level of background immunity may be present from natural exposure that may complicate the evaluation of ring vaccination campaigns although there are no data to support this supposition.

The *impact* of FMD on milk yield, clinical mastitis and culling observed in this study may be similar to that in other large-scale farming systems. The impact of disease is likely to be different in areas where zebu breeds predominate and where beef ranches are more common, where the effects on weight gain also need to be considered. In the Nakuru County area, where dairy production is common, the smallholder farms often own exotic (i.e. grade or European) breeds of cattle due to their higher milk production. In fact cattle are often purchased from the large-scale farms depending on their proximity to these farms although the true extent of this practice is unknown (Figure 11.2). Smallholder cattle may be on a lower plane of nutrition compared to the large-scale farms which may affect milk yield. The author’s experience of working with smallholders in Nakuru County suggests that, depending on the area, they may have good access to high protein/energy concentrated feeds through locally organised co-operatives (Figure 11.3). Therefore the levels of nutrition may be comparable to the larger commercial farms. Given the similar genetics from purchasing cattle from the local large-scale farms, there is unlikely to be much difference in the rate of FMD-attributable clinical mastitis although decisions related to culling are likely to be different in a smallholder setting. At the household level, the socio-economics of disease are likely to be substantially different and require investigation.



Figure 11.2. Photograph of a smallholder farm on the outskirts of Nakuru. These cattle are zero grazed and fed a combination of concentrated feed and Napier grass. They were sourced from a nearby large-scale farm.



Figure 11.3. Photograph of a Co-operative society based in Esageri, Baringo County. It is through co-operatives such as this that smallholder farmers can get access to concentrated feed that may be in part exchange for milk supplied to the co-operative society.

This thesis only considered cattle and none of the other FMD-susceptible species such as *sheep*, *goats* and *pigs*. The reasons for this include:

- a) In Kenya FMD vaccination is generally performed only in cattle
- b) Disease impact is considered low among small ruminants due to the tendency for them to have subclinical infections or mild disease
- c) The pig population is relatively small (Figure 1.4).

Separate studies would be required for these species to evaluate the role vaccination may have in decreasing the transmission of FMDV and additional impacts which this might bring at the national level. It has been demonstrated that oil adjuvanted vaccines are more efficacious in pigs compared to aqueous (Anderson et al., 1971), so pig vaccination may become more common with the new vaccine.

11.3.2 Vaccine effectiveness estimate

These studies indicate low vaccine effectiveness of a particular FMD vaccine in cattle in an endemic setting and are the first such analyses based on data from Africa. An absolute vaccine effectiveness estimate was not possible from these data, and the specific reasons for the poor vaccine performance were not conclusively shown. As stated previously, the collinearity between age and lifetime number of doses received, means this important confounder cannot be adjusted for in effectiveness estimates. This highlights an important limitation when evaluating any vaccine on a farm that uses a routine whole herd vaccination strategy.

11.3.3 Accuracy of the case definition and lack of serological data from outbreaks

There are also concerns over the sensitivity of the case definition particularly for Farm 1/SAT2. NSP antibody testing can be used in vaccine effectiveness studies, increasing the sensitivity of the case definition. Under some circumstances this can provide a means of evaluating the effectiveness of the vaccine at preventing infection, as shown by Knight-Jones et al. (2014) among smallholders in Turkey. NSP antibody surveys were not considered appropriate in these outbreaks, as the vaccine was not NSP purified. The serology results would have been difficult to interpret due to the presence of maternal antibody in young, non-vaccinated animals and in the case of Farm 1/SAT2, animals being vaccinated at a young age before maternal antibody would have waned. There are some unpublished observations that when a non-NSP purified

vaccine is used a higher percentage inhibition cut-off can be used for defining those that were infected (for example 75% compared to the usual 50%) (C. Bartels, Personal Communication). Such an approach might be utilised in Kenya although validation studies would be required to assist interpretation under these circumstances. Although complementary laboratory testing is preferred in these investigations, their absence makes these studies very cheap to perform and the results from this thesis demonstrate that laboratory tests (for example vaccine matching) are not essential to begin vaccine effectiveness investigations. Field studies are appropriate for under-resourced endemic settings where more expensive sample submission can be problematic or significantly delayed.

It could be that the failure to observe a difference in milk yield impact between reported cases and non-cases was due to poor recording, poor sensitivity of the case definition or subclinical infection. This necessitated the use of a predictive analysis which introduces greater uncertainty to the results. This may indicate that in a herd affected with FMD, comparisons between cases and non-cases in affected groups are not an appropriate form of analysis. Focus may be better directly towards comparing affected and non-affected groups but problems of comparability will arise. On this farm, all lactating groups were affected so this approach was not possible. Comparisons between several farms is an alternative approach although this would need to consider the difference in management approaches between farms that will affect milk yields, most importantly nutrition, fertility and genetics.

11.3.4 Vaccine matching

Vaccine matching (VM) data were lacking in these investigations which limited our ability to explain the reasons for vaccine failure. Vaccine matching is performed by a limited number of laboratories including the WRL in Pirbright, UK, where samples from Kenya have historically been sent. Although some VM results have been produced in recent years for Kenyan field isolates, the vaccines to which they were matched were not being used in Kenya thus limiting their usefulness. The WRL currently supports the processing of up to 50 FMD viruses isolated per country per year without charge. Financing the shipment to the WRL can be problematic due to limited governmental resources although funding may be sourced through research projects or agencies like EuFMD.

Staff at the Kenyan National FMD Laboratory have received training on VM in recent years, funded through a reciprocal arrangement with EuFMD related to the Kenya based FMD training courses. Despite this, it is not routinely performed as more training is currently

needed. The process of delivering this training is ongoing, but until the laboratory becomes fully accustomed to this technique, there will be an ongoing need to send virus isolates to the WRL for VM. Even once VM has started in Kenya, there will be a continual need for external validation particularly in the early stages which can also be provided by the WRL.

As detailed in the literature review in Chapter 2 (section 2.3), there are limitations to the inferences that can be made from VM results in isolation. However, alongside field evaluations they would strengthen the ability to interpret an observed level of vaccine effectiveness. At the time of writing, the WRL have the original strains currently used in the KEVEVAPI FMD vaccine with complementary BVS recently supplied by the Kenya National FMD laboratory, but require the currently used vaccine seed strains in order to provide reliable VM results. Through activities related to the research presented here, an agreement was signed in July 2014 between KEVEVAPI and the WRL to share these strains under certain conditions respecting their commercial value. KEVEVAPI have also been advised to send other candidate vaccine seed strains and their complementary BVS so that matching to strains not currently used in vaccines can be similarly evaluated in case their inclusion is warranted. These samples are awaiting shipment, alongside some selected samples taken as part of national surveillance.

11.3.5 “Outbreak bias”

Clearly if the vaccine performed on all farms in Kenya like that reported in these outbreaks, one could not recommend its use and it is unlikely to be a cost effective control measure. However, a major limitation of using herds where vaccination has failed as the basis for evaluating a vaccine is that the approach may be biased, by focusing on farms where vaccination has been ineffective. This is a form of *selection bias*. It is possible that there are other farms that have not had disease despite exposure and where the vaccine has been successful in preventing infection or disease. Such farms are not included in the vaccine evaluation as no disease outbreak has been detected to alert a relevant investigation. This “outbreak bias” effect will tend to bias vaccine effectiveness estimates towards null. An assessment of the extent of outbreak bias could be performed by surveying large-scale farms on their experience with FMD combined with collecting information on vaccine management practices. Other study designs would be required to address this bias to establish whether the performance seen in these outbreaks is typical or atypical for other farms in Kenya (see “Recommendations”, section 11.4.3).

11.3.6 Vaccination reducing disease impact

For the disease impact analysis on Farm 1/SAT2, the routine vaccination appears to have been ineffective in preventing clinical disease, but a reduction in the severity of disease due to vaccination cannot be ruled out. For example, vaccination may have led to less days not producing milk or decreased the risk of culling. Although ideally one would measure the impact of FMD in an unvaccinated population, based on the author's field experience very few large-scale farms in Kenya do not vaccinate.

11.3.7 Follow-up period

An impact on clinical mastitis and culling was apparent, although the statistical evidence for the latter was weak. The analysis was performed one year after the outbreak started and a 12 month follow-up period chosen as a consequence. Longer term analysis might have been more informative, particularly for animals that fail to get pregnant, for which a prolonged time period may elapse before a culling decision is made. The choice of follow-up period for culling will depend on the individual farm and its management policies. A shorter follow up period could be chosen for analysing the impact on mastitis in future studies. A 12 month follow-up was used in this analysis so that all available data were used.

11.3.8 Source of VNT data

The VNT data were supplied by the vaccine manufacturer using the QA reports that they provide to a commercial farm that receives free vaccine in exchange for permission to allow blood sampling. The precise methodology used in the VNT is unknown, and some external quality control would be appropriate so that the results can be validated. A protective titre of 1.36 is used by the manufacturer although no data have been seen to support this assumption. The presence of subclinical infection on the farms cannot be ruled out, particularly for SAT1, such that cross reacting neutralising antibody may have affected the results.

11.4 Recommendations

This work generates several recommendations for FMD control in Kenya related to individual farm outbreak management, methods of vaccine evaluation and general FMD control policy as outlined below. Further areas of research that should be prioritised in his area have also been identified.

11.4.1 Farm contingency plans

The epidemiological analyses suggest several areas that could be addressed on large-scale dairy farms to limit the spread of infection, and that individual contingency plans should be developed. Several high risk events were identified on these study farms that are likely to have led to widespread exposure and infection transmission. The experience from these outbreaks is that it is unlikely that isolation alone will be effective in containing an outbreak. Other experience, from attending outbreaks among smallholder farms in Kenya, suggests that close proximity does not necessarily lead to transmission. On this basis, it may be possible for large-scale farms to contain outbreaks particularly if there is sufficient level of background immunity from vaccination requiring high virus exposure to initiate infection. Specific plans will vary depending on the individual farm. Some generic recommendations can be made on the basis of these findings to limit the spread between groups of animals and to prioritise the protection of the lactating groups among whom the impact is likely to be greatest. Due to the extensive sharing of equipment and personnel with lactating animals, once disease is in one of these groups, infection is likely to have already spread to all other lactating groups.

Farm plans should have sections on “Preventing introduction” and on “Minimising spread and impact”. Recommendations are given here, in no particular order.

Preventing introduction:

1. Lactating groups should not be grazed in direct contact with other livestock groups or near the farm perimeter
2. Farm personnel should be regularly trained on FMD recognition and the contingency plans
3. If livestock personnel own their own livestock on a site off the main farm premises and FMD is in the area, they should notify the farm and not come to work. The farmer should then notify the veterinary authorities who should investigate the claim since

FMD is a notifiable disease. This will then act as a check that the employee's report has a genuine foundation and reduce the number of false reports that may arise with such a policy.

4. Separate clothing and footwear should be available for farm workers that have contact with livestock areas. These must never leave the farm, where they should also be laundered.
5. Visitors (people, vehicles) should be denied access to animal holding areas
6. Purchased animals should be isolated from the main herd with no direct contact for a minimum two week period. They should be thoroughly checked for clinical signs during this period. Designated staff and equipment should be assigned to this quarantine section or at the very least these animals should be attended to after the other groups.
7. Small ruminants should not be co-grazed with cattle as subclinical infection is more common in these animals so infection may circulate unknown to the farm workers.
8. Vehicles that work with livestock should remain within the farm and not go to other farms or areas that non-farm livestock may be present. If they do, they should be thoroughly cleaned and disinfected before being allowed back on the farm.

Minimising spread and impact:

1. Selection of isolation paddocks should be *ad hoc* depending on the location of the index case. Ideally no movement will be required. If it is, it should be through areas that other animals will not be walking through or grazing. This may necessitate the opening up of paddocks to avoid the use of roads.
2. After a first case is observed in a group, the whole group should be isolated. Individual animals may be further isolated to reduce the exposure of non-diseased vaccinates. This route of isolation must be undertaken with no risk of contact with unaffected groups.
3. Groups in contact with affected groups should be considered as part of an infected cluster and should be similarly isolated.
4. Designated farm staff and equipment should be used for each group until two weeks after the onset of the last case. Such equipment should be disinfected before being used again after the outbreak.
5. Separate paddocks should be created for cows that are calving to minimise their movements during the outbreak (i.e. movement of these animals to the main lactating

groups should be avoided. Hand milking may be required for these cows unless a portable milking unit is available).

6. If FMD is known to be in the area, youngstock should be regularly inspected with oral examinations to ensure they are not affected and isolated if appropriate.
7. Contact should be minimised between farm personnel that are working with infected groups and other livestock workers. A central manager on the farm who does not contact any animals (diseased or non-diseased) should co-ordinate activities. A separate office should be used by staff that are working with the affected groups.
8. Equipment (e.g. for treatment) for the affected groups must be stored separately and not shared.
9. Affected groups should be clearly indicated on farm maps but also at entry to affected paddocks.
10. Paddocks where a diseased groups had been previously should not have unaffected groups placed into them. The length of time after which a movement may occur is not based on field evidence but a minimum of four weeks is suggested.

Foot and wheel baths are often recommended for minimising the risk of FMDV transmission to or from a farm (Figure 11.4). Foot baths may also be recommended once an outbreak has begun. There are several difficulties with these biosecurity approaches that limit their usefulness so they are not part of the recommendations given above. The major criteria for effective use are:

1. Regular, frequent changing of solution to maintain effective levels of disinfectant
2. Removal of organic matter to allow effective disinfection
3. Cost and availability of appropriate disinfectants
4. Access to sufficient water with methods for distribution on the farm

When used for preventing the introduction of virus, substantial effort (and cost) must be allocated to ensure the criteria above are met presuming sufficient infrastructure is already in place. Concerning wheel baths, for farms with large numbers of vehicles entering the premises, the disinfectant may become rapidly ineffective particularly in dusty environments as in the dry seasons in Kenya when FMDV typically circulates from increased movements of pastoralist herds. Although knowledge on various disinfection types is well established (DEFRA, 2014), the data available are from laboratory measures of virus inactivation and there are few published studies on their effectiveness in the field (Brennan and Christley, 2012). Extended

contact times (for example 30 minutes) are often suggested that are simply impractical for field conditions and are unlikely to be used where FMD is endemic.

A wheel bath was present on Farm 1/SAT2, but due to the large numbers of vehicles and required maintenance it had not been used for a number of years (Figure 11.4). With eight years between outbreaks on the farm in an endemic area with regularly reported outbreaks, it is difficult to justify continued use. After the study outbreak, the farmer did not reinstate the wheel bath due to the problems described above. Cost-effectiveness analyses would be useful but could not be performed as part of this research project and their effectiveness would be difficult to evaluate in the field. Similar arguments can be made for foot baths if used to prevent infection being introduced to the farm. Their use during an outbreak may be more worthwhile for individuals working with individual groups. If they are used, separate foot baths should be present for each affected and non-affected group.



Figure 11.4. Photograph of wheel bath present on Farm 1/SAT2. The bath is not in use with only rainwater being present.

11.4.2 Surveillance

Currently, all FMD outbreaks in Kenya are supposed to be notified to a local government veterinary officer by either a farmer, veterinary paraprofessional (for example an animal health assistant [AHA]) or private practitioner. In the first instance, a government veterinary officer should visit the farm to see if the clinical signs are consistent with FMD and take a sample so that viral antigen detection tests may be performed by the National FMD laboratory. If FMD is suspected, a notification should be sent to the central Department of Veterinary Services (DVS).

Confirmed outbreaks between 2003 and 2013 were presented in Chapter 1 (Figure 1.5). This graph does not include all reported outbreaks (i.e. where no virus was detected) and from talking to government veterinary officers in the field, it is unclear whether all outbreaks are consistently reported (for example if no sample could be taken or where due to being under-resourced outbreaks were not attended) and also how an outbreak is defined. The National FMD laboratory is beginning to use PCR based methods for antigen detection which is likely to improve the sensitivity of the diagnosis for those samples being submitted. However, it is clear that more resources are needed in order to improve reporting of FMD outbreaks. This should include the regular training of field veterinarians to ensure they are aware of the systems in place. Many of these areas have been highlighted in the government's national FMD control strategy published in 2012, although as yet no changes have been observed in the field. Part of this strategy is to implement an electronic notification system that may improve reporting and dissemination of information.

With the plans for FMD-free zones, the government also aims to increase targeted surveillance through regular inspections of the free/protection zones and compulsory vaccination areas. Targeted surveillance should also include performing regular serosurveys, also identified in the national FMD control strategy, to increase the understanding of FMD epidemiology in Kenya. Although a Kenyan FMD serosurvey was published in 2013 (Kibore et al., 2013), this refers to sera collected in 2010 and there are deficiencies in the data presented complicating inference (see Chapter 1, section 1.4.3.2). Therefore it is suggested that a nationwide serosurvey is repeated by trained government epidemiologists with risk factor analysis based on NSP serology, possibly with guidance from the FAO. Through these activities, the implications of using a non-NSP purified vaccine on NSP titres may be evaluated provided accurate and detailed data are collected on animals sampled (i.e. age, total number of lifetime doses received, date since last vaccination). Large-scale farms in the first instance may be more

useful as they are more likely to have readily accessible data on the animals present, good handling facilities and the convenience of having all the animals in one location. The QA farms used by the vaccine company would also provide a good opportunity to meet this objective.

11.4.3 Vaccine and impact evaluation

When an FMD outbreak notification occurs, data on recent vaccination are usually collected on a standardised submission form used by the government-employed veterinary surgeon. This should automatically trigger notification of the vaccine manufacturer and government epidemiologists as a possible vaccine failure. Appropriate vaccine effectiveness evaluations should then be performed. This will establish an evidence base for vaccine effectiveness and ultimately inform resource allocation.

The strategy of vaccine application on Kenyan farms depends on the production system. On large-scale farms, routine vaccination is typically used whereby all animals in the herd are vaccinated at the same time as on the farms described in this study. For smallholders, ring vaccination is more common, being used as part of government efforts to contain a confirmed outbreak. In the field there are also reports of private animal health assistants offering vaccine to smallholders although the extent of this activity is not clear. With different strategies of vaccination, different forms of evaluation are necessary to evaluate effectiveness. These investigations also provide opportunities to measure disease impact for different production systems.

For large-scale farms experiencing an outbreak despite regular vaccination, it is recommended that individual animal data are collected in order to carry out vaccine effectiveness and disease impact assessments. These data include:-

1. ID of animal affected
2. Sex
3. Date of birth
4. Date affected
5. Group ID
6. Clinical signs
 - a. Salivation
 - b. Oral lesions
 - c. Foot lesions (and number of feet affected)

- d. Teat lesions
 - e. Abortion
 - f. Clinical mastitis
 - g. Death
7. Date (and batch number) of last vaccination
 8. Total number of vaccine doses received

Recording clinical signs is necessary for establishing the case status using an appropriate definition, and also so that the impact of vaccination on disease severity may be analysed in an economic analysis. Most large-scale farms use individual animal identification system that should facilitate such data collection. Large-scale farms should be incentivised to report all suspect cases to the vaccine manufacturer and be given recording sheets to be used in the event of an outbreak. Giving regular and relevant feedback to these farms, including notifications of disease events in the surrounding area, is likely to improve reporting.

For smallholder farmers, equivalent data on individual animals would ideally be collected but may be less readily available due to a lack of vaccination records and individual animal identification systems. Therefore to evaluate the effectiveness of ring vaccination in such settings, a different approach may be required. Where villages have been identified as having an outbreak and ring vaccination has occurred in the previous six months, data may be collected after the outbreak has finished evaluating the effectiveness of this strategy using a historical cohort study approach. "Cases" can be defined at the household level (i.e. did the household have any sick animals consistent with FMD during the recent outbreak). Vaccine effectiveness may therefore be defined at the household level (i.e. if a household used vaccination in the last six months, was this associated with the household being protected from disease). The number of households to interview should be based on a sample size to detect the minimum acceptable vaccine effectiveness estimate (e.g. 60%) and the estimated household coverage. Farms should be randomly selected by establishing a sampling frame through the use of satellite images or local knowledge of farm locations and performing a census. Data will also need to be collected on confounding variables that will indicate exposure (e.g. use of communal dip). Pilot studies among smallholders would be necessary before beginning such surveys to inform aspects of study design and sample size. These studies are not traditional vaccine effectiveness evaluations and are limited in their inference about the vaccine for which individual animal data are required. They may be able to indicate the influence of the ring vaccination campaign which is important to inform control policy.

Standardised approaches to investigating apparent vaccine failures will be required for consistency and transparency and such investigations should be performed independent of the vaccine company. This is particularly important when vaccines are subsidised by the government so that allocation of resources can be justified.

A major problem with field evaluation of veterinary vaccines is the lack of vaccine records in small farms and the absence of unvaccinated controls on large farms. Also, both of the described investigation methods are subject to the “outbreak bias” (discussed in section 11.3.5). The problem with evaluating any vaccine is that it is only truly tested when exposed to the pathogen it is meant to be protecting against. This exposure is only guaranteed on farms where vaccine failure is apparent and appropriate samples have been taken. A less biased evaluation would be to visit a series of randomly chosen large-scale farms or villages (or pre-defined high risk sentinel populations) in endemic areas and do periodic surveys of disease incidence independent of an outbreak being reported. Similar investigations could be routinely performed to get an idea of vaccine coverage at the individual and household level. In areas where a non-NSP purified vaccine is used, post vaccination monitoring of herds or villages may be used to complement these investigations. Some insights may still be gained by doing this where a non-NSP purified vaccine is used if appropriately validated as described in the previous section.

Alongside these investigations, attempts at virus isolation should always be made in line with current national policy and regular VM tests performed. However, such testing is not essential to begin vaccine evaluations and a lack of resources to do this should not prevent conducting field investigations or reported vaccine failures. If during the passive surveillance process no samples are taken from vaccinated populations, a subset should still always be tested for VM so that possible problems with matching can be identified as early as possible.

11.4.4 National policy implications

The FMD Control Strategy for Kenya published in January 2012 involves the establishment of three zones based on FMD status: an infected zone where reactive vaccination is practiced (similar to that described in section 1.4.2); a protection zone where there is intensive mass vaccination; and a FMD free zone where there is no vaccination. From the latter, the hope is to open up potential markets by allowing the export of meat and meat products. Vaccination is an important component of this strategy and therefore it is timely to recommend that Kenya instigates the monitoring of vaccine performance.

FMD is a notifiable disease in Kenya so the Government and the veterinary epidemiologists in their employ have a key role in its control. Currently, ring vaccination is used and is restricted to cattle. Large-scale farms that use routine vaccination may source their vaccine directly from the manufacturer although local government officials must be notified. Ring vaccination was not evaluated in this study, but evaluations like those recommended above should be part of the national control strategy. Ring vaccination campaigns take place by either farmers bringing their animals to a common location (for example a communal dip [Figure 11.5] or a person's homestead that has appropriate handling facilities) or teams going from household to household offering vaccine. This typically takes place after a period of publicity such as visiting cattle dips, churches, schools and area chiefs, where the dates and locations of upcoming vaccination are advertised.





Figure 11.5. Photograph of government-subsidised reactive vaccination campaign in Ngata, Nakuru County, January 2012. Vaccination is occurring at a communal dipping facility.

Since there may be problems with vaccine performance in the field, it is recommended that government vaccination teams collect routine data to assist vaccine effectiveness evaluations in particular data on vaccine **coverage**. Currently the date, number of doses of vaccine administered at each location and batch number is the only information recorded along with some relatively subjective information from the team regarding impressions on turnout and problems encountered. The following information should be collected and fed back to appropriately trained government epidemiologists:

1. Name of owner
2. Address (either at sub-location level or preferably referring to a map)
3. Number of cattle vaccinated
4. Number of cattle owned that were not vaccinated
5. Age of animals vaccinated and non-vaccinated
6. Batch number of vaccine
7. Last occurrence of FMD in herd

After a vaccination campaign, appropriately designed surveys of the area to establish the proportion of vaccinated households and animals are recommended. Such post vaccination coverage assessments are more likely to give an accurate indication of actual coverage than simply dividing the number of doses by the livestock census figures. Implementing a system of individual animal identification, as used in Europe and elsewhere, is not currently considered feasible in this setting. A standardised record card with vaccination details is more feasible and has been used to some extent already in Kenya. Figure 11.6 shows a record card used in regular vaccine campaigns carried out in the former Central Province organised by the Kenyan Veterinary Association (KVA). The KVA incentivised farmers to keep the card by charging 10 KSH for a new card each time if they did not bring it with them to the next round of vaccination. A standardised card for use nationally could be developed and would help with studies on vaccine coverage and effectiveness.

LIVESTOCK VACCINATION CERTIFICATE

10/=  11/ *SEARCH NUMBER* 

NAME OF FARMER: First Middle Last.....
 ADDRESS DISTRICT
 DIVISION LOCATION.....
 SUBLOCATION..... CRUSH.....

DISEASE INVENTORY

DISEASE	SPECIES	NO. VACC	BATCH NO	DATE VACC	FEES	NAME OF VACCINATOR	SIGNATURE
ANTHRAX/BQ							
LUMPY SKIN							
FOOT & MOUTH							

NB: This is an important document. Please keep it safe. It may be required as proof that the animals have been vaccinated, for example when buying or selling or when procuring movement permit. Please bring this certificate with you when procuring booster vaccination.

BOOSTER VACCINATIONS

DATE DUE	VACCINE	NO. VACC	BATCH NO	DATE VACC	FEES	NAME OF VACCINATOR	SIGNATURE

FOR: KENYA VETERINARY ASSOCIATION. CENTRAL REGION BRANCH P. O. BOX 270 NYERI

BOOSTER REGULARLY AS ADVISED BY YOUR VET

Figure 11.6. Vaccination card used by the Kenyan Veterinary Association for regular campaigns carried out in what was the Central Province (now Nyeri County).

In the course of doing field work for this research, conversations were held with many smallholder farmers and government employees directly involved with FMD control and vaccination. One problem that was reported was that vaccination often takes place in areas of an ongoing FMD outbreak. Some farmers will take their animals to be vaccinated at a common location even if disease is present in their animals either with the hope that others in the herd will then be protected or due to confusion over the difference between vaccination and treatment. This is not necessarily due to their ignorance of spreading disease as farmers typically know the main route of FMD transmission – animal to animal contact. Additionally vaccination teams may move from farm to farm with no appropriate biosecurity measures in place. These high risk practices may make vaccinated households at increased likelihood of exposure and therefore disease. This will likely result in farmers believing the vaccine is ineffective and thereby undermines vaccination campaign efforts. Conversations with farmers reveal this to be the case and vaccine evaluations are near impossible in these circumstances. Rigorous active surveillance occurring immediately before the vaccination campaigns is essential to ensure that the areas vaccinated are not already affected. Additionally a suitable sized buffer zone must be established within which vaccination should not occur, with

surveillance in this zone to ensure disease is not present. If this action is not possible due to limited resources, the role of ring vaccination in national policy should be questioned and even withdrawn from the national control strategy.

In order to maintain the cold chain, cool boxes must be used (Figure 11.7). A survey of temperature monitoring in cool boxes would be worthwhile to ensure current practices are satisfactory. Such monitoring does not currently occur but should become routine practice.

In order to carry out these recommendations, it is important that there are a sufficient number of adequately trained veterinary epidemiologists that are either employed by the government or alternatively employed on a consultancy basis. A national FMD consultant should also be appointed. This will likely require investment by the government.



Figure 11.7. Photograph taken inside of cool box during government subsidised vaccination campaign in Ngata, Nakuru County, January 2013.

11.4.5 Further research priorities

- Further field evaluation

Research should be directed towards further field evaluations of vaccines in line with the recommendations above. A cluster-randomised trial would be the ideal choice since this study design will incorporate both direct and indirect protection from vaccines. Such a study has never been performed for evaluating FMD vaccines, and would require significant financial investment, so may not be feasible for Kenya at this time.

- Assessment of different vaccination schedules

Different schedules for youngstock need to be evaluated which in the first instance can be applied to the manufacturers QA farms before being assessed in the field. The ongoing VNT work should continue although external validation, either within or outside Kenya, is suggested as part of quality control. As part of this work, NSP antibody should be routinely measured so that the levels induced from non purified vaccines can be assessed. This will in turn inform the interpretation of serological surveys.

- Assessment of SAT2 vaccine

Assessment of the SAT2 vaccine should be addressed as a priority including the quantification of antigen. Given the known stability issues with SAT2 vaccines, cold chain studies analysing the degradation of antigen under field conditions would also particularly worthwhile for this serotype.

- Oil-based vaccines

As the oil based, NSP-purified vaccine becomes available, it is important to evaluate the kinetics of antibody responses after different schedules, including long term waning of antibody. Vaccine match between the field and vaccine strain will still need to be monitored and these vaccines will still be susceptible to inactivation through insufficient maintenance of the cold chain. The recommendations in this thesis including the uptake of vaccine effectiveness evaluations are therefore still relevant with a change in vaccine adjuvant. The NSP-purification will also allow more informative sero-surveillance to be performed.

- Socioeconomic impact studies

The need for further socio-economic impacts studies among the same and different stakeholders is essential to inform resource allocation. Particularly on large-scale dairy farms, the impact of FMD on fertility has never been fully evaluated and is worthy of investigation. Economic assessments are needed to inform the cost-effectiveness or cost-benefit of different control strategies including the use of vaccination.

11.5 Dissemination of results

The results of these studies have been disseminated in various ways during the course of the project in addition to the publications.

A presentation was given on the disease impact work at a PCP workshop in Nakuru, August 2014 to government representatives from Kenya and Uganda. A separate presentation was given during this workshop on the vaccine evaluation work to representatives from the Kenyan National FMD laboratory and KEVEVAPI. Two presentations were given at the EuFMD Open Session in Cavtat, Croatia, in October 2014 (Appendix F).

An interim report was provided to the Kenyan Department of Veterinary Services at their request in May 2013. Three copies of the final version of the thesis will be sent to the Ministry of Science and Higher Education in line with the research permit agreement.

11.6 Conclusion

This thesis presents the results from epidemiological investigations and vaccine evaluations on two large-scale farms in Nakuru County, Kenya, where large FMD outbreaks occurred despite histories of repeated vaccination. On one of these farms, the impact of FMD on milk yield, clinical mastitis and culling was evaluated. This work was complemented by analysis of quality control serological data made available from the vaccine manufacturer.

The results provide insights into FMD epidemiology in this setting and are used to inform the development of farm contingency plans in the event of future outbreaks. Evidence for poor vaccine effectiveness was presented for O and SAT2 serotypes. Particular concerns are apparent over the very low effectiveness of the SAT2 vaccine supported by poor seroconversion rates and waning of antibody titres. Evidence was provided for limited

effectiveness for serotype O vaccine but only after several doses of vaccine. Modifications to the vaccine schedule among youngstock are suggested as is the possibility of using additional or alternative vaccine strains after appropriate vaccine matching studies have been performed. The impact analysis provides unique insights into the impact of FMD on milk yield, clinical mastitis and culling rates.

Although the studies are limited in their generalisability by only including two farms and the specific reasons for apparent vaccine failure were not fully elucidated, the approaches used to evaluate the vaccine can be generalised to other circumstances. These approaches should be encouraged and performed alongside more conventional vaccine quality assessments the results of which are severely limited in their applicability and validity for field settings. With the control strategy for Kenya looking towards the establishment of disease free zones without vaccination alongside intensively vaccinated protection zones, effective vaccination is essential for the future of FMD control in Kenya.

Chapter 12. References

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Appendix A

Consent forms for participating farms

Informed consent form for research study on Foot-and-Mouth disease

Informed consent form for _____
Farmer name

Study information

We are veterinarians from the UK working in collaboration with the Department of Veterinary Services (DVS), doing research on foot-and-mouth disease (FMD) in cattle in the Rift Valley Province/Nakuru County. The purpose of the research is to understand how the disease spreads, the effectiveness of vaccination and the financial impact on farms that suffer disease.

We would like to use your farm as part of this research using data from a recent outbreak on your farm, which we previously attended with the DVS. This will involve using the data from this initial outbreak investigation alongside using individual animal and production data, and relevant maps. Where appropriate, samples already taken by the DVS may be used.

These data will be used as part of a PhD thesis performed at the London School of Hygiene and Tropical Medicine (LSHTM) and Royal Veterinary College (RVC), London, UK. Upon completion, the thesis will be published online. Additionally, articles may be published in peer-reviewed academic journals. All collected data will be securely stored and no data will be shared without your permission. In any publications you and your farm will be anonymous although if you wish to be acknowledged, then you can waive your right to anonymity by ticking the box below.

Contact details

For any information relating to this research, call Nick Lyons on 0700126250 (Kenya) or +44(0)7976554031 (UK) or alternatively email nicholas.lyons@lshtm.ac.uk.

Consent

Please tick the relevant boxes and sign below.

I confirm that I understand the information given in the information sheet.

I agree to sharing data on questions answered relating to the animals I own including health issues, their vaccination history and the financial income they contribute.

I understand that my participation is voluntary and am free to withdraw at any time without giving a reason.

I agree to waive my right to anonymity

Print name of consenting farmer _____

Address of consenting farmer _____

Signature of farmer _____

Date (dd/mm/yyyy) _____

Appendix B

Research Permits

Research authorisation from National Council for Science and Technology

(Incorporates local ethical approval)

REPUBLIC OF KENYA



NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telephone: 254-020-2213471, 2241349
254-020-310571, 2213123, 2219420
Fax: 254-020-318245, 318249
When replying please quote
secretary@ncst.go.ke

P.O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref:

NCST/RCD/10/012/35

Date:

21st November 2012

Nicholas Anthony Lyons
University of London
UK.

RE: RESEARCH AUTHORIZATION

Following your application dated *9th November, 2012* for authority to carry out research on "*Field evaluation of vaccine effectiveness for Foot and Mouth Disease in Kenya*," I am pleased to inform you that you have been authorized to undertake research in **Rift Valley Province** for a period ending **30th September, 2013**.

You are advised to report to the **Provincial Commissioner, the Provincial Director of Education and the Provincial Director of Livestock, Rift Valley Province** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR M.K. RUGUTT, PhD, HSC.
DEPUTY COUNCIL SECRETARY

Copy to:

The Provincial Commissioner
The Provincial Director of Education
The Provincial Director of Livestock
Rift Valley Province.

Research Permit



REPUBLIC OF KENYA

PERMIT FOR RESEARCH CLEARANCE

EXCAVATION, FILMING AND COLLECTION OF BIOLOGICAL SPECIMENS

IN THE DISTRICT OF ...

SECTION 1

1.1. You must report to the District Commissioner and the District Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit.

2. Government Officers will not be interviewed with-out prior appointment.

3. No questionnaire will be used unless it has been approved.

4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.

5. You are required to submit at least two (2) / four (4) bound copies of your final report for Kenyans and non-Kenyans respectively.

6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice.

GPK6055t3mt10/2011

PAGE 2

PAGE 3

THIS IS TO CERTIFY THAT:
Prof. Dr. /Mr./Mrs./Miss/Institution
Nicholas Anthony Lyons
of (Address) University of London
has been permitted to conduct research in
Location
District
Province
Rift Valley
on the topic Field evaluation of vaccine
effectiveness for Foot and Mouth Disease
in Kenya



Research Permit No. NCST/RC/CD/10/012/35
Date of issue 21st November, 2013
Fee received KSH 34,000

Applicant's Signature
Nicholas Lyons
National Council for Science & Technology

for a period ending 30th September, 2013.

Letter from Provincial Commissioner for Rift Valley Province

OFFICE OF THE PRESIDENT

Telegrams: "PROVINCER", Nakuru
Telephone: Nakuru 2216566/2216523
When replying please quote



PROVINCIAL COMMISSIONER
RIFT VALLEY PROVINCE
P.O. BOX 28
NAKURU.

ADM.15/1/5 VOL.IV/(75)

14th December, 2012

TO WHOM IT MAY CONCERN

RESEARCH AUTHORIZATION – NICHOLAS ANTHONY LYONS

This is to introduce to you the above named from the University of London (UK) who has been authorized by the National Council for Science and Technology to carry out research on "Field Evaluation of Vaccine Effectiveness for Foot and Mouth Disease in Kenya". The research will be carried out in Rift Valley Province for the period ending 30th September, 2013.

Kindly accord him all the necessary assistance.

A handwritten signature in black ink, appearing to read 'Boaz K. Cherutich'.

BOAZ K. CHERUTICH
FOR: PROVINCIAL COMMISSIONER
RIFT VALLEY PROVINCE

CC. The Council Secretary
National Council for Science & Technology
P. O. Box 30623-00100
NAIROBI - (Your Authority NSCT/REC/10/012/(35) of
21st November, 2012)

Letter from FAO representative in Kenya



OFFICE OF THE FAO REPRESENTATIVE IN KENYA

United Nations Office at Nairobi,
United Nations Avenue
Block P, 3rd Level,
Gigiri,
P.O. Box 30470 - 00100,
NAIROBI

E-mail: FAO-KE@fao.org
Fax: +254 7625921

Tel: +254 762 5920

Our Ref: IN11

10th December 2012

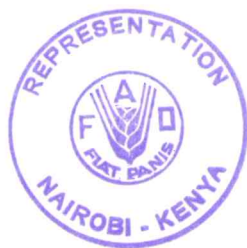
Dear Sir/Madam

This is to certify that Dr. Nicholas Anthony Lyons holder of British National Passport no. 099278223 is a veterinary surgeon and researcher from the London school of Hygiene and Tropical Medicine.

Dr. Lyons has been invited by Food and Agriculture Organization of the United Nations to train participants from Kenya, Department of Veterinary Services (DVS) and delegates from the European Union in workshops on Food and Mouth Disease funded by FAO/European Union, Foot and Mouth Commission- (EU-FMD). The workshops are taking place in Nakuru from 3rd December 2012 to 25 January 2013. Dr. Lyons in collaboration with DVS under an existing Agreement will also be required to collect data related to the Foot and Mouth disease in the Rift Valley between the training sessions and before his departure from the Country.

He arrived in the Country on 28th November 2012 and was issued with one month visa on arrival at the Jomo Kenyatta International Airport although he is expected to depart on 16th February 2013. It would be appreciated if his visa for Kenya would be extended up to 16th February 2013 to facilitate the training and collection of the data.

Your cooperation and assistance would be highly appreciated.



Yours Sincerely

Dan Rugabira
FAO representative in Kenya

Principal Immigration Officer
Immigration Department
Nyayo House
Nairobi

Letter from the European Commission for the Control of Foot-and-Mouth Disease (EuFMD)



Our Ref.:

Your Ref.:

To whom it may concern

Nicholas Lyons is a veterinary surgeon and researcher based at the London School of Hygiene and Tropical Medicine (UK). He is coming to Kenya to train delegates from the European Union and Kenya in workshops on Foot and Mouth Disease in association with the European Union Foot and Mouth Disease Commission (Eu-FMD) based in United Nations agency Food and Agricultural Organization (FAO).

As part of a pre-existing memorandum of understanding (MOU) between the FAO and Department of Veterinary Services (DVS), he will also be collecting data in collaboration with the DVS in the Rift Valley Province relating to foot and mouth disease for which a research permit has been granted.

The workshops will take place in Nakuru between the 3rd and 7th December and 14th to 25th January. Between these courses he will be collecting the aforementioned data in collaboration with the DVS. He will leave Kenya on the 2nd March 2013.

All assistance to Dr. Lyons will be very much appreciated.

Faithfully

Keith Sumption
Secretary, European Commission
for the Control of Foot-and-Mouth Disease
Animal Health Service

Appendix C

Data management – Farm 1/SAT2

Routine farm data

Each individual animal had a unique ear tag identification number associated with all recorded data for that individual either on paper or electronically. As events occurred, for example an animal being served or an animal having clinical mastitis, records were initially made on paper and then periodically taken to a central farm office where they were entered into InterHerd. The InterHerd database was backed up at the end of each day and a file stored remotely using Dropbox.

Data extraction and cleaning

InterHerd records were accessed using Microsoft Access on which the program is based. Appropriate links between tables were established with the assistance of people experienced in using the program. Specific queries were run depending on the analysis being performed. These results were exported into a Microsoft Excel spreadsheet and imported into Stata 12.0 for further cleaning. A sample of animals was periodically checked to ensure consistency with the original Interherd database.

All variables were recoded and labelled as appropriate. Ear tag identification numbers were checked to ensure they were unique. Where dates were missing or implausible, an enquiry was sent to the farm so that original paper records could be checked (paper records are kept on the farm for several years before being disposed). Occasionally dates of birth were mistakenly recorded under the date of registry variable in InterHerd.

Outbreak data

A list of all animals on the farm was printed using Interherd which formed the basis of data recording. As an animal became affected, the date was written alongside the respective animal's ear tag number. Sometimes for convenience the animal's ID number was written down if it could not be quickly found. A single employee was responsible for this process.

Photographs of these records were taken and data entered into Microsoft Excel by the author (NL). These outbreak data were merged in Stata 12.0 with animal data from the InterHerd database. Entries that could not be merged for example due to inaccurate recording of the animal ear tag number were investigated and checked with the farm staff as necessary. For analysis, a separate entry was included for each animal's time within a group so that group-level incidence rates could be calculated.

A sketch of the farm including the labelling of named bomas/paddocks was made during the outbreak period. A shapefile was created by drawing polygons using Google Earth, labelled with the name of the boma/paddock and a unique ID number. A few months after the outbreak had subsided, a return visit was made to the farm to establish the accuracy of this map. Once this was verified with the farm staff, the data were saved as a kml file and converted to a layer in ArcGIS 10.2 using the appropriate conversion tool in ArcToolbox. This was then exported as a shapefile and formed the basis of all maps produced on Farm 1/SAT2.

Survival analysis

All health events were extracted alongside the date they occurred (124,932 event records). Events were excluded if irrelevant to the analyses or the date was missing or inconsistent (for example if accidentally recorded in the future). Due to some inconsistency in how disease events were recorded in InterHerd, several variables were checked to ensure all relevant data were captured including the content of free text entries.

Milk yields

Cows were milked twice daily and the recording from each was weighed and manually recorded onto standardised paper sheets according to the cows ID number. Several people were entering weights at this stage. A single person was responsible for data entry into a computer in the main farm office. Each morning and afternoon weight was entered into a Microsoft Excel data sheet and the daily mean was calculated for each cow based on a week of yield data (Sunday to Saturday), using appropriate program formulae for each individual cow. If a cow was only lactating for part of a week, a daily mean was only based on the number of days that animal was lactating. These daily means were entered into InterHerd every week attached to the cow's ID number. The date for the entry corresponds to the last day of each week.

Weekly milk records began being consistently recorded from 11th April 2005 onwards. These 71,916 entries were accessed through a specifically designed query in Microsoft Access. All milk records for animals calving after the outbreak period were removed. Impossible or extreme milk yield values were checked and recoded as missing if they could not be confirmed using the original milk records in Excel.

Appendix D

Data management – Farm 2/O

Routine farm data

Apart from bull calves that were euthanased shortly after birth, each individual animal had a unique ear tag identification number and name which all events were associated with for that individual. Each animal had a record card (Figure D.1) on which health and fertility events are manually recorded. As events occur, for example an animal is treated for being lame, they are recorded in a notebook by staff working with the cows and this is given periodically to a single person who is responsible for transferring this information to the animal's record card that are stored permanently in the farm office.

Data extraction and cleaning

On the 29th November, whilst the outbreak was ongoing, the farm was visited and the identities of all animals present on the farm since the date of the index case were entered into a Microsoft Excel spreadsheet. Using the record cards, sex, dates of birth, parity at outbreak onset, and current group was entered alongside the identification number of the animal. The dates and animal identifications relating to inter-group movements since the index case were also recorded. After this visit, as animals were born or died and when individuals moved groups, the farm manager periodically emailed these events including the date and identification numbers of the animals which was used to update the spreadsheet.

Data were later imported into Stata 12.0. All variables were recoded and labelled as appropriate. Ear tag identification numbers were checked to ensure they were unique. Where dates were missing or were implausible, an enquiry was sent to the farm so that original paper records could be checked

Outbreak data

As animals became diseased, the identification number and date was recorded in a notebook. This was kept by the farm primarily so that a list of animals treated was kept. These data were inputted into the same spreadsheet described in the previous section, also supplemented by regular updates from the farmer. For analysis, a separate entry was included for each animal's time within a group so that group-level incidence rates could be calculated.

No :	NAME :	DATE OF BIRTH :							
DESCRIPTION :		REG. No /STUD BOOK No :							
SIRE :		LACTATION RECORD							
		Date of Calving	Calf	Days	Kgs.	B.F.		C.I.	
						%	Kgs.		
	DAH :								
SERVICE RECORD									
YEAR	DATE	BULL	DATE	BULL	DATE	BULL	DATE	BULL	Due to Calve

Figure D.1. Individual Record card used on Farm 2/O for recording animal identification, health and fertility events. Continued on next page.

VETERINARY RECORD

DATE	ILLNESS	REMEDY/ REMARKS	DATE	WORMING	DATE	WORMING	DATE	VACCINATION	DATE	VACCINATION

Figure D.1. Continued from previous page.

Appendix E

Photographs of clinical cases from Farm 1/SAT2 and Farm 2/O

Farm 1/SAT2



Photo 1.1. Hyperptyalism demonstrated by cow X158 on Farm 1/SAT2. This is a second parity cow that also developed clinical mastitis subsequent to having reported FMD and was culled as a consequence.

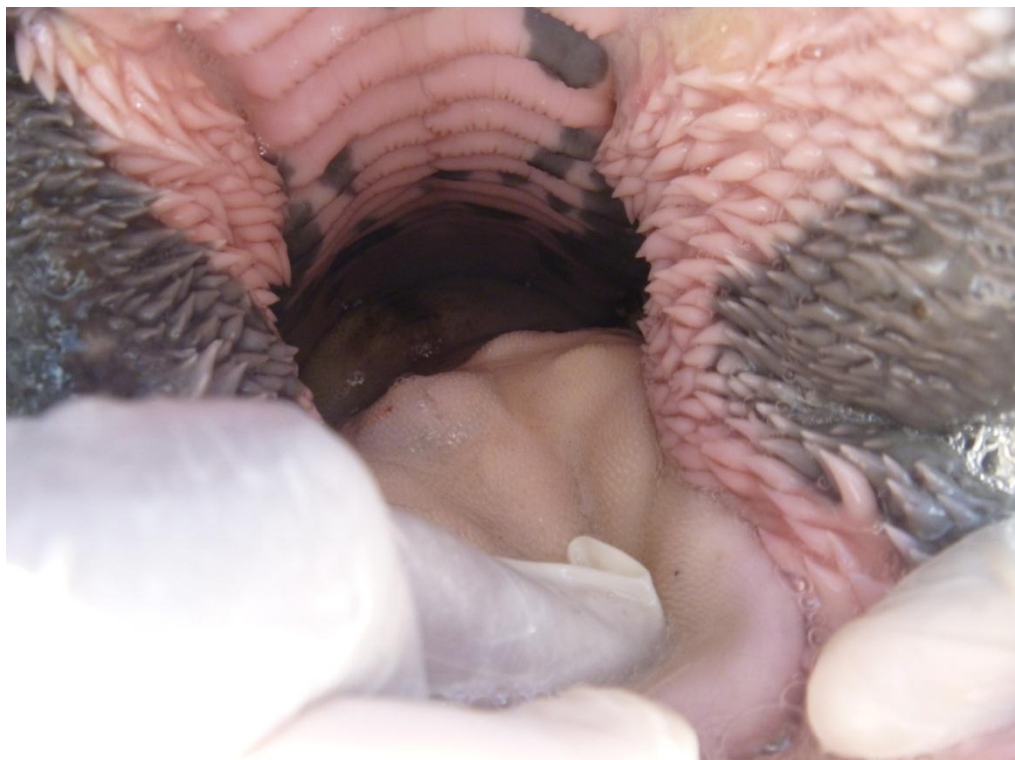


Photo 1.2. Vesicles on the dorsum of the tongue in an animal with reported FMD on Farm 1/SAT2. This cow had an epithelium sample taken confirming FMD due to serotype SAT2.



Photo 1.3. Examining animals with suspected FMD on Farm 1/SAT2.



Photo 1.4. Taking a sample of epithelium from an animal with suspected FMD on Farm 1/SAT2.



Photo 1.5. Vesicles on the teats of a cow affected with suspected FMD on Farm 1/SAT2.



Photo 1.6. Teat cannulas being used on a cow with suspected FMD on Farm 1/SAT2. The pain associated with the teat lesions meant the cow could not be milked normally so cannulas were used to allow milk let-down.

Farm 2/0



Photo 2.1. Jersey cows with suspected FMD presented for examination on Farm 2/O.



Photo 2.2. A Jersey cow with suspected FMD on Farm 2/O. This animal was demonstrating the classic “lip smacking” sound heard in cows affected with FMD.



Photo 2.3. A Jersey cow with suspected FMD on Farm 2/O demonstrating extreme hyperpyalism.



Photo 2.4. Examining oral lesions in a cow with suspected FMD on Farm 2/O.



Photo 2.5. Close-up of older suspected FMD lesions in a cow with FMD on Farm 2/O. This lesion was estimated to be 7-10 days old.



Photo 2.6. Close up of an oral lesion consistent with FMD in a Holstein-Friesian cow on Farm 2/O. This lesion was estimated to be 4-5 days old.



Photo 2.7. A recently burst vesicle on the ventrolateral aspect of the tongue in a Jersey cow examined on Farm 2/O, suspected as FMD.



Photo 2.8. A lesion consistent with FMD seen in the interdigital space of a Jersey cow.



Photo 2.9. Close-up of an interdigital lesion consistent with FMD in a Jersey cow examined on Farm 2/O.

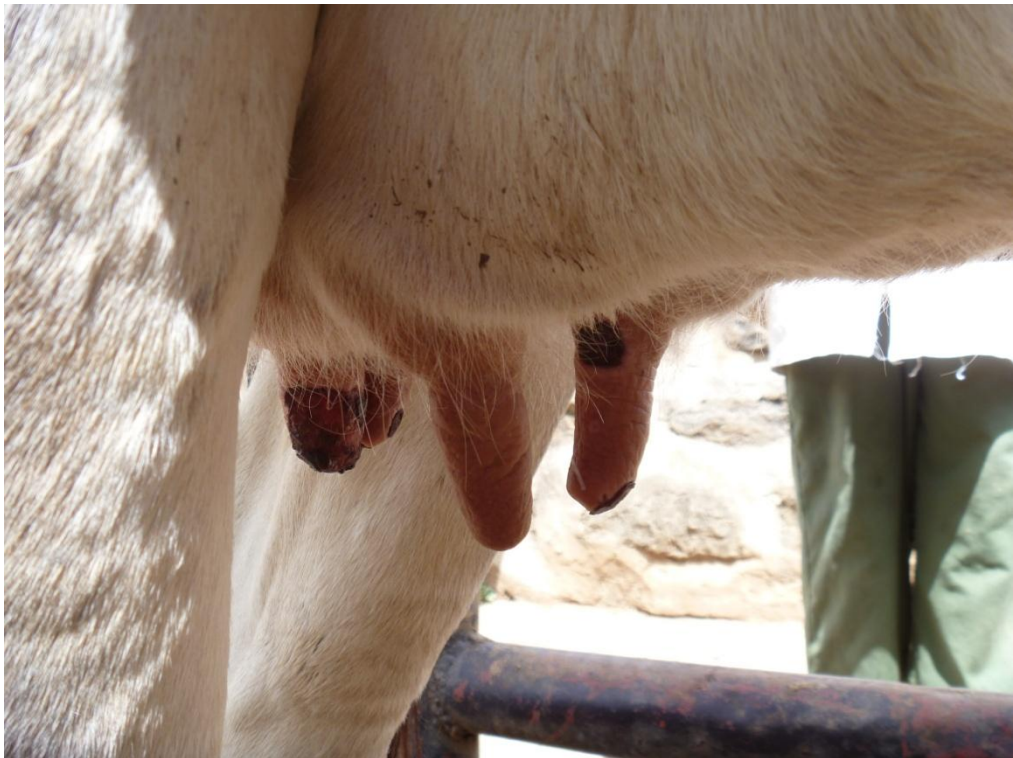


Photo 2.10. Teat lesion seen in a cow on Farm 2/O with suspected FMD.

Appendix F

Presentations given at the EuFMD Open Session , Cavtat, Croatia, October 2014.



Open session of the Standing Technical and Research Committees of the EuFMD

Cavtat (Croatia), 29-31 October 2014



Impact of FMD on milk yield, mastitis, fertility and culling on a large-scale dairy farm in Kenya

Nick Lyons
London School of Hygiene and Tropical Medicine



Background – FMD Economics

Lack of objective field data looking at FMD impact particularly in **endemic** settings

Tendency to rely upon **expert opinion** and **assumptions**

More data needed to inform **cost-benefit** analyses of **control** measures (e.g. vaccination strategies, culling and compensation measures)

Need data from **different people involved in the system** as outlined in the PCP stage 1

Background

Objective: to quantify the **impact** of FMD on a large-scale dairy farm in Kenya focussing on:-

- **Milk yield**
- **Clinical Mastitis**
- **Culling**
- **Fertility**



Outbreak

KENYA



NAKURU COUNTY

4

Farm background

Dairy Herd: 650 mainly Holstein-Friesian
Milking around 250 cows
Calving all year around
Artificial insemination only

All cows uniquely identified



Record daily milk yields, health and fertility events, sales etc in InterHerd (InterAgri, School of Agriculture, University of Reading, UK).

5

Outbreak – August/September 2012

Serotype SAT2, lasting 29 days

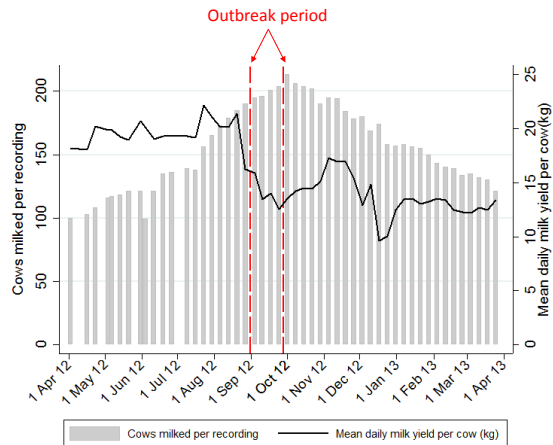
Case definition: Hypersalivation with any other sign indicative of FMD: decreased milk yield, decreased feed intake, oral/interdigital/teat lesions, pyrexia



Vaccine: Limited/no vaccine effect in preventing clinical disease. Overall Attack rate: 400/644 (62.1%)

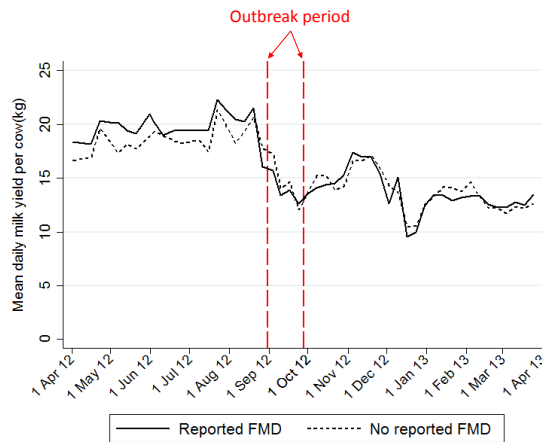
6

Milk yield – overall impact



7

Milk yield – Reported FMD cases versus non-cases

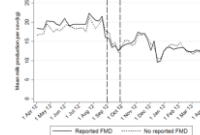


8

Milk yield – No difference?

Possible reasons:

1. Poor/inaccurate recording of cases
2. Insensitive case definition
3. Subclinical infection



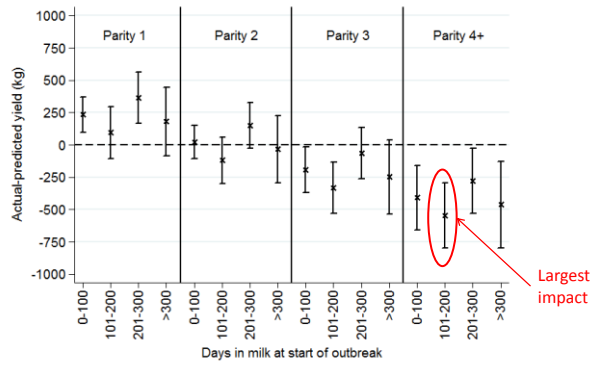
Next approach:

Predict yield for all individuals based on historic farm records accounting for parity, days in milk, and season (GEE model with a AR1 autocorrelation matrix)

Compare production from beginning of outbreak to end of 305 day lactation irrespective of disease status

9

Milk yield – Actual vs Predicted



Impact dependent on parity and lactation stage when diseased

10

Clinical mastitis and culling – Survival analysis

Follow up: 12 months from beginning of outbreak

Statistics: Cox proportional hazard regression

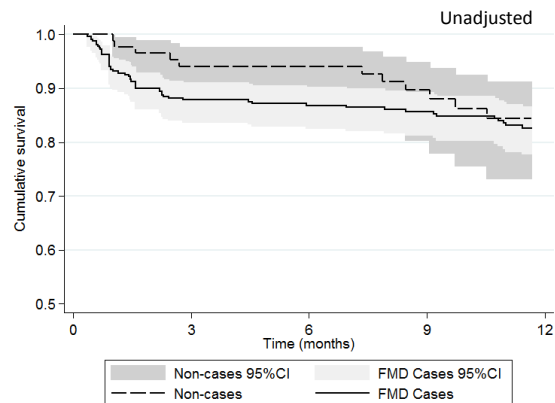
Adjusted for any non-proportional hazards by incorporating time varying effects

Study population: Culling - All animals

Mastitis - ≥ 18 months old

11

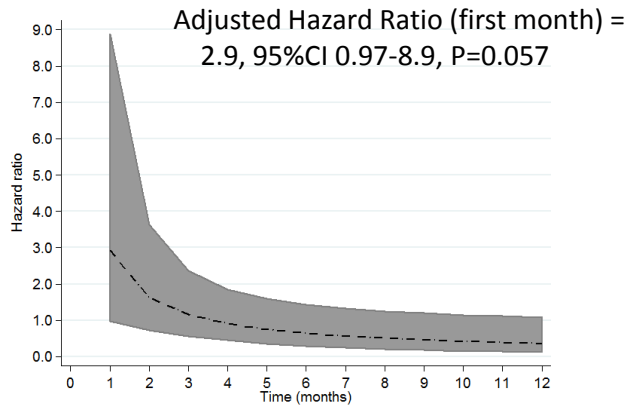
Clinical mastitis



Study population restricted to animals over the age of 18 months at start of outbreak

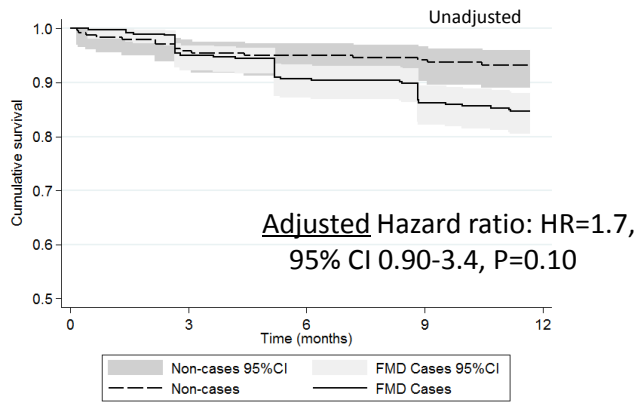
12

Clinical mastitis



13

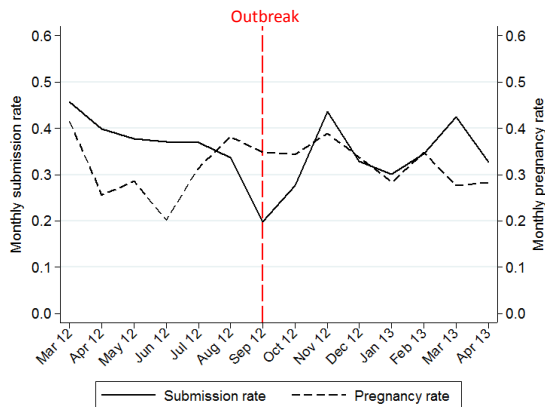
Culling



Culling is defined as exiting the herd for any reason associated with a adverse health event

14

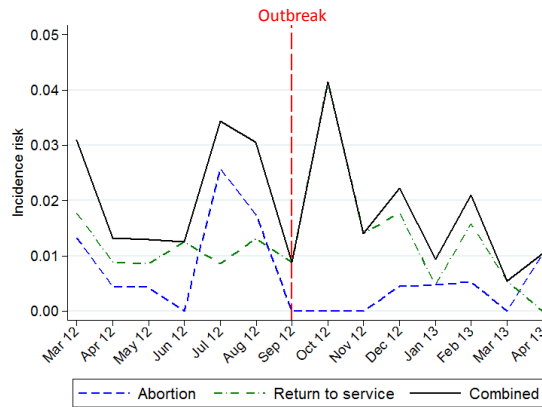
Fertility – Submission rate, Pregnancy rate



Submission rate decreased, but pregnancy rate not affected

15

Fertility – Abortion and Early Embryonic Death



No obvious effect on abortion, but increased returns to service

16

Summary - overall

- Milk yield – Depends on parity and lactation stage
- Clinical mastitis – 3 times the hazard in first month
- Culling – 1.7 times the hazard over 12 months
- Fertility – impact on submission rate, returns to service

Data may be used in developing cost analyses

Limitations

Generalisability

(Smallholders produce \approx 70% milk output)

Lack of statistical power

Vaccination mitigating impact

17

Conclusions - summary

Great need for **rigorous evaluations** of disease impact

There needs to be **investment in data collection** on **disease losses and costs** so that we can move away from relying on expert opinion and assumptions

Essential to reliably quantify impact for allocating **limited resources** in animal disease control

More need for field data from **different farming systems** in **different settings**

18

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EuFMD

MSD Animal Health, Royal Veterinary College (London)




Open session of the Standing Technical and Research Committees of the EuFMD
Cavtat (Croatia), 29-31 October 2014



Vaccine evaluation on large-scale dairy farms using routine prophylactic schedules for FMD

Nick Lyons
London School of Hygiene and Tropical Medicine





1

Background – Vaccine effectiveness

Vaccine effectiveness: % reduction in incidence among vaccinated individuals attributable to vaccination, measured under *field* conditions

Reasons for poor FMD vaccine performance in the field:

1. Poor **potency**
2. Lack of vaccine **match**
3. Break in the **cold chain**
4. Sub-optimal **coverage**
5. Interference by **maternally derived antibody** (MDA)
6. Incorrect **schedule**



2

Farm A -Background

Kenya, Nakuru County

Dairy Herd: ≈350 mainly Jersey

Last known outbreaks: March 2004 (SAT2),
December 2010 (NVR)

FMD Vaccination

Vaccinates animals every 4 months with a locally available quadrivalent (A, O, SAT1, SAT2) vaccine

Only vaccinates animals over 6 months old

Aqueous-adjuvanted, Non-NSP purified vaccine.
Only ≥6.0 PD₅₀ vaccines are approved for use.

3

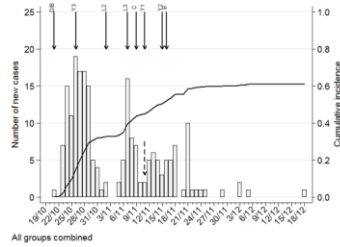
Farm A - Outbreak

Serotype O

October-December 2013

Last dose 3 months before

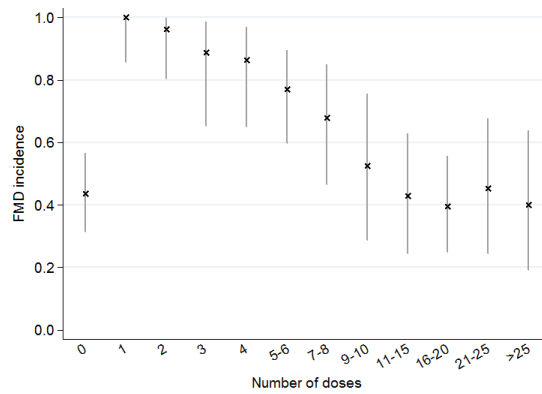
Probable source: Farm workers



4

Farm A - Vaccine

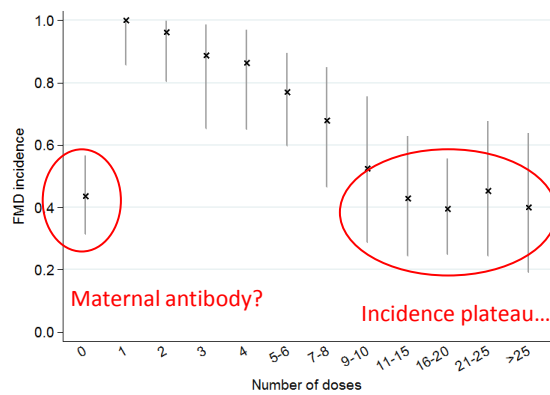
“Incidence risk” versus **“Number of lifetime doses”**



5

Farm A - Vaccine

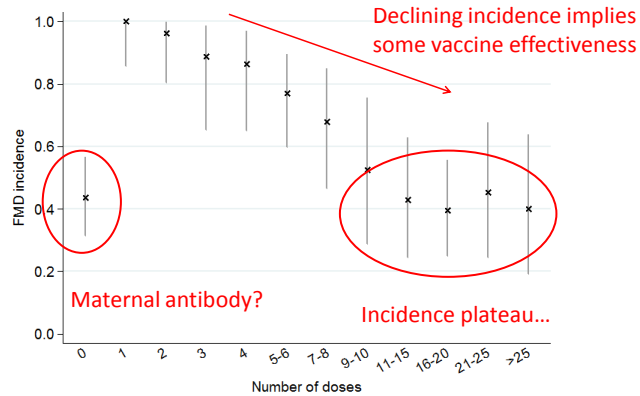
“Incidence risk” versus **“Number of lifetime doses”**



6

Farm A - Vaccine

“Incidence risk” versus **“Number of lifetime doses”**



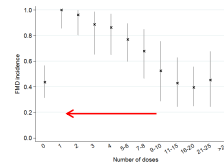
7

Possible reasons for incidence pattern on Farm A

40% incidence in multiply vaccinated clearly reveals a problem....

- Potency?
- Match?
- Cold chain?

Suboptimal schedules as well?



Can have multiple reasons for poor VE!

8

Farm B - Background

Kenya, Nakuru County

Dairy Herd: ≈650 mainly Holstein-Friesian



Last known outbreak in 2004 (unknown serotype)

FMD vaccination

Vaccinates animals every 4-6 months with a locally available quadrivalent (A, O, SAT1, SAT2) vaccine

Vaccinates all animals irrespective of age

9

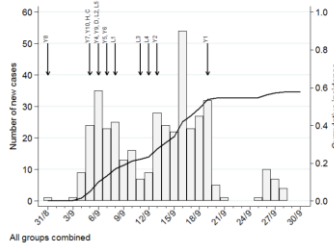
Farm B - Outbreak

SAT2

August – September 2012

Last vaccination 3 months before

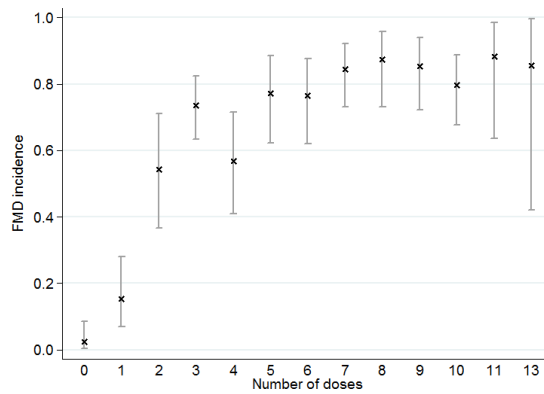
Probable source: Farm workers



10

Farm B - Vaccine

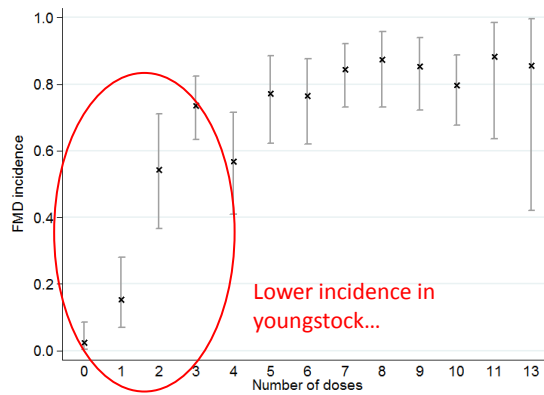
“Incidence risk” versus **“Number of lifetime doses”**



11

Farm B - Vaccine

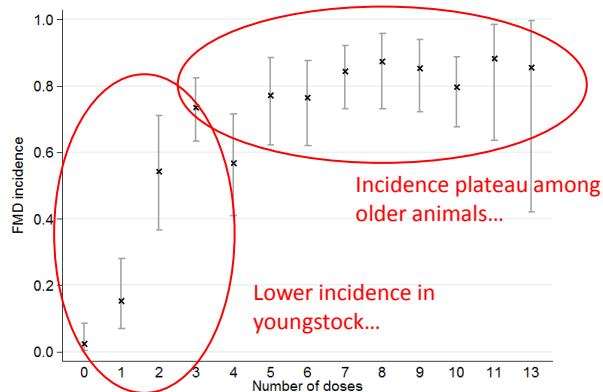
“Incidence risk” versus **“Number of lifetime doses”**



12

Farm B - Vaccine

“**Incidence risk**” versus “**Number of lifetime doses**”



13

Possible reasons for incidence pattern on Farm B

Low incidence in **youngstock** – poorer reporting?
less severe disease?, less transmission/exposure?
(like Netherlands, 2001?)

Vaccine **match**? (SAT2 VP1 sequence 13% difference
to that reported by Sangula et al, 2010)

Vaccine **potency**?

SAT2 – known to be less stable antigen requires
higher antigen dose than other serotypes

Cold chain?

14

Farm C - Background

Iran, Shahriar County, Tehran Province
Dairy herd: 3,500 cattle, Holstein-Friesian



Last known outbreak 4 years previously (Not Asia-1)

FMD Vaccination

Every four months with trivalent (Asia-1 Shamir, O, A).
Calves >2 months old get two doses one month apart
as a primary course.

High potency, NSP purified vaccine.

15

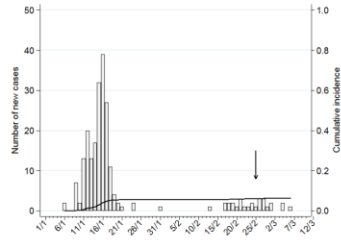
Farm C - Outbreak

Asia-1

January-March 2011

Last dose 11 weeks before

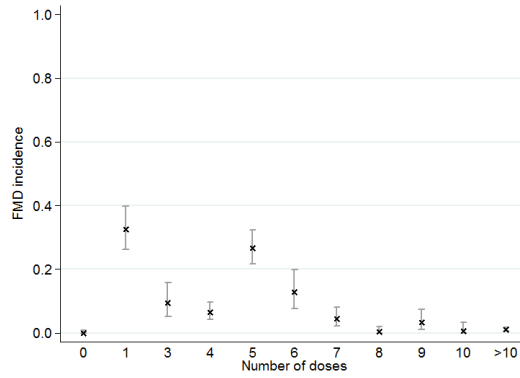
Probable source: Local semi-nomadic sheep/goat herds



16

Farm C - Vaccine

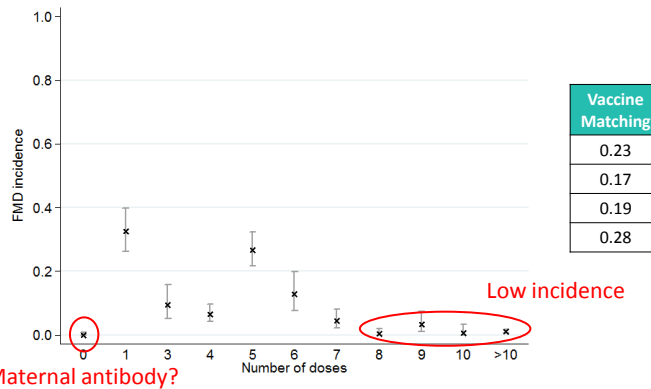
“Incidence risk” versus **“Number of lifetime doses”**



17

Farm C - Vaccine

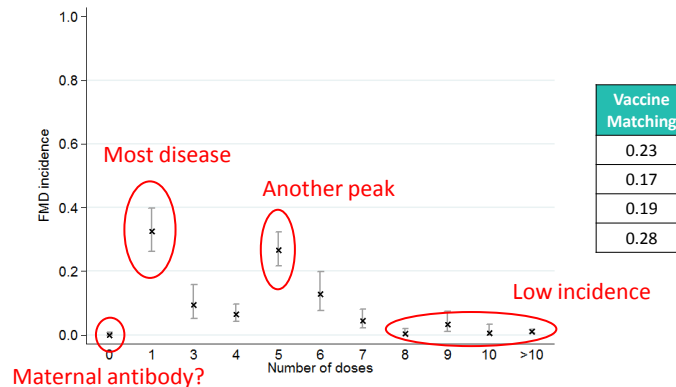
“Incidence risk” versus **“Number of lifetime doses”**



18

Farm C - Vaccine

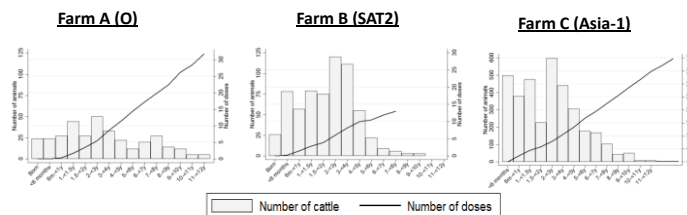
“**Incidence risk**” versus “**Number of lifetime doses**”



19

Vaccine protection and coverage

- Does appear to be evidence of some **cumulative protection** with number of doses (Farms A and C)
- Cannot rule out **other age related effects** (although not due to exposure in these outbreaks)
- **Age distribution** in the herd affects **coverage!**



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Summary and conclusions

Generalisability?

Field-based vaccine performance assessments provide **additional information** on the effectiveness of a control **policy**

Conventional laboratory-based evaluations should be performed **alongside field evaluations**

Standardised **protocols** for monitoring vaccine performance and investigating apparent low effectiveness are needed

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