Serological response of cattle vaccinated with
a bivalent (SAT 1 and SAT 2) foot-and-mouth disease vaccine
in Gaza Province, Mozambique

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C. Abbreviations

Al(OH)$_3$	Aluminium hydroxide
BHK-21	21-Baby hamster kidney cell culture
BVI	Botswana Vaccine Institute
CFT	Complement fixation test
CVL-IIAM	Central Veterinary Laboratory of Mozambican Agrarian Research Institute
EITB	Enzyme-linked immunoelectrotransfer blot
ELISA	Enzyme-linked immunosorbent assay
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
IB-RS	Pig kidney cell line
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LNP	Limpopo National Park
LPBLE	Liquid-phase blocking ELISA
MPV	Month post-vaccination
NSP	Non-structural viral proteins test
OIE	World Organization for Animal Health
OVI	Onderstepoort Veterinary Institute
PBS	phosphate buffered saline solution
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction
SADC	Southern African Development Community
SAT	Southern African Territories
SPCE	Solid-phase competition ELISA
VI	Virus isolation test
VNT	Virus neutralization test

1. SUMMARY

Serological response of cattle vaccinated with a bivalent (SAT 1 and SAT 2) foot-and-mouth disease vaccine in Gaza Province, Mozambique

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Foot-and-mouth disease (FMD) is a viral disease endemic in Africa, the Middle East, South America, Asia and parts of Eastern Europe. It is a major constraint to international trade in livestock and livestock products in many African countries. In southern Africa, African buffaloes (*Syncerus caffer*) are reservoirs of the South African Territories (SAT) 1, SAT 2 and SAT 3 FMD viruses, and cattle raised in the vicinity of wildlife conservation areas are at constant risk of becoming infected with FMD viruses. In Mozambique, control of FMD is fundamentally based on vaccination of cattle in zones around protected areas. However, the vaccination protocol recommended by the vaccine producer (two primary vaccinations followed by four- to six-monthly boosters) is logistically impractical and financially not suitable for most countries. As a result, the double primary vaccination is usually not implemented.

A commercially available bivalent FMD vaccine, containing the SAT 1 and SAT 2 serotypes, was assessed for its ability to induce and sustain immunity in cattle for at least 6 months following a single primary inoculation. The study was conducted with cattle reared in Limpopo National Park (LNP), Mozambique, and adjacent areas. One hundred and seventy five seronegative cattle aged between 6 and 18 months were vaccinated and 42 others from the same areas were left unvaccinated, as controls. A group of 39 vaccinated cattle were revaccinated 4 months after initial vaccination and 63 others were revaccinated 6 months after initial vaccination.

The vaccinated and unvaccinated cattle were bled at predefined intervals (at vaccination, and at 1, 4, 5, 6, 8, 10 and 12 months post vaccination) and sera were tested with a liquid phase blocking ELISA to measure the antibody level against FMD virus.

A high proportion (73%) of vaccinated cattle had seroconverted ($\log_{10}$ titre ≥ 1.6 for any SAT serotype) at one month after vaccination with a single primary dose and there was no

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significant difference between the proportions of animals that seroconverted to SAT 1 compared to SAT 2. A higher proportion of animals within LNP (82%) than outside LNP (50%) had seroconverted at one month after vaccination (P = 0.001). Overall, however, only relatively few animals (27% for SAT 1, 35% for SAT 2 and 7% for SAT 3) had protective antibody titres ($\log_{10}$ titre ≥2).

At 4 months after vaccination, a very low proportion (8.3%) of vaccinated cattle had antibody titres ≥1.6 to any of the SAT serotypes, and there was no significant difference between the proportions of animals with antibodies to SAT 1 (2.1%) compared to SAT 2 (7.3%) (P = 0.17). No cattle had a protective titre (≥2) to SAT 1 at 4 months and only 4.2% to SAT 2.

The revaccination at 4 months after initial vaccination elicited antibody titres ≥1.6 in 22% of vaccinated animals at one month after revaccination; this rose two months later to 90% and remained high (91%) at 10 months post first vaccination before dropping to 65% at 12 months. However, only 15% of cattle had protective titres (≥2) to any of the SAT serotypes at 12 months. For cattle revaccinated at 6 months after first vaccination the percentage of cattle that had a titre ≥1.6 two months after revaccination was also high (80%), remained high (89%) at 10 months post first vaccination and dropped to 54% at 12 months after first vaccination. Only 11% of cattle had protective titres (≥2) at 12 months.

The research findings indicate that, although the vaccine is able to induce production of antibodies against SAT 1 and 2 in a significant percentage of cattle within one month after a single primary vaccination, these antibodies are short lived and have largely disappeared by 4 months post vaccination. This suggests that a protocol of a single primary vaccination is inadequate in naïve animals, even if revaccination takes place four months later. Revaccination improved the immune response for a longer period, resulting in detectable titres in the majority of animals for 6-8 months post revaccination. This can be used in disease control programmes to ensure some protection of cattle particularly when it is applied immediately before identified high risk periods of occurrence of FMD outbreaks. However, it seems unlikely that six-monthly revaccination is sufficient to maintain adequate levels of protective immunity.

The study highlighted several difficulties associated with the vaccination of livestock under field conditions and the conduction of field trials. These included difficulties with cold chain maintenance, poor infrastructure for animal handling, and loss of follow-up due to loss of animal identification or poor owner/herder compliance.

2. INTRODUCTION

2.1. Literature Review

Foot-and-mouth disease (FMD) is a highly contagious acute viral infection, affecting almost exclusively ruminants and pigs and it is characterized by high morbidity and low mortality and by vesicles and erosions in the mucosa of the mouth and skin of the interdigital spaces of the feet and coronary bands (Sobrino et al., 2001; William et al., 2002; Kitching et al., 2007).

Livestock plays an important role in the livelihood of the people of Africa by providing animal protein when used for human consumption, a source of income, and animal traction to till land and harvest crops and for transport. It is a form of investment and it plays an important social role, in most African communities (Timberlake, 1981; Perry et al., 2003).

Animal diseases are one of the main constraints for efficient livestock production (Perry et al., 2003). Disease directly affects animal productivity through morbidity and mortality, but also constitutes a major source of risk to development of livestock-centred enterprises. Of particular importance in this respect are the highly infectious transboundary animal diseases, such as FMD (Hunter, 1998; Perry et al., 2003), which severely limits the access of certain products to local and international markets.

In the SADC region, livestock have been recognized as one of the most important and valuable assets that rural communities have for the improvement of their livelihood (Thomson, 1995; Perry et al., 2003; Thomson et al., 2004). Foot-and-mouth disease is the major constraint for trade between SADC member states, and for access to the high value markets for animals and animal products (Thomson et al., 2004). Because of these factors, there is a need for integrated strategies for FMD control in the region. Foot-and-mouth disease is enzootic in many parts of the world. Regions that traditionally have been free of the disease are Australia, New Zealand, Japan, and Central and North America. Foot-and-mouth disease was introduced into South America during the middle of the 19th century by the importation of European breeding stock.

As soon as vaccines were developed, vaccination was gradually introduced into Europe in the form of ring vaccination or regional programmes, often in combination with slaughter of animals in infected farms, in order to limit the dissemination of the disease. These measures

reduced the number of outbreaks in Europe, limiting the disease to only a few thousand farms. With the advances made in vaccine technology, general vaccination became possible in Europe and most countries were able to achieve freedom from the disease in recent decades (Sutmoller et al., 2003).

2.1.1. Aetiology

2.1.1.1. Causal agent

Foot-and-mouth Disease virus (FMDV) belongs to the family *Picornaviridae*, and is one of the two members of the genus *Aphthovirus*. It has seven immunologically distinct serotypes, designated serotypes A, O, C, SAT 1, 2 and 3, and Asia 1 (Sobrino et al., 2001, Thomson et al., 2004).

2.1.1.2. Characteristics of FMD virus

The virus contains a single-stranded RNA genome of approximately 8500 nucleotides. The capsid shows the classic structural organization of the *Picornaviridae*, consisting of a non-enveloped capsid with icosahedral symmetry, 28–30 nm in diameter, composed of 60 asymmetrical protomers (Sáiz et al., 2002). The virion exists as approximately 70% protein and 30% RNA as well as small quantity of lipid, with a relative molecular mass of about 8.5 x 10^6 and sedimentation constant of 146S (Sobrino et al., 2001).

Despite showing structural similarities with other picornaviruses, FMDV presents distinctive features. The capsid surface is relatively smooth, exhibiting a main protruding element, the G-H loop in VP1 (Sáiz et al., 2002; Sobrino, et al., 2001; Thomson et al., 2004). The G-H loop spans about 20 residues around positions 140–160 and can adopt different conformations without apparent perturbations of the rest of the capsid (Sáiz et al., 2002). This large loop contains a highly conserved Arg–Gly–Asp (RGD) triplet, a universal cell-recognition site also present in various extracellular proteins, which interacts with cell surface receptors. The immunogenicity of FMDV particles is associated with amino acid residues that are well exposed on the surface of the capsid (Sáiz et al., 2002; Paton et al., 2005).

Picornaviridae in general are stable at pH between 3 and 9. The FMDV is distinguished from other members of the *Picornaviridae* by its lability at pH below 7-9 and relatively high density in CsCl (1.41-1.45 g/ml) (Mason et al., 2003). The virus is labile in mildly acidic solutions. At pH 6.0 the rate of inactivation is 90% per minute while at pH 5.0 it is 90% per second (Sáiz

et al., 2002). At pH 10.0, it loses 90% of its infectivity every 14 hours. Different FMDV strains show variation in their lability to low or high pH; in general, a low pH inactivates the virus faster than a high pH (Sáiz et al., 2002; Paton et al., 2005).

In contrast to the effect of pH, FMDV is relatively resistant to the effects of heat and considerable variation is seen in different virus types and strains (Thomson et al., 2004). Generally, temperature above 43°C causes rapid destruction of the virus in an aerosol, while it survives well in aerosols at cool environmental temperatures with relative humidity above 60% (Sobrino *et al.*, 2001*;* Thomson et al., 2004).

2.1.2. Epidemiology

2.1.2.1. Distribution of FMD

Foot-and-mouth disease is endemic and highly prevalent in many African countries, Middle East and Asia. It is also present in parts of South America and Europe (Kitching, 1999; World Organization for Animal Health (OIE) on World Animal Health Status, 2010).

The geographic distribution of the seven serotypes of FMDV is heterogeneous. Serotypes A and O are prevalent in South America, Europe, the Middle East and parts of Africa. Serotype Asia 1 is confined to the Middle East, Far East and Balkans. The SAT types are restricted to sub-Saharan Africa (Vosloo et al., 2005; Kitching et. al.*,* 2007). Foot-and-mouth disease outbreaks caused by serotype C viruses have not been reported for many years, and they may be extinct (Vosloo et al., 2002).

In Africa, all serotypes except Asia 1 are present, but due to under reporting, the information available is incomplete. The serotypes O, SAT 1, and 2 are widely distributed and serotype A and C are less so, while SAT 3, despite having been identified in buffaloes in East Africa, has never been reported outside the southern Africa region (Thomson et al., 2004).

In southern Africa, the three SAT 1, 2, and 3 serotypes are most prevalent (Vosloo et al.*,* 2002). With the exception of a few occurrences of exotic serotypes in Angola, Mozambique, Namibia and South Africa, all outbreaks of FMD have been caused by viruses belonging to the three SAT serotypes (Vosloo et al.*,* 2002; Kitching et al.*,* 2007).

In Mozambique, the three SAT serotypes and type O prevail and were responsible for the outbreaks recorded in the country between 1950 and 2011. A total of 21 outbreaks were

recorded of which 43% were caused by SAT 2, 29% by SAT 2, 10% by SAT 3, O and untyped SAT viruses. (Pinto, 1991; Thomson, 1995; Direcção Nacional dos Serviços de Veterinária, 2011). The outbreaks were located close to the major wildlife conservation areas, particularly those populated by African buffaloes (Limpopo National Park, Marromeu Buffalo Reserve and Gorongoza National Park). The type O FMDV was brought into the country by cattle imported from Brazil in 1974. A survey conducted in 2010 in buffaloes in Marromeu Buffalo Reserve found serological evidence of the presence of type O serotype in this population (Onderstepoort Veterinary Institute (OVI) - Transboundary Animal Diseases Programme 2011). Between 2002 and 2003 and 2010 and 2011 the province of Gaza was affected by FMD outbreaks. SAT 1 was isolated, as well as SAT 2 from later outbreaks. The 2010 outbreak was detected in September of the same year (OIE on World Animal Health Status, 2010; Direcção Nacional dos Serviços de Veterinária, 2011).

The viruses belonging to these serotypes have high levels of geographically-specific intratypic genetic variation. These variations, called topotypes, are genetically reflected in the gene encoding the VP1 protein one of the four structural proteins of the virus capsid which is involved in virus neutralization. Because of these intratypic variations, also reflected in antigenic changes, vaccines containing different topotypes to those prevailing in certain regions do not necessarily protect animals against FMD caused by different strains to those contained in the vaccine. This fact affects the control of FMD by means of vaccination and vaccine matching is required to evaluate the suitability of the use of the vaccine to protect animals against certain FMDV (Hunter, 1998; Vosloo et al., 2002; Bastos et al., 2003).

The epidemiology of FMD in Africa is influenced by two different patterns: a cycle in which wildlife, especially African buffaloes (*Syncerus caffer*), plays a role in maintaining and spreading the SAT serotypes to other susceptible domestic and wild cloven-hoofed animals, and a cycle that is maintained within domestic animals without involvement of wildlife species (Thomson et al., 2004, Vosloo et al., 2005).

Cattle are efficient hosts, maintaining FMDV via active infection, which plays an important role in maintaining and spreading FMDV within the cattle population and other domestic cloven-hoofed animals. This is mainly if control is ineffective (Thomson et al., 2003). From 1983 to 1989 Zimbabwe reported isolated FMDV outbreaks in cattle, which probably were related to and maintained within cattle populations (Thomson, 1995).

2.1.2.2. Transmission of FMD virus

Foot-and-mouth disease virus may be transmitted from infected to susceptible animals either by direct or indirect contact. Transmission most commonly occurs by direct contact (Sutmoller et al., 2003; Thomson, 1994), although the virus can be mechanically disseminated by animals, animal products, farmers, farming equipment, and during animal transport (Sáiz et al., 2002; Sobrino et al., 2001). Long-distance, airborne transmission has also been documented to occur in other parts of the world, but in sub-Saharan Africa there is no evidence that this occurs, as environmental conditions are not favourable for virus survival. In addition it might be due to relatively low densities of pig and cattle populations (Thomson, 1994; Thomson, 1999).

The infective dose required for the establishment of infection has been studied in domestic animals and is variable. It depends on factors such as species, breed, route of infection and the strain of virus involved (Thomson et al., 2003). Cattle belonging to the *Bos taurus* type are more susceptible than local indigenous breeds or *Bos indicus* type cattle. Young animals of all susceptible species are more affected by FMDV infection (Thomson et al., 2003).

During natural infection, the main route of virus entry is the respiratory tract (Orsel et al., 2007), and the disease can be produced when as few as one to ten infective particles are inhaled (Sobrino et al., 2001), whereas by the oral route a 10,000 times higher infective dose is required (Thomson et al., 2003).

During the acute phase of the disease, the movement of infected animals and of contaminated products of animal origin are the most common ways of dissemination of FMDV (Sáiz et al., 2002). Sub-clinically infected animals, particularly ruminants, are often responsible for the spread of FMD (Thomson et al., 2003). Small ruminants (sheep and goats), in which lesions are often difficult to discern (Alexanderson et al., 2002), pose a particular risk in terms of FMDV dissemination (Sutmoller et al., 2003).

2.1.2.3. Reservoirs of FMD virus

Foot-and-mouth disease viruses are able to cause a persistent infection, after an acute phase of disease, in cattle, sheep, goats, African buffaloes and some wild cloven-hoofed ruminants (Sutmoller et al., 2000; Alexanderson et al., 2002). The animals in which the infection persists for more than 28 days after recovering from acute infection are called

"carrier" animals (Alexanderson et al., 2002). The mucosa of the soft palate, pharynx and cranial oesophagus are the site in which FMDV replicates and persists.

In general terms and for many other diseases, some authors assign the term "carrier" to animals able to disseminate an infection without clinical signs of disease (Sutmoller et al., 2003). In the case of FMD, carrier animals do not seem to be contagious, carrier status is long and transmission from carriers to susceptible animals has not been proven to occur experimentally (Thomson et al., 2004), except in the case of African buffaloes (Sutmoller et al., 2003). In this document, the term "persistently infected" will be used, as "carrier" refers to animals in which FMDV persists more than 28 days after recovering from an acute infection.

The duration of persistence of infection in domestic animals is variable (Vosloo et al., 2005). In cattle it varies between 2 and 5 years, in sheep up to 9 months and in goats 4 months. In pigs FMDV infection does not induce a carrier state (Sutmoller et al., 2000). In many cloven-hoofed wild animals with exception of African buffaloes, the persistence of infection is of short duration and the kudu (*Tragelaphus strepsiceros*) is the only species in which the maintenance of FMDV in a carrier state for significantly long periods of time (140 to 160 days) has been proven (Sutmoller et al., 2003).

The prevalence of carrier animals in the population is influenced by the epidemiological situation of the area (Alexanderson et al., 2002*).* In FMD endemic areas and after outbreaks, the prevalence is generally higher *(*Thomson, 1995), and drops quickly to 34% and 5% after 6 and 12 months respectively (Hedger, 1968).

Several studies have addressed the role of persistently infected animals in the maintenance and transmission of FMDV (Sutmoller et al., 2003; Vosloo et al., 2005*).* The mechanism leading to the establishment of persistence of FMDV in animals is not well understood (Alexanderson et al., 2002*)* and the ways in which carrier animals transmit the infection to other animal are still not clear (Thomson, 1999; Thomson et al., 2003).

Transmission of FMD has only been demonstrated experimentally in buffaloes where infected buffaloes and susceptible cattle were housed in close contact during a relatively long period of time (2 years), but even then it was a rare occurrence (Bengis et al., 1986). It was demonstrated that in areas where FMD control programmes were successfully implemented the persistently infected cattle were not a regular source of infection to other cattle (Thomson et al., 2003).

In sub-Saharan Africa, African buffaloes (*Syncerus caffer*) play a central role in the epidemiology of the FMD due to their ability to maintain and transmit FMDV of SAT 1, 2 and 3 serotypes for significantly long periods of time (Condy, et al. 1985; Bastos, et al, 2003). The duration of the persistence status has been studied by various researchers. Condy et al. (1985) reported that an individual buffalo can maintain all three SAT serotypes at the same time and may carry FMDV for at least five years. In an isolated herd, the infection can be maintained for over 24 years. The manner in which FMDV is maintained within African buffalo populations is still not clear (Vosloo et al., 2002). The maintenance of serotypes other than SAT strains in buffaloes has not been proven to occur (Thomson, 1999). However, serological evidence of the maintenance of serotype O in wild buffaloes in Marromeu Buffalo Reserve was observed in a recent survey carried out under the SADC TADs project, where three young buffaloes in a sample of 25 animals had antibody titres against serotype O (OVI, personal communication, 2011). This was 30 years after an outbreak caused by serotype O of FMDV occurred in the cattle population in this area in 1980.

In the majority of the cases, FMDV is transmitted by direct contact between acutely infected and susceptible individuals, and occasional transmission between carrier buffalo and susceptible individuals (Thomson et al., 2004; Vosloo et al., 2005). Two theories have been postulated for maintenance of FMDV in buffaloes populations: The most plausible theory is that FMDV is perpetuated by regular 'epidemics' among young animals and, during this period large amounts of FMDV are excreted and transmitted to other susceptible buffaloes within the herd. At such times, virus may be transmitted to other susceptible species that come in close contact with buffalo herds, including cattle (Thomson, 1994). The possibility of sexual transmission has also been described, but it is probably a rare event (Bastos et al., 1999; Donaldson and Alexanderson, 2002)

Many other species of cloven-hoofed wild animals have been reported as having been infected with FMDV, but their role in maintenance and spread FMDV in southern Africa is not well understood (Vosloo et al., 2005; Vosloo et al., 2009) .

A better understanding of the epidemiology of FMD is needed, with particular emphasis on:
a) the mechanisms leading to the establishment of persistence of infection in domestic and wild ruminants, b) the transmission mechanisms from the carrier buffaloes to susceptible animals, c) the way in which domestic animals maintain FMD without involvement of wild species and d) the immunity mechanism induced by FMDV infection which stimulates the production and maintenance of higher levels of protective antibodies against FMDV for long periods of time.

As wildlife in Africa plays a central role in maintaining and spreading FMD there is an urgent need to address the issue of control the FMD in wild animals, in order to lower the risk posed by these species to the livestock industry (Thomson et al., 2004).

2.1.3. Pathogenesis

In domestic ruminants and African buffaloes, acquisition of the infection is by means of inhalation and the sites of initial replication are presumably the lung bronchioles. The pharynx and dorsal soft palate are the predilection sites for FMDV replication and persistence (Thomson et al., 2004).

The virus spreads from the first replication sites to all organs via the lymphatic and blood systems (Alexanderson et al., 2002). The FMDV is present, in measurable quantities, in all body secretions and excretions of viraemic individuals (Thomson et al., 2004). The FMDV can be also recovered, in high quantities, in lymph nodes, adrenal glands, myocardium, pancreas, thyroid and mammary glands (Thomson et al., 2004).

The virus is eliminated within a few days (14 days) in most organs and secretions as soon as the production of antibodies starts (Alexanderson et al., 2002). In cattle and pigs detectable viraemia, resulting from infection by SAT types, does not last longer than 3 days (Thomson et al., 2004).

The interaction between viruses with receptors present in host cells is a requirement to initiate the infection. Studies of FMDV components have demonstrated that the G-H loop of capsid protein VP1, particularly its RGD motif, is critically involved in virus interaction with integrin (Sáiz et al., 2002). The replication and translation of FMDV RNA occurs in the cytoplasm of an infected cell in association with the cell membrane (Sáiz et al., 2002).

Foot-and-mouth disease virus is externalized by lesion material, by the body secretions and excretions such as saliva, milk, faeces, urine, semen, nasal discharge and exhaled air, via the urogenital tract and nasal passages. These secretions and excretions contain a significant level of virus in an infected animal (Thomson et al., 2003).

2.1.4. Virulence of FMD

The severity of the clinical disease caused by FMDV varies depending on: a) the species affected, b) the immune status of the animals, c) dose, and d) the phenotype of the infecting virus (Kitching et al., 2007). Little is known about virulence and host-range determinants of FMDV *in vivo*.

Non-structural proteins of the virus (particularly 3A) are likely to play a role in the pathogenesis of FMDV in natural hosts, since changes in 3A have been linked to differences in severity of the clinical signs in cattle for several viral strains and serotypes (Sáiz et al., 2002).

2.1.5. Clinical Signs and Pathology

The clinical outcome of the disease may vary among the host species and the infecting virus strain (Sáiz et al., 2002). Foot-and-mouth disease is characterized by a rapid spread of infection with short incubation periods, generally ranging from 2 to 8 days, with high morbidity, approximating 100% (Thomson, 1994). However observations in indigenous cattle in southern Africa have indicated low morbidity (0.2%) (Baipoledi et al., 2004).

The earliest signs of FMD infection are: fever, anorexia, fall in milk production, cessation of rumination and excessive salivation. The development of characteristic vesicular lesions in the mouth and feet, the main characteristic of FMD, is the result of infection of squamous epithelium and persistent irritation or friction of the infected organs sites. These lesions produce salivation as consequence, serous nasal discharge, lameness and disinclination to stand. In domestic species; young animals may die before showing clinical signs (Thomson, 1994).

In cattle and pigs, fever and viraemia usually start within 1 to 5 days after infection, leading to viral spread into different organs and tissues and the production of secondary vesicles, preferentially in the mouth and feet. The acute phase of disease lasts about 1 week, and declines gradually. In sheep and goats, symptoms are frequently less severe and may make the detection of the disease difficult (Sáiz et al., 2002).

2.1.6. Diagnosis

The ability to diagnose FMD easily and accurately is a prerequisite for efficient control (Reid et al., 1999). Because FMDV is able to spread rapidly, it causes severe economic losses, and can be mistaken for other diseases with similar symptoms; these infectious agents should be handled in laboratories with high levels of bio-security (OIE, 2011a).

The diagnosis of FMD is dependent upon clinical assessment, identification of FMDV or viral antigens and genomic material and serology (OIE, 2011a).

2.1.6.1. Serological tests

There are two types of serological tests for FMD (OIE, 2011a): those detecting antibodies to viral structural proteins (SP) and those detecting antibodies to viral non-structural proteins (NSP). The SP tests in use are: the complement fixation test (CFT), the virus neutralization test (VNT), the solid-phase competition ELISA (enzyme-linked immunosorbent assay) (SPCE), the liquid-phase blocking ELISA (LPBE) and the enzyme-linked immunoelectrotransfer blot assay (EITB). The LPBE has high sensitivity and tends to produce more false positives, while the SPCE has a high specificity (Thomson et al., 2004).

The ELISAs have more advantages compared to VNT because the ELISAs are serotype specific, quicker to perform, and do not depend on tissue culture systems and the use of live virus, whereas VNT requires the use of cell culture facilities and the results are only obtained within 2 to 3 days (Smitsaart et al., 1998; Sutmoller et al., 2000).

The LPBE is an indirect ELISA and prescribed by OIE to monitor immunity conferred by vaccine in the field or for matching the vaccine with the field strain. LPBE is a blocking assay, based on using serotype-specific monoclonal antibodies. The test antigen composition should be closely related to the circulating strain or vaccine strains. The antigens are prepared from selected strains of FMDV and grown in monolayer BHK-21 cells (OIE, 2011a).

In recent years NSP tests have been developed and used to differentiate between vaccinated and infected animals (Sutmoller et al., 2000). At present, it is difficult to reliably estimate and compare the diagnostic performance of existing and newly developed NSP-based tests, because there is no 'gold standard' test for positive serum to NSP (Goris et al., 2007b).

Generally, the NSP tests are recommended as screening tests on a herd basis, because some inactivated and non-purified vaccines might have a high level of NSP, which may induce the production of antibodies, particularly after repeated vaccination, resulting in false positive results if applied to individual animals (Kitching et al., 2007). The sensitivity of the NSP tests can be improved through retesting false-negative animals or using a combination of tests (Brocchi et al., 2006; Goris et al., 2007a).

2.1.6.2. Virus isolation

Virus isolation (VI) requires careful handling of specimens within high bio-safety laboratories (Roeder et al., 2007). Virus isolation can be used to confirm the presence of infection in individual animals (Thomson, 1994; OIE, 2011a). It is reliant on cell cultures such as primary or secondary calf thyroid, pig kidney and lamb kidney cells. Other cell cultures routinely used are baby hamster kidney (BHK) and pig kidney (IB-RS-2) cell lines, but these could be less sensitive for primary isolation (OIE, 2011a).

2.1.6.3. Current research into new diagnostic techniques

Polymerase chain reaction (PCR) offers an alternative diagnostic method for FMD. The performance of the PCR tests varies under different circumstances and with different serotypes (Reid et al., 2003). The nucleic acid detection techniques offer potential advantages such as requiring a small portion of sample to reduce handling and conservation problems in the field. Risks for false negative results are limited (Reid et al., 2003). Despite significant advantages, the real time PCR (RT-PCR) tests have low sensitivity, and a narrow spectrum of reactivity compared to the results of ELISA tests (Reid et al., 1999). The sensitivity of RT-PCR tests have been improved through the design of primers covering a wide variety of topotypes and strains (Reid et al., 1999, 2003).

Worldwide increased use of PCR for diagnosis has facilitated phylogenetic analysis of all seven FMDV serotypes, and generated epidemiological information more rapidly (Reid et al., 1999). Therefore, RT-PCR methodologies need further evaluation to ascertain their ability to detect FMDV in milk and probang samples (Reid et al., 2003).

2.1.7. Control

2.1.7.1. History of FMD control

Over the centuries protection of cattle herds against FMD has been a concern for cattle breeders. In the past aphthisation and injection of immune serum were methods used separately or in combination to control outbreaks (Lombard et al., 2007). Aphthisation is an immunization method used in the past that involved deliberately infecting all healthy animals in the herd using material from lesions of sick animals once the first case of disease was identified on the farm or neighbouring farm (Lombard et al., 2007).

Historically, FMD prevention and control have been based on zoo-sanitary measures, stamping-out policies and separation of infected and susceptible animals (Anderson et al., 1974; Barnett et al., 2002). In many parts of the world the application of zoo-sanitary measures (biosecurity and segregation) has prevented the dissemination of infectious agents and contributed to the elimination of diseases (Sutmoller et al., 2003).

Vaccines and vaccination allow countries to reduce the incidence of FMD by reducing the number of susceptible individuals in the herd, and by preventing the transmission of infections within the herd and between herds (Lombard et al., 2007). European and South American countries have extensively used vaccination to lower the incidence of FMD, leading to eradication in combination with other control strategies (Thomson, 1994). In southern Africa, South Africa, Botswana and Namibia have eliminated FMD in extensive areas of their countries using combined control strategies (Brownlie, 2001; Derah et al., 2005).

2.1.7.2. FMD control measures currently in use

The methods used to control FMD are: a) stamping-out, b) separation between wildlife and susceptible domestic animals through fences and c) vaccination (Sáiz et al., 2002). The strategies are chosen based on the situation of FMD in the country, and the relative importance of animals for international trade (Vosloo et al., 2002). Each method has its advantages and disadvantages. In most southern African countries, FMD control strategies are a combination of different control methods, including separation of infected wild species and domestic animals through fences, restricted movement of animal and animal products and vaccination in adjacent areas of the fenced wildlife conservation areas (Thomson et al., 2003).

In Mozambique FMD control is based on routine vaccination at 6-monthly intervals (which is, however, not fully observed) and restriction of movement of animals and animal products through use of an animal movement permit system. Routine vaccination is carried out as a prophylactic measure with the objective of preventing re-introduction of FMDV into livestock populations within the Limpopo National Park (LNP) and adjacent to conservation areas along the border with Zimbabwe and South Africa. Routine vaccination against FMD in Mozambique is administrated to cattle above 4 months of age using a bivalent vaccine (SAT 1 and SAT 2). The vaccination regime adopted consists of a single primary dose application with revaccination at 6 month intervals. Ring vaccination and a ban on in-country movement of susceptible species and its products are applied to control outbreaks. The routine and ring vaccinations are provided free of charge by government. The FMD control zone where routine vaccination is practised is located along the eastern boundary of LNP, along the border with Zimbabwe from the south up to Manica province, and the eastern border of the Kruger National Park on the Mozambique side, covering an area approximately 60 km wide along the borders.

2.1.7.2.1. Stamping-out

Stamping-out refers to the killing and disposal of all susceptible livestock on infected and adjacent farms followed by cleaning and disinfection of the premises (OIE, 2011b). Stamping-out is the control method often selected in the countries traditionally free from FMD. It is usually successful, at least if the disease is not yet widespread and if it is efficiently performed and adequate compensation policies are in place (Brownlie, 2001).

In most countries in sub-Saharan Africa, the eradication of FMD by the implementation of stamping-out is impractical due to: a) the vastness of the countries, b) limitations of infrastructure, c) the need for mobilization of a high number of field staff, d) the need for utilization of diagnostic laboratory facilities for testing, e) lack of identification of animals and f) the problems involved in destruction of a large number of animals. Additionally, in most countries funds for compensation of farmers are often unavailable (Hunter, 1998; Patil et al., 2002b). Stamping-out is disruptive for all the farmers and feared by everyone more than FMD itself (Sutmoller et al., 2003).

2.1.7.2.2. Fences and control of animal movement

The World Organization for Animal Health presently considers that any territory in which infected buffaloes occur is "infected". According to Chapter 8.5 of the Terrestrial Animal Health Code of the OIE (2011b), parts of countries or zones can be recognized as free of FMD in countries infected by FMD when susceptible species in the FMD free zone can be separated by a buffer zone or by physical or geographical barriers from the rest of country or neighbouring countries with a different animal health status.

In southern Africa, where wildlife maintains FMDV, the approach adopted is the separation of wild species from domestic animals by means of fences (Thomson, 1999; Vosloo et al., 2002). The type and the characteristics of fences depend on the sort of wildlife species predominant in the areas (Thomson et al., 2003) In general, the game-proof fence used is a 1.8 to 2.4 m high one-line fence, electrified or not (Condy et al., 1985; Jori et al., 2009 and 2011).

The protection of the wildlife heritage is a priority in the sub-Saharan African region, particularly in east and southern Africa (Vosloo et al., 2005). Wildlife conservation areas are being established and restocking programmes are being carried out associated with anti-poaching law enforcement. These actions have enabled an increase of wildlife populations in this region. Despite these efforts to protect wildlife, the majority of the conservation areas have no appropriate infrastructure to ensure effective separation between wildlife and domestic animals. Moreover, the maintenance of these infrastructures, where they exist, is not regularly done because of the high cost of erecting and maintaining the fences, and only countries that have access to profitable markets for their livestock products that compensate the costs can afford to establish these infrastructures (Vosloo et al., 2002).

Some countries in southern Africa have used this method in combination with vaccination in adjacent areas to control FMD with success (Condy et al., 1985). Despite this, the efficiency of the fences in their role of separation between livestock and wildlife is not always guaranteed for all wildlife species and varies among locations (Hargreaves et al., 2004; Jori et al., 2009 and 2011), resulting in an increased interaction between these species.

Fences have been severely criticized by wildlife conservationists because fences block migration routes or access of wildlife to water points. These facts highlight the need to explore alternatives that are ecologically acceptable but adequate to control FMD, as it is difficult to control by vaccination alone (Thomson et al., 2003, Vosloo et al., 2005).

2.1.7.2.3. Vaccination

2.1.7.2.3.1. History of vaccination

The development and introduction of FMD vaccines gradually changed the disease control strategies and the scenario of the disease in the world (Lombard et al., 2007; Roeder et al., 2007). The first semi-industrial FMD vaccine that was produced was based on a concept developed by the Vallée-Schmidt-Waldman team (Lombard et al., 2007). The large scale production of FMD vaccines was only possible after Frenkel's discovery of the ability of FMD virus to grow *in vitro* in fragments of surviving bovine tongue epithelium maintained in suspension culture (Sutmoller et al., 2003).

Modern vaccines against FMD are prepared from cell culture-derived inactivated virus. The immunogenicity of the inactivated FMD antigen is poor, requiring the addition of adjuvants to enhance its activity (Sáiz et al., 2002) and frequent revaccination. Two basic types of adjuvant are generally used: a) aluminum hydroxide ($Al(OH)_3$) with saponin and b) various mineral oil formulations (Sobrino et al., 2001). The latter can also be used in pigs whereas alhydrogel/saponin formulations are ineffective in this species (Doel, 1999; Sutmoller et al., 2003). The protection that these vaccines provide to ruminant species is not sterile, and vaccinated animals may still become infected (Hedger, 1970). However, it is clear that vaccination programmes, properly and extensively applied in association with other zoosanitary measures, can assist in reducing disease prevalence to a sustainable level or to the point of eradication (Brownlie 2001; Doel, 2003).

2.1.7.2.3.2. Important aspects of vaccination campaigns

Different FMD vaccine formulations are needed to provide protection against all circulating serotypes of FMDV and their strains (Brownlie, 2001; Kitching et al., 2007). Correct use of FMD vaccine is important for controlling the disease. Some factors should be observed to ensure that vaccination campaigns deliver the required protection of vaccinated animals. These factors are maintenance of the cold chain, appropriate injection of vaccine as recommended by the manufacturer and the use of vaccine strains that protect vaccinated animals against virus to which they are likely to be exposed (Kitching et al., 2007; Roeder et al., 2007).

Administration of alhydrogel-saponin FMD vaccines should be done as follows: a primary inoculation dose followed by a second dose from 2 to 8 weeks. Regular revaccination should be conducted every 4 to 6 months until at least 2 years of age, then biannual or annual revaccination (Thomson et al., 2004).

This schedule is not followed by many countries, as they prefer to apply a single first dose followed by biannual or annual vaccination. However, this schedule seems to be satisfactory in areas where infections are not severe (Thomson et al., 2004). In fact, a second dose 2 - 8 weeks after the primary dose is hardly ever applied, due to practical difficulties (Thomson, 1995).

In most southern African countries, the vaccination coverage is not high (Thomson, 1995) and for effective control of FMD and to reduce disease transmission the herd immunity should be between 70-80% (Roeder et al., 2007; Smitsaart et al., 1998).

Control of FMD in wild species using conventional vaccinations has been proposed and attempted, but with no conclusive results (Moutou, 2002; Thomson, 1996). The main constraints are related to conventional FMD vaccine formulations not preventing infection and persistence post infection, high cost of application, and insufficiently tested vaccine. As a result, vaccination of free living wildlife is currently not recommended (Thomson, 1996).

2.1.7.2.3.3. New vaccines

Novel vaccines are being developed based on genetically engineered technologies to produce empty FMD capsids and synthetic peptide antigens for FMDV (Brownlie, 2001*;* Sáiz et al., 2002; Wang et al., 2001). Deoxyribonucleic acid (DNA) vaccines and modified live virus vaccines containing analogues of virus genes are capable of expressing viral antigens in host cells, and are able to induce an immune response with specific FMD neutralizing antibodies (Mason et al., 2003*).* Novel FMD vaccines, currently under investigation, are based on reverse genetics, where specific motifs are changed or adenoviruses expressing FMDV antigens are used (Grubman and Mason, 2002*;* Yao et al., 2008)

The FMDV encoding region involved in capsid assembly (P1, 2A and 3C) is being used to develop new vaccines based on empty FMDV capsids delivered by live virus vectors (Grubman and Mason, 2002). The vaccines based on human *Adenovirus* vectors are able to induce specific FMD neutralizing antibodies. These vaccines have been shown to protect pigs and cattle against clinical disease and prevent FMDV replication. Despite these

promising results, the *Adenovirus* vaccines require more detailed research (Grubman and Mason, 2002).

The new vaccines have certain advantages compared to the conventional vaccines, because a wide spectrum of protection might be induced against antigenic serotypes and strain variants (Brownlie, 2001; Sobrino et al., 2001). Peptide vaccines have been shown to provide reliable protection against disease in swine, even at small doses (Wang et al., 2001).

Immunization with DNA vaccines administered intramuscularly provided a solid cellular immune response by processing endogenous antigen (Yao et al., 2008). The administration of DNA vaccines usually requires a gene gun, which is expensive and of limited use and not yet applied under field conditions (Yao et al., 2008).

2.1.7.2.3.4. Quality of the vaccines and their efficacy

Vaccines should ideally be capable of stimulating rapid and potent long-lasting immunity after a single vaccination (Cox et al., 2003), This has been achieved by some conventional vaccines (Doel, 2003*;* Sáiz et al., 2002). Since the 1980s certain southern African countries such as Botswana and RSA have used segregation of infected wildlife and susceptible domestic animals to control FMD. Animal segregation has been combined with regular vaccination and resulted in successful control of FMD for a long period. This success could in part be attributed to the improved quality of the FMD vaccines locally produced by the Botswana Vaccine Institute (BVI) and Onderstepoort Veterinary Institute (OVI) (Thomson, 1995).

The quality of the vaccines is mainly associated with the safety and potency of the vaccine (Doel, 1999). The safety is the ability of the vaccine not to cause disease or transmit infectious agents (Doel, 1999). Conventional FMD vaccines are produced from virulent FMD virus that can cause an outbreak if the inactivation is not properly done (Doel, 1999*;* Sutmoller et al., 2003). To ensure reliable inactivation, FMD vaccine manufacturers follow strict guidelines and quality control (Höhlich et al., 2003). The potency of the vaccine depends on quantity and quality of antigen, antigenicity of strains (which should cover a wide range of viruses prevailing in the area), the adjuvants and the formulation of the vaccine (Garland, 1999; Doel, 2003). In some developing countries it is difficult to assess the quality of the vaccine due to lack of specialised personnel and financial resources (Roeder et al., 2007).

The genetic and resultant antigenic diversity of SAT type viruses within each serotype and topotype, particularly SAT 2, has implications for FMD control based on vaccination. The vaccine should be immunologically related to the virus strain circulating in the field (Bastos et al., 2003; Paton et al., 2005), which is a challenge to vaccine producers (Doel, 2003).

Considering the facts mentioned above, the production of an effective vaccine requires intensive and continuing research to identify the circulating viruses and develop more effective vaccines containing homologous SAT strains (Condy et al., 1985).

2.1.7.2.3.5. Longevity of immunity provided by vaccines

Protection of individuals recovered from infection or vaccination is mediated by antibodies, particularly the neutralizing antibodies immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) (Doel, 2003). In infected cattle the first neutralizing antibodies produced are IgM. These antibodies are detectable within 3 to 4 days post exposure and reach the peak level between 10 and 14 days post infection (Sobrino et al., 2001).

The duration of antibodies induced by infection is influenced by different factors such as strain, species affected, age, breed, nutritional condition, and combined infection (Thomson et al., 2004). Generally, protection against re-infection with homologous viruses varies from 1 to 3 years after exposure (Thomson et al., 2003; Lombard et al.2007).

In endemic areas, the colostral ingestion of maternal antibodies, which is essential in the early stages of life, provides protection for 2 to 5 months (Sutmoller et al., 2003). This immunity compromises the protection provided by vaccination, because the maternal antibodies interfere with the immunity induced by vaccination in the early stages of life (Thomson et al., 2004).

Many research projects have investigated the ability of aqueous FMD vaccines to induce and maintain high level of neutralizing antibody titres against FMDV. Different vaccination protocols were used but none of them obtained satisfactory results (Cloete et al., 2008; Hunter, 1996)

2.2. Problem Statement and Research Questions

The vaccines currently used in southern Africa are inactivated and formulated in aluminium hydroxide with saponin adjuvants. The guidelines of the vaccine manufacturer (BVI) recommend subcutaneous inoculation as follows: the first dose, consisting of two successive applications, i.e. the first inoculation followed by a second three to four weeks later, should be followed by a revaccination every four to six months, depending on epizootic situations and local legislation of the country. The vaccine must be conserved at temperatures ranging between 2 to 8°C because these vaccines are heat labile (Kitching et al., 2007).

Regular boosters are needed because immunity induced by conventional FMD vaccines only lasts for 3 to 6 months (Cloete et al., 2008; Hunter, 1998). However, in many countries the manufacturer's recommendations have not been observed because of lack of resources and logistical difficulties to implement a massive vaccination programme (Hunter, 1996; Kitching et al., 2007). Most countries prefer to apply a single first dose followed by biannual or annual revaccination (Bruckner et al., 2002; Kitching et al., 2007).

Since the vaccination protocols adopted by the countries are not what the manufacturer recommends, there are no studies indicating how long vaccinated animals take to raise protective antibody levels and how long immunity lasts after a single primary vaccination, rather than the recommended double primary vaccination.

This study therefore aims to provide information on the period required for vaccinated animals to produce antibodies, on the duration of immunity in cattle inoculated with FMD vaccines, and on the effect of revaccination four or six months later. This aim will be achieved through investigating the following questions:

1. How long does it take for vaccinated cattle to start producing a detectable and protective level of antibodies after a single inoculation of bivalent (SAT-1 & SAT-2) FMD vaccine by the subcutaneous route, under field conditions in Mozambique?

2. How long do the detectable and protective antibody titres last in cattle vaccinated with a single primary inoculation?

3. What is the antibody titre response to revaccination at four months or at six months after primary vaccination, and does it differ between the two?

4. Is the antibody titre response different in high risk areas (where interaction with wildlife is more frequent) compared to low risk areas?

The results of this survey will assist the veterinary authorities of Mozambique to design and implement efficient control policies for FMD, avoiding unnecessary expenses and maximizing benefits through correct use of the vaccine for FMD prevention and control. This project will also provide information that will contribute to the overall knowledge of the efficiency and effectiveness of the FMD bivalent vaccine currently used in Mozambique, and that can be useful for other countries in the SADC region.

2.3. Objectives of the Study

The objectives of the study were the following:

1. To monitor the antibody response to SAT 1, 2 and 3 in cattle receiving a single primary inoculation of bivalent FMD vaccine, in cattle revaccinated at four or six months, and in unvaccinated control animals, to determine the proportion of animals that seroconvert across the 12 months' duration of the study.

2. To determine the level and the duration of protection provided by the bivalent FMD vaccine by monitoring antibody titres to SAT 1, 2 and 3 in vaccinated and control livestock.

3. To compare immunity induced by the vaccine in two different areas: one where there is close contact between livestock and wildlife, and one where there is a greater distance between the two animal populations.

3. MATERIALS AND METHODS

This was a prospective, controlled field study in which vaccinated and control animals were observed over time and repeatedly sampled and assessed using direct observations and laboratory tests.

3.1. Study area

The study areas were selected in accordance with the perceived risk that cattle may get infected with foot-and-mouth disease virus (FMDV) as a result of contact with wildlife, particularly buffaloes. The distribution of buffaloes and their proximity to the villages were used as selection criteria.

The study was conducted in two separate areas, Limpopo National Park and Mabalane District of Gaza province.

The Limpopo National Park (LNP) was created in 2001, covering 11,233 square kilometres, and is located about 380 km north of the capital Maputo. The park boundaries extend into three districts, Chicualacuala, Mabalane and Massingir. The park limits are: the Limpopo River in the north and east, the Elephant River in the eastern part of the southern border and Kruger National Park, South Africa in the west. This area has large settlements of human populations dependent on livestock and is also densely populated by wildlife species including wild African buffaloes (Whyte et al 2006).

In this park three villages, with crush pens located at latitude S 23.443 and longitude E 31.741 in Chimangue, latitude S 23.562 and longitude E 31.921 in Machamba and latitude S 23.642 and longitude E 32.099 in Bingo were selected for the study.

The Mabalane study area is located about 280 km north of the capital, Maputo, and limits are the Limpopo River to the south, Chicualacuala District in the west, Chigubo District in the north and two districts on the east, Guijá and Chokwè. In this district the study was conducted in cattle from two crush pens located at latitude S 24.017 and longitude E 32.663 in Pfukwè and latitude S 23.847 and longitude E 32.623 in Covela. Figures 1 and 2 show the locations of the study sites.
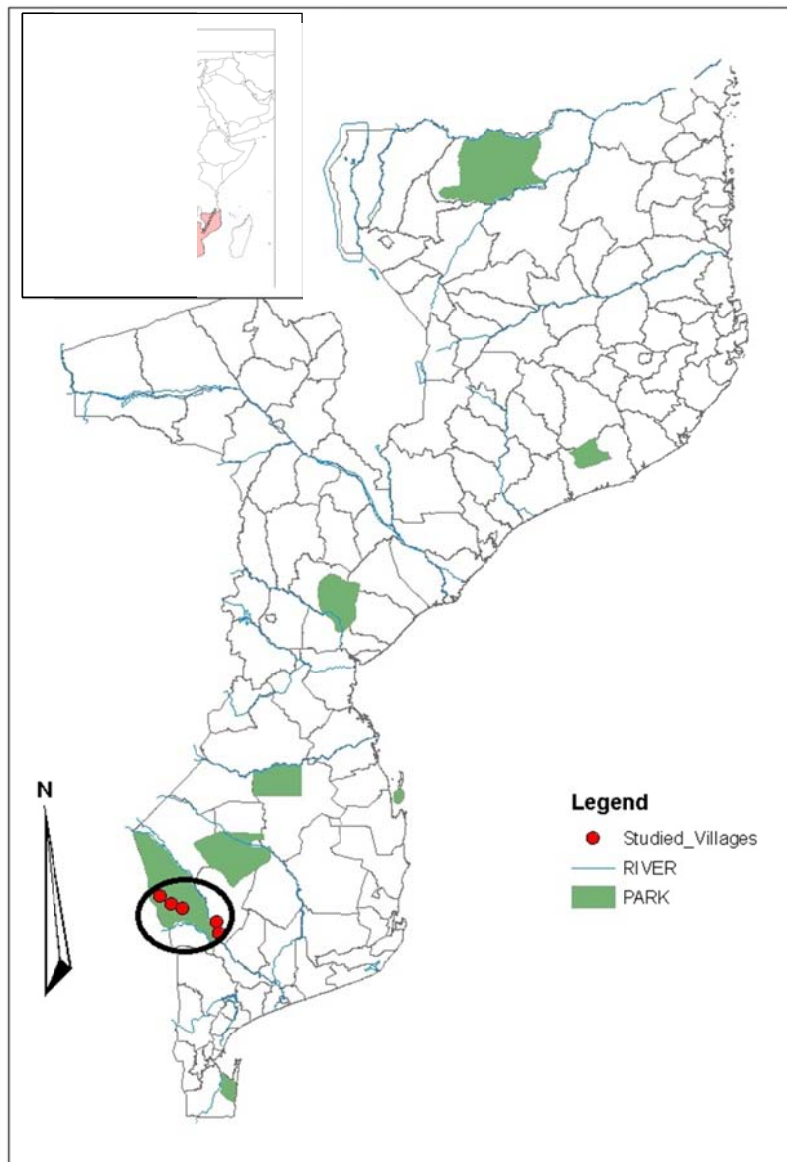
Figure 1. Map of Mozambique showing the location of Limpopo National Park and the study areas.

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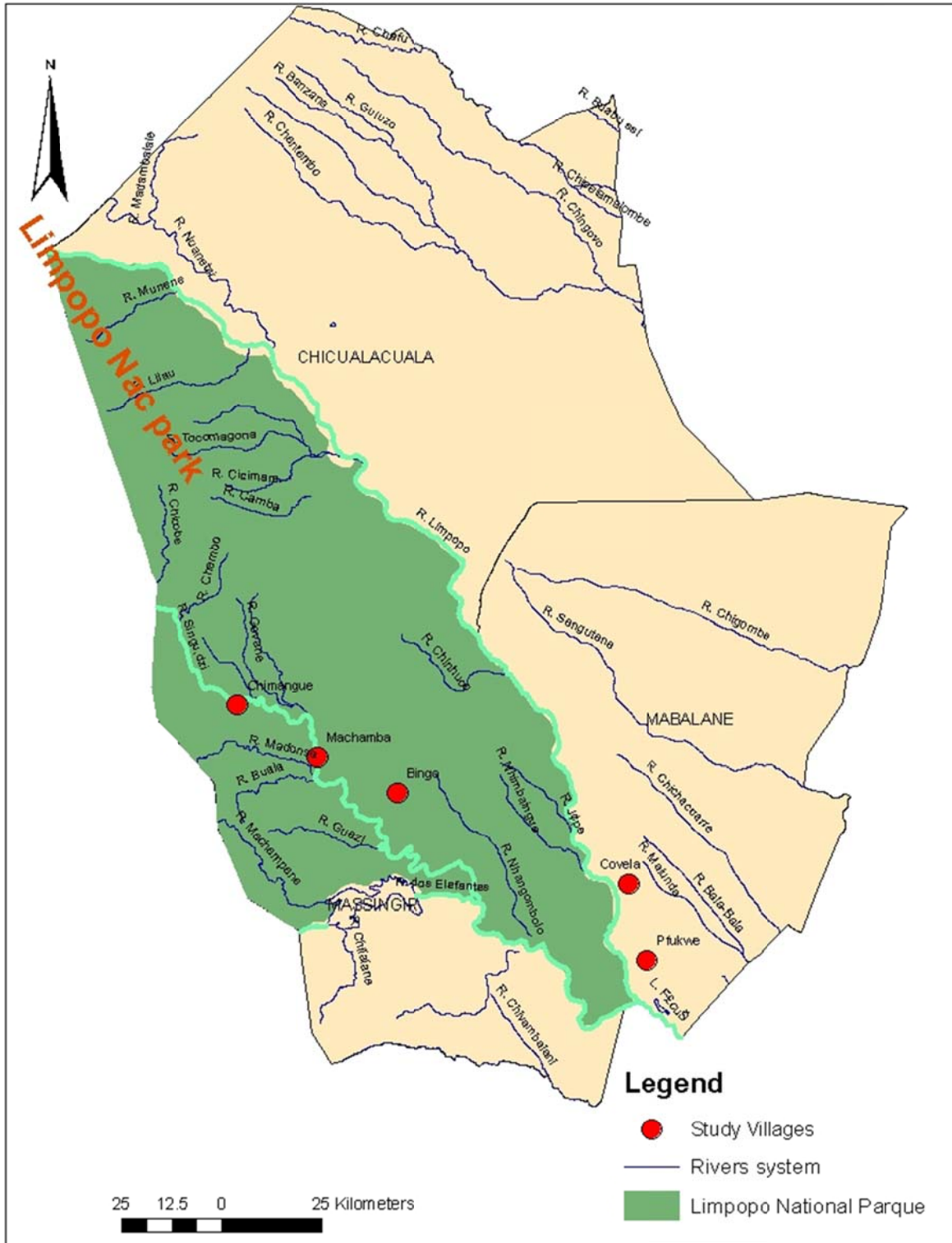
Figure 2 Map of Limpopo National Park and Chicualacuala, Mabalane and Massingir Districts, showing the location of the study sites.

3.1.1. Study area background

Mozambique is one of the southern African countries that is embarking on the conservation of biodiversity through the establishment of conservation areas and involvement in transfrontier conservation initiatives. Although significant positive achievements have been obtained in the conservation of biodiversity in the conservation areas, animal health issues pose great challenges to the veterinary authorities managing animal diseases, particularly those transmitted between wildlife, livestock and humans, since people and their livestock are living in most conservation areas in the country.

3.1.2. Climate and vegetation

The climate of Mozambique comprises two distinct seasons, summer and winter. Summer is a hot, rainy season from October to March while winter is a relatively cool and dry season from April to September.

The climate of the study area is tropical semi-arid, with an average annual rainfall of 200 - 400 mm. The rainfall is irregularly distributed during the year with cyclical droughts every 2 to 3 years. The maximum temperature during summer varies between 31 and 42°C and during winter between 15 and 21°C.

Savanna woodland is the main vegetation type, dominated by *Colophospermum mopane* and *Acacia* spp. Different species of grass occur in the area, with the following being the most abundant: *Panicum maximum, Hyparrhenia dissulata, Trachypogon spicatus, Elionurus argenteus, Brachiaria mutica, Urochloa mosambicensis* and *Themeda triandra.*

3.1.3. Livestock population and husbandry in the study area

Livestock husbandry is a common activity in the study area. The livestock population in the districts where the study was carried out was 62,607 cattle, 23,242 goats, 9,075 sheep and 2,413 pigs (Livestock census Direcção Nacional dos Serviços de Veterinária, 2009). The study villages host an estimated domestic animal population of about 6,350 cattle, 8,000 goats, 3,100 sheep and 500 domestic pigs (Serviços Provinciais de Pecuária de Gaza, 2009). Most of the local inhabitants breed Nguni (Sanga type) cattle (Rocha et al., 1991; Kotze et al., 2000).

In the area cattle and small ruminants are kept in corrals at night, particularly in the rainy season (October to March) when crops are in the fields. During the dry season (April to

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September) most animals roam and graze freely, but oxen continue to be corralled in the evening as they are used for different purposes, such as transport and ploughing.

Many watercourses are found across the study area in both districts that are all seasonal and water flows only for a short period. During the dry season the Shinguedzi River is the main source of water for wildlife and livestock in the study area. The Shinguedzi flows through the three rural villages in the Massingir district. The rural villagers in the Mabalane district rely mainly on the Limpopo River and the Pfukwe dam as the main water sources for livestock.

3.2. Selection of the study animals

The sample size was determined using Win Episcope 2.0. The expected seroconversion prevalence was 70%, at a 95% confidence interval and a precision of 10%. This resulted in a required sample size of 81 animals per district.

Considering expected loss to follow-up, the final sample size was increased to 100 cattle per district. Thirty additional animals in each district were included and kept unvaccinated as controls. Table 1 shows the detailed distribution of the sample size between districts and villages.

Table 1. Planned sample size for the study and its distribution by district and village.

District	Village	Vaccination	Control	Total
Massingir	Bingo	34	10	44
	Chimangue	33	10	43
	Machamba	33	10	43
	Subtotal	**100**	**30**	**130**
Mabalane	Covela	50	15	65
	Pfukwe	50	15	65
	Subtotal	**100**	**30**	**130**
Total		**200**	**60**	**260**

Cattle aged 6 to 18 months old were selected based on the assumption that cattle from an endemic or FMD vaccinated area aged less than 6 months have maternal antibodies, and

animals over 18 months old might have been vaccinated at least once and therefore be seropositive at the beginning of the study.

To ensure compliance with the inclusion criteria the following age estimation methods were used for the selection of animals: the calving season, the state of the horns, the weaning age in the study area in the traditional system, and the state of the dentition.

The main calving season in the southern region of Mozambique is influenced by the abundance and quality of pasture, which is directly influenced by the rainy season. In this part of the country the rainy season is from October to March, and most cows conceive between November and April and their calving takes place from late July to January (Rocha et al., 1991).

Generally the weaning season is from April to May, when calves are approximately seven or eight months old. At this age, the majority of calves have visible horns. Local cattle owners use the state of horn development to decide when to wean their calves if they have not ceased to suckle by themselves.

The state of dentition was also used to determine the age. The first pair of permanent incisor teeth erupts fully from 18 to 24 months of age.

Based on the above criteria, calves between 6 and 18 months of age were selected for inclusion in the study.

The selection of cattle started in March 2009, following a preparatory phase from November 2008 to March 2009 during which 300 cattle were identified for potential inclusion in the study. It was agreed between the owner and the local authority that these animals would be made available for the duration of the study.

Cattle were regularly traced by the local veterinary staff during the tick control programme, provided free of charge by the government at intervals of 15 to 20 days. The exemption of charges to the cattle owners was an incentive to motivate their participation in the study. The tick control programme was conducted between November 2008 and April 2010. As a result some animals selected for the study were sold, exchanged or slaughtered and others were not made available at the start of the study.

A total of 217 cattle was ultimately available at the start of the study. Of the 217 cattle selected and included in the study 121 were in Massingir and 96 in Mabalane. All cattle selected were identified by ear tag and branded with a hot iron. The branding was done in June 2009, after it was noted that the numbers on the ear tag were fading. The animals were divided into the following four groups:

1. **Vaccinated once**: 73 animals that were vaccinated once with a single dose of bivalent FMD vaccine,
2. **Revaccinated at 4**: 39 animals that were vaccinated once and revaccinated four months later,
3. **Revaccinated at 6**: 63 animals that were vaccinated once and revaccinated six months later, and
4. **Control**: 42 animals that were not vaccinated

3.3. Experimental procedures

3.3.1. Vaccination

The vaccine used in this study was a bivalent vaccine containing SAT 1 and SAT 2 serotypes produced by BVI in February 2009, batch no. 92304, and purchased by the Mozambican Ministry of Agriculture. Information on the strains used as vaccine antigen was not available.

The cold chain facilities for vaccines were ensured from Maputo to the vaccination points and also during the inoculation. The vaccine was kept refrigerated at approximately 3°C in a portable refrigerator operating with 12 V DC and 220 V AC. The vehicle battery was used to supply electrical power to the cool box during the field trips. Once back in town the electrical power source was switched from 12 V DC to 220 V AC. The temperatures were monitored by two thermometers, one registering the minimum temperature and another for maximum temperatures. These thermometers were placed inside the refrigerator and read at intervals of between 1 h and 1h30.

A total of 175 animals (99 cattle in LNP and 76 cattle outside LNP) were vaccinated. A small number of the study cattle, 42 cattle (22 in LNP and 20 outside LNP), were left unvaccinated as controls.

During September 2009, four months after the first vaccination, a total of 39 cattle from the vaccinated group, 27 in LNP and 12 outside, were revaccinated (referred to as Revaccinated at 4). In November 2009, six months post first vaccination, a group of 60 vaccinated cattle, 364 of them from LNP and 27 from outside, were revaccinated (Revaccinated at 6). Table 2 shows the distribution of cattle between study groups and study areas.

Animals in the vaccination groups were inoculated with a single dose of 2 ml of FMD vaccine, via the subcutaneous route in the neck at time zero in late May 2009. The group of animals revaccinated at 4 or at 6 months after initial vaccination were inoculated with 2 ml of the vaccine at 4 MPV and 6 MPV.

Of the cattle that were vaccinated, 42% were vaccinated only at time zero (vaccinated once), 22% were vaccinated twice, at time zero and at four months post vaccination (vaccinated at 4) and 36% were also vaccinated two times at time zero and six months post first vaccination (vaccinated at 6).

Table 2. The final distribution of vaccinated, revaccinated and control animals by study area and village.

Study area	Villages	Number of cattle				
		Control	Vaccinated at time zero	Vaccinated once	Revaccinated at 4	Revaccinated at 6
Massingir	Bingo	12	33	9	10	14
(High risk area)	Chimangue	5	39	20	9	10
	Machamba	5	27	7	8	12
	Subtotal	**22**	**99**	**36**	**27**	**36**
Mabalane	Covela	6	41	15	5	21
(Low risk area)	Pfukwe	14	35	23	7	6
	Subtotal	**20**	**76**	**38**	**12**	**27**
Total		**42**	**175**	**74**	**39**	**63**

3.3.2. Sample collection

The sampling schedule was: the first blood samples were collected on the first vaccination day, defined as day zero (t0). The samples collected on this day were used to determine the immune status of the animals before vaccination. Animals were then again sampled one, four, five, six, eight, ten and twelve months after the first vaccination. Table 3 summarizes the number of samples collected at each sampling time.

Of the 176 animals vaccinated at beginning of the study 139 (79%) were sampled at one month post-vaccination (MPV), 116 (66%) at 4 MPV, 102 (58%) at 5 MPV, 103 (59%) at 6 MPV, 92 (53%) at 8 MPV, 89 (51%) at 10 MPV and 98 (56%) at 12 MPV.

In the control groups, of the 42 cattle unvaccinated at beginning of the study, 23 (55%) were sampled at 1 MPV, 16 (38%) at 4 MPV, 22 (52%) at 5 MPV, 16 (38%) at 6 MPV, 10 (24%) at 8 MPV, 11 (26%) at 10 MPV and 12 (29%) at 12 MPV. Table 3 shows the number of cattle monitored per vaccination schedule per sampling time and per study area.

Table 3. Number of cattle monitored per vaccination status per district.

Area of Study	Study Group	Months post Vaccination							
		Zero	1 MPV	4 MPV	5 MPV	6 MPV	8 MPV	10 MPV	12 MPV
Massingir	Control	22	13	6	11	9	8	7	11
	Vaccinated once	36	29	17	17	8	18	18	19
	Vaccinated at 4	27	26	27	15	23	22	20	22
	Vaccinated at 6	36	33	19	25	34	25	23	25
Mabalane	Control	20	10	10	12	7	2	4	1
	Vaccinated once	37	11	8	14	1	3	4	2
	Vaccinated at 4	12	7	9	8	7	1	2	4
	Vaccinated at 6	27	16	16	22	26	15	14	10

The blood was collected from the study cattle directly from the jugular and coccygeus veins, using vacutainer tubes containing a clot activator gel. The tubes with blood were left to stand until the complete clot was formed, and the serum was decanted into Eppendorf micro tubes.

The sera were transported from the field in a refrigerated cooler box to the Veterinary Central Laboratory of the Mozambique Agricultural Research Institute (VCL), in Maputo. The samples were stored frozen at -70°C at VCL prior to shipment to the Onderstepoort Veterinary Institute (OVI). The samples were sent to OVI on dry ice (Hargreaves, et al., 2004; Hedger, 1968). A total of 1066 serum samples were obtained and submitted to OVI for testing.

3.3.3. Laboratory tests

The laboratory tests were performed at Transboundary Animal Diseases Programme, OVI, an OIE reference laboratory for FMD.

The samples were tested using a liquid-phase blocking ELISA (LPB-ELISA; Hamblin et al., 1986) screening test using reagents developed in-house, one of the diagnostic tests recommended by OIE (OIE, 2011a), to monitor antibodies against SAT 1, 2 and 3 serotypes of FMD, and a commercially developed NSP test, the PrioCHECK® FMDV NS, was used to detect antibodies to non-structural proteins of FMDV and active virus infection.

The test principle of the LPBE is a blocking ELISA and is serotype-specific. It is based on blocking of liquid phase FMD antigen by antibody in the test serum. The test uses rabbit antiserum that is raised to serotype-specific FMDV which is coated onto microplates and used to capture the FMD type-specific antigen.

The LPBE test was performed as follows: ELISA plates were coated with rabbit antisera homologous to the 146S antigen being tested for, then incubated for 1 hour at 37°C, after which the test plates were washed with PBS solution. Viral antigen that is homologous to the rabbit antisera used to coat the plates was added and mixed and then the plates were incubated at 37°C for 1 hour. After that the mixture of antigen and serum was transferred from the carrier plates to the rabbit-serum coated ELISA plates, incubated for 1 hour at 37°C on a rotary shaker and then washed. After that the guinea pig antiserum, homologous to the viral antigen used in the previous step and pre-blocked with normal bovine serum diluted in washing buffer-tween, was added to the test plates and incubated at 37°C for 1 hour on a

rotary shaker, then the plates were washed again. A chromogen solution was added and left for 15 minutes to allow colour development before addition of the stopping solution.

The results were read at 450 nm on a spectrophotometer connected to a computer which received the reading from the spectrophotometer and transferred the results to a spreadsheet file. The antibody titres were calculated as that dilution at which the reaction of the test sera results in an optical density equal to 50% of the median optical density of the reaction of the control wells.

The NSP test principle is a blocking ELISA. The test plates were coated with 3ABC specific monoclonal antibody, followed by incubation with antigen (3ABC protein). Finally, the test plates of the kit containing FMD non-structural (NS) antigen captured by coated monoclonal antibodies was read in the spectrophotometer attached to the computer.

The test was performed by dispensing the test samples into the wells of the test plates. After incubation the test plates were washed and the conjugate was added. The FMDV NS specific antibodies against the non-structural proteins that may be present in the test samples will bind to the 3ABC protein and block the binding of the monoclonal antibodies conjugated to HPRO. After incubation, the plate was washed and the chromogen substrate was added to all wells of the test plates. The test samples were incubated at room temperature (22±3°C) and the colour development was stopped by addition of the stop solution.

The reaction was read at 450 nm on spectrophotometer connected to the computer. The percentages of inhibition (PI) of the control and test sera were calculated according to the following formula:

$$PI = 100 - \left(\frac{OD_{450} \text{ test sample}}{OD_{450} \text{max}}\right) \times 100$$

Interpretation of the percentage of inhibition was done as follows:

1. PI <50% is negative (antibodies against NS protein of FMDV are absent in the test samples)
2. PI ≥50% is positive (antibodies against NS protein of FMDV are present in the test samples)

For the LPBE ELISA titres ≥ 1.6 $\log_{10}$ were considered positive and titres ≥ 2.0 $\log_{10}$ were considered protective.

In our study we used heterologous reagents as we did not have access to reagents homologous to the vaccine strains.

3.3.4. Other observations

The animals in the study were clinically examined every 30 days during the study period for detection of FMD signs and lesions through inspection of the mouth, tongue and feet as described by Cloete et al. (2008).

3.3.5. Data analysis

Animal identification, sex, age, study group, vaccination date, sampling dates and the laboratory testing results for each animal in the study were kept in a Microsoft Access database.

Descriptive data analysis was performed with EpInfo™ (Centers for Disease Control and Prevention) and Stata 11 (StataCorp, College Station, TX, U.S.A.) software packages.

The proportion of animals with detectable (≥ 1.6 $\log_{10}$) and protective (≥ 2.0 $\log_{10}$) titres to each of the three SAT serotypes was calculated for each time point, by vaccination group and study site. Exact binomial 95% confidence intervals were calculated for each proportion. Changes in $\log_{10}$ antibody titres over time were summarized in graphs and tables.

The two-tailed Fisher's exact test was used to compare the percentage of sero-conversion between groups and between study sites at each sampling time. A 5% level of significance was used.

4. RESULTS

The study was conducted between May 2009 and June 2010. The first five sampling points were covered between May and November 2009 and the last three between January and June 2010. The first part of the study period was during the dry season and cattle were not corralled. Many of the study cattle were not monitored as scheduled because it was very difficult to gather them in one place at a single time. Despite the tick control programme provided by the Veterinary Service that was meant to stimulate farmers to bring their cattle for assistance, this did not improve the monitoring process.

In the group of cattle revaccinated at 4 MPV, 3 animals from outside LNP were excluded from the analysis because they were accidentally revaccinated at 6 MPV and 3 others (2 in LNP and 1 outside LNP) in the group of cattle revaccinated at 6MPV were also excluded from the analysis because they were wrongly identified.

Even though the vaccine contained only SAT 1 and SAT 2 antigens, because there is a permanent risk of SAT 3 introduction into the region due to the presence of carrier buffalo, sera were tested against SAT 3 as well.

With the exception of the results presented in section 4.3 the other results are presented as combined (aggregated) from both study areas.

The study results show that no antibody titres against any SAT serotypes or NSP antibody titres were found in any animals at the beginning of the study (Table 4 and Figures 3 and 4). At one month after vaccination with the single primary vaccination dose, antibodies against SAT serotypes were observed in 72.9% of vaccinated cattle. This proportion dropped to 8.3% at 4 months after vaccination as illustrated in Table 4. The proportion of animals that had seroconverted with antibody titres ≥2.0 against any of the SAT serotype observed at one month after initial vaccination was 43.4% in vaccinated animals; this proportion dropped to 4.2% at 4 MPV (Table 5).

In the unvaccinated control group (Table 4 and Figure 3), 21.7% of the cattle had antibody titres ≥1.6 to any of the SAT serotypes at 1 MPV and 8.7% of these animals had antibody titres ≥2.0 (Table 5). The proportion of animals with antibody titres ≥1.6 and 2.0 dropped to 6.3% at 4 MPV as illustrated in Tables 4 and 5.

The serological response of animals in the vaccinated group was statistically significantly different to the response of animals in the unvaccinated control group, with a higher proportion of vaccinated cattle producing a response ($P < 0.05$).

Between 22.9 and 59.8% of cattle in the vaccinated group and between 4.3% and 21.7% of cattle in the unvaccinated control group had antibody titres ≥1.6 to each of SAT serotypes at one month after vaccination (Table 4 and Figures 2), while between 7.4% and 35.2% of cattle in the vaccinated group and 8.7% in the unvaccinated control group had protective levels of antibody titres at 4 MPV (Table 5).

In general, the results show that the vaccine induced production of antibodies in the majority of vaccinated animals within one month after initial vaccination with one single primary dose. However, only a small proportion of these animals had protective levels.

Table 4. Percentage of cattle showing antibody titre ≥1.6 to SAT 1, 2 and 3 foot-and-mouth disease virus after single primary vaccination with bivalent (SAT 1 & SAT 2) vaccine and control animals.

Group	Serotype	Time post-vaccination					
		0		1 MPV [1]		4 MPV	
		(n) [3]	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)
Vaccinated groups	SAT 1		0.0 (0;2.1)		57.4 (48.1;66.3)		2.1 (0.3;7.3)
	SAT 2	175	0.0 (0;2.1)	122	59.8 (50.6;68.6)	96	7.3 (2.9;14.4)
	SAT 3		0.0 (0;2.1)		22.9 (15.8;31.4)		0 (0;3.8)
	Combined [2]		0.0 (0;2.1)[a]		72.9 (64.2;80.6)[a]		8.3 (3.7;15.8)[a]
Control	SAT 1		0.0 (0;8.4)		0 (0;14.8)		0 (0;20.5)
	SAT 2	42	0.0 (0;8.4)	23	17.4 (4.9;38.8)	16	6.3 (0.2;30.2)
	SAT 3		0.0 (0;8.4)		4.3 (0;18.8)		0 (0;20.5)
	Combined [2]		0.0 (0:8.4)[a]		21.7 (7.5;43.7)[b]		6.3 (0.2;30.2)[b]

Within each time point, percentages with no superscripts in common differ significantly (*P* < 0.05).

[1] MPV = months post-vaccination

[2] Combined = Antibody titre ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested

Table 5. Percentage of cattle showing antibody titres ≥2.0 to SAT 1, 2 and 3 foot-and-mouth disease virus after single primary vaccination with bivalent (SAT 1 & SAT 2) vaccine and control animals.

Group	Serotype	Time post-vaccination					
		0		1 MPV [1]		4 MPV	
		(n) [3]	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)
Vaccinated groups	SAT 1		0.0 (0;2.1)		27.0 (19.4;35.8)		0.0 (0;3.8)
	SAT 2	175	0.0 (0;2.1)	122	35.2 (26.8;44.4)	96	4.2 (1.1;10.3)
	SAT 3		0.0 (0;2.1)		7.4 (3.4;15.5)		0.0 (0;3.8)
	Combined [2]		0.0 (0;2.1)[a]		43.4 (34.4;52.7)[a]		4.2 (1.1;10.3)[a]
Control	SAT 1		0.0 (0;8.4)		0.0 (0;14.8)		0.0 (0;20.6)
	SAT 2	42	0.0 (0;8.4)	23	8.7 (1.1;28.0)	16	6.3 (0.2;30.3)
	SAT 3		0.0 (0;8.4)		0.0 (0;14.8)		0.0 (0;20.6)
	Combined [2]		0.0 (0;8.4)[a]		8.7 (1.1;28.0)[b]		6.3 (0.2;30.3)[b]

Within each time point, percentages with no superscripts in common differ significantly (*P* < 0.05).

[1] MPV = months post-vaccination

[2] Combined = Antibody titre ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested
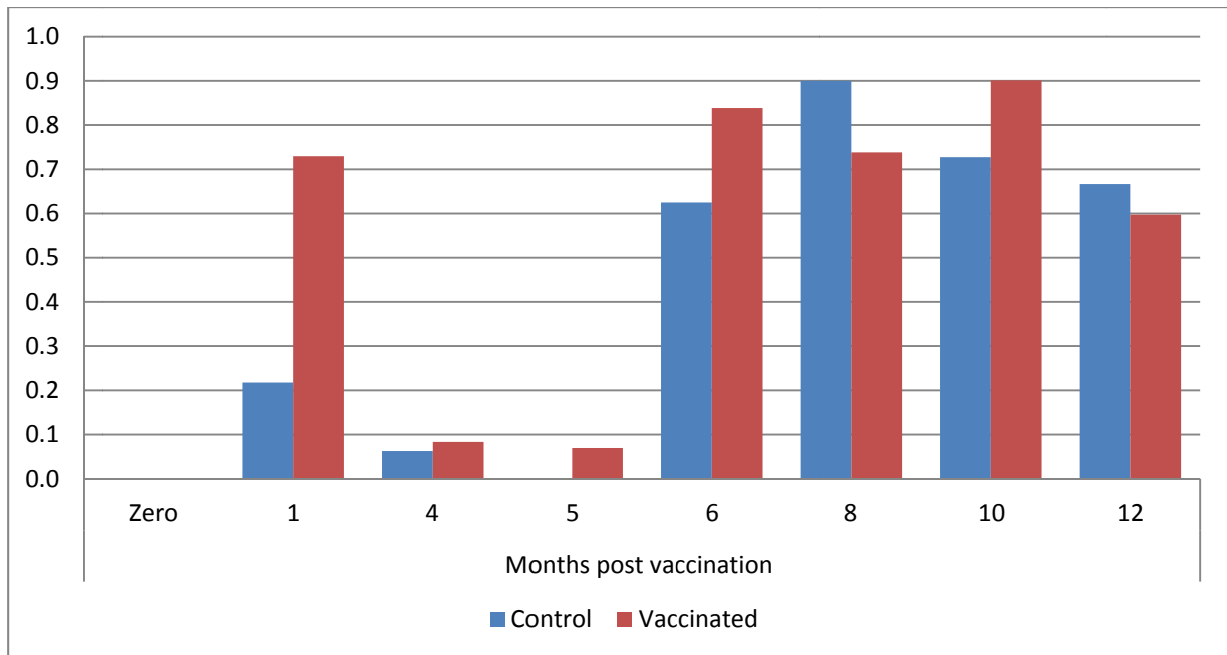
Figure 3. Proportion of cattle with antibody titres ≥1.6 against any of the SAT serotypes in the three vaccination groups combined and the unvaccinated controls for both study areas.
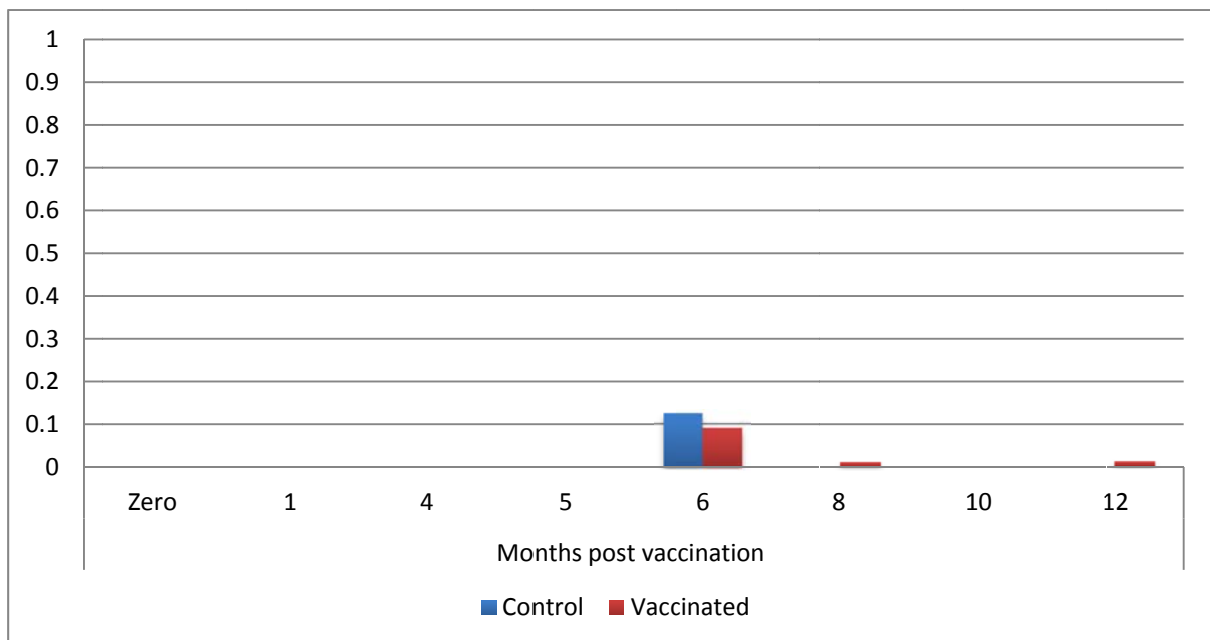
Figure 4. Proportion of cattle with antibodies to non-structural proteins of foot-and-mouth disease virus.

4.1. Results by serotype (vaccination groups combined)

The patterns of the seroconversion observed for each of the SAT serotypes were more less the same, varying in terms of the proportion of animals that showed antibody titres at each time point (Figures 5). In general, the proportions of vaccinated animals with antibody titres ≥1.6 to each of the SAT serotypes observed at one month after vaccination were between 22.9% and 59.8% and dropped to between zero and 7% at 4 MPV (Figure 5). In the unvaccinated control group the proportion of animals that had antibodies to each of the SAT serotypes varied between zero and 17.4% at 1 MPV and between zero and 6.3% at 4 MPV.

For individual SAT serotypes (Figures 5 and 7) the percentage of vaccinated animals (in both study areas combined) with antibody titres ≥1.6 to SAT 1 rose from zero at beginning of the study to 57.4% at one month after initial vaccination then dropped to 2.8% at 4 MPV. The proportion of vaccinated cattle that seroconverted to SAT 1 with antibody titres ≥2.0 was 27% at 1 MPV but none of them had protective levels at 4 MPV. For SAT 2, the percentage of cattle with antibody titres ≥1.6 at 1 MPV in the group of cattle vaccinated also rose to 59.9% and then dropped to 7.3% at 4 MPV, while protective titres were observed in 35.2% at 1 MPV and in 4.2% at 4 MPV. For SAT 3, the percentage of animals that had antibody titres ≥1.6 at 1 MPV was 22.9% and then this percentage dropped to zero at 4 months after initial vaccination, while protective levels of antibodies to SAT 3 were observed in a small proportion of vaccinated cattle, 7.4% at 1 MPV, but at 4 MPV no cattle were found with antibody titres ≥2.0 to SAT 3 in the vaccination group.

For cattle in the unvaccinated control group (Figures 6 and 8), 17.4% had antibody titres ≥1.6 to SAT 2 and 4.3% to SAT 3 at 1 MPV. The proportion dropped to 6.3% for SAT 2 and zero for SAT 3 at 4 MPV. The proportion of cattle in the control group with antibody titres ≥2.0 to SAT 2 rose to 8.7% at 1 MPV then dropped slightly to 6.3% at 4 MPV. For the other two serotypes (SAT 1 and 3) there were no cattle in the unvaccinated control group with antibody titres ≥2.0 at 1 and 4 MPV (Figure 8).

The serological response to SAT 1 and SAT 2 in the vaccinated group showed similar patterns, although the proportion that were seropositive (≥1.6) was greater for SAT 2 than for SAT 1 at 1, 4, 5, 8 and 10 MPV ($P < 0.001$). There were significant differences between SAT 1 and SAT 2 in the proportions with protective titres (≥2.0), with a higher proportion for SAT 2 than SAT 1 at 1, 4, 5, 8, 10 MPV and a higher proportion for SAT 1 than SAT 2 at 6 and 12 MPV.
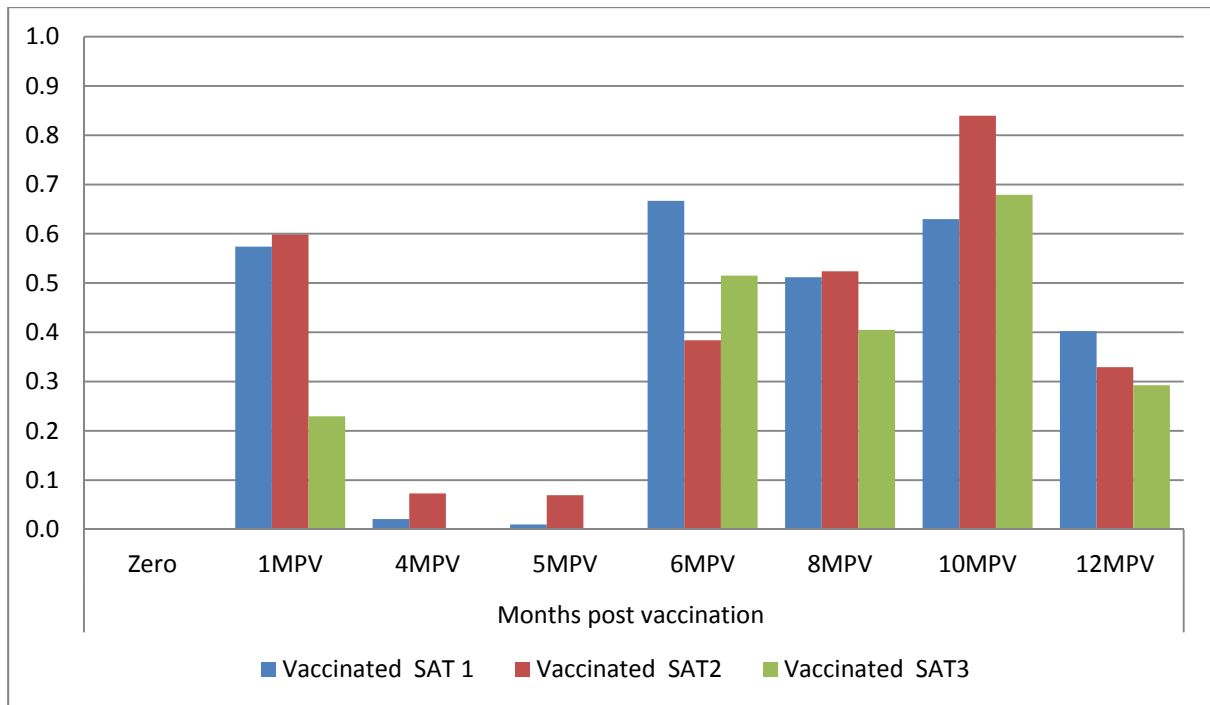
Figure 5. Proportion of combined vaccinated cattle with antibody titre ≥1.6 to SAT 1, 2 and 3.
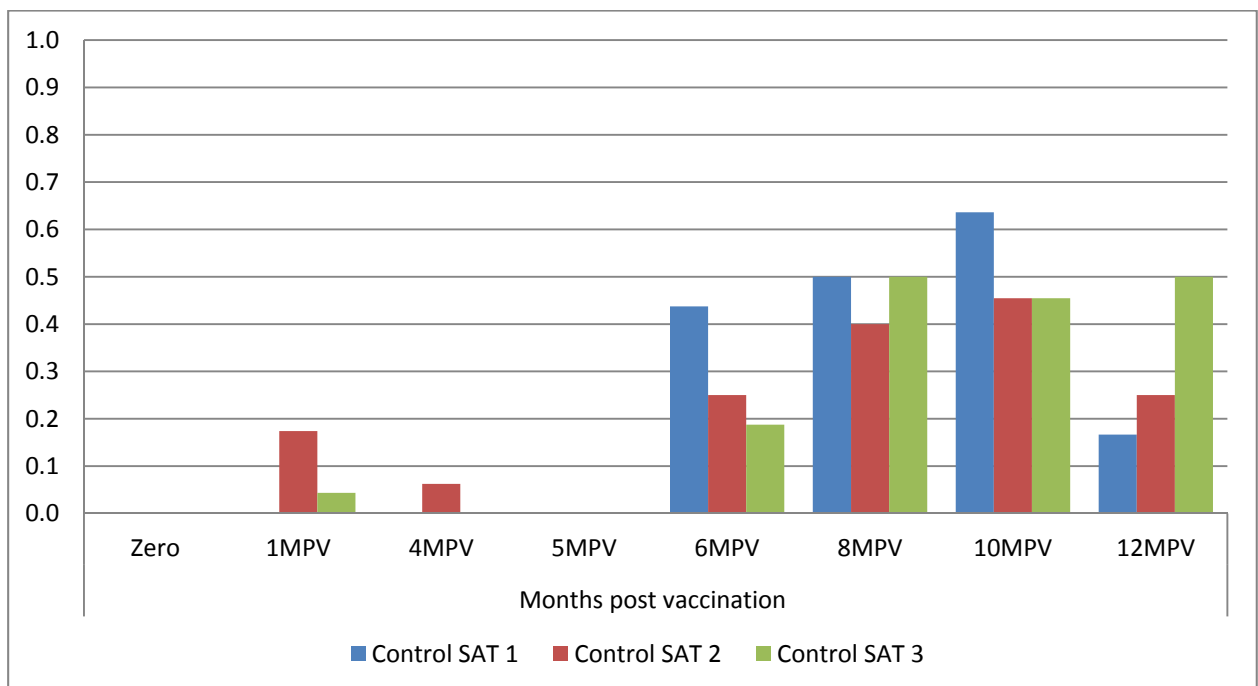
Figure 6. Proportion of unvaccinated control cattle with antibody titre ≥1.6 to SAT 1, 2 and 3.
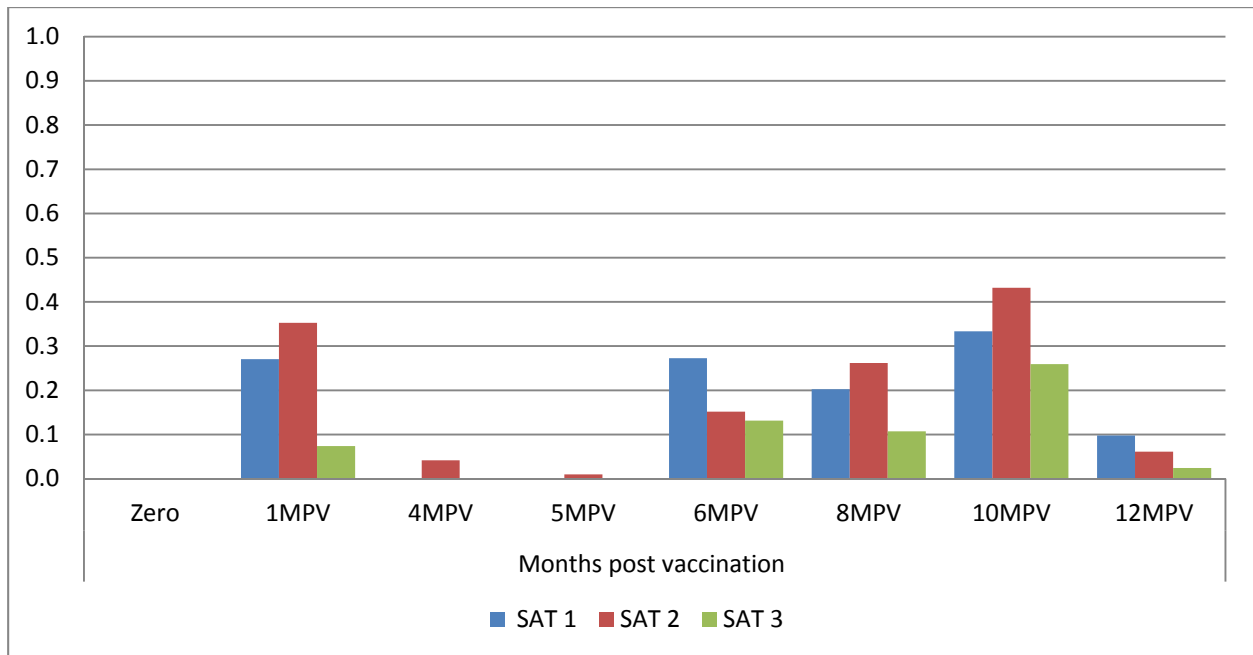
Figure 7. Proportion of combined vaccinated cattle with a protective level of antibody (titres ≥2.0) to SAT 1, 2 and 3.
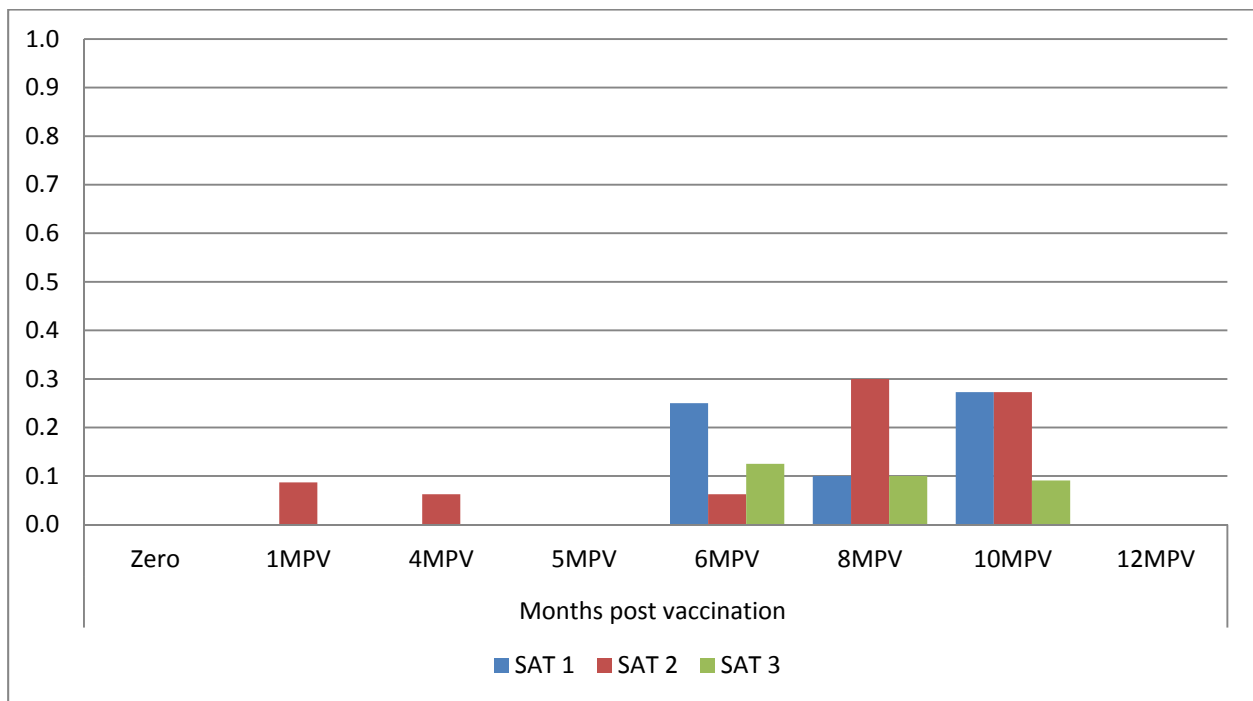
Figure 8. Proportion of unvaccinated control cattle with antibody titre ≥2.0 to SAT 1, 2 and 3.

4.2. Results by vaccination group

Between 63.3% and 81.8% of cattle in the three vaccination groups had antibody titres ≥1.6 at 1 MPV (Table 6). These proportions dropped to between 5.6% and 12% at 4 MPV. Cattle revaccinated at 4 months after initial vaccination showed a slight increase in the proportion of animals with antibody titres ≥1.6 at 5 MPV while the other two groups (vaccinated once and revaccinated at 6 months) showed proportions between zero and 4.3% at the same time point. The proportions in the three vaccination groups rose at 6 MPV to values between 80% and 90%, then dropped slightly at 8 MPV and again rose to 89% - 90% at 10 MPV and then started falling to proportions between 54% and 65% at 12 MPV (Table 6 and Figure 9).

In general, the proportion of animals with antibody titres ≥2.0 in each vaccination group showed a similar pattern to that observed for antibody titres ≥1.6 in each vaccination group, although the proportion of animals that had antibody titres ≥2.0 was relatively small (Table 7). The proportion of animals vaccinated in each group with a protective level of antibody (titres ≥2.0) at 1 MPV was between 37.5% and 48.5%, then dropped to between 2.9% and 5.6% at 4 MPV. At 5 MPV 4.3% of the cattle in the group revaccinated at 4 MPV were the only cattle that had antibody titres ≥2.0. From 6 to 10 MPV the proportion of animals with protective levels of antibody rose again and remained high in all the vaccinated groups, between 33.5% and 55.5% at 6 MPV, with a high proportion in the cattle vaccinated once, while at 8 MPV the proportion observed was between 34.8% and 42.5%. At 10 MPV between 50% and 63.6% of cattle in the vaccinated groups had antibody titres ≥2.0. This proportion fell to 10% - 24% at 12 MPV.

The serological responses to SAT 1 and SAT 2 showed similar patterns, although the proportion seropositive (≥1.6) was greater for SAT 1 than for SAT 2 at 6 MPV ($P < 0.001$) and greater for SAT 2 than for SAT 1 at 10 MPV ($P < 0.004$). There were no significant differences between SAT 1 and SAT 2 in the proportions with protective titres (≥2.0) in the three vaccination groups.

The proportion of animals with antibody titres ≥1.6 in the unvaccinated control group was 22% at 1 MPV and then dropped to 6% and zero respectively at 4 and 5 MPV. The proportion rose to 62.5% at 6 MPV and 90% at 8 MPV and then fell to 73% at 10 MPV and 67% at 12 MPV (Table 6). The proportion of animals that seroconverted and had antibody titres ≥2.0 was 8.7% at 1 MPV then dropped to 6% and zero respectively at 4 and 5 MPV, then rose to 31% at 6 MPV, after which the proportion steadily dropped to 30% at 8 MPV, 27% at 10 MPV and zero at 12 MPV.

The serological response to any of the SAT serotypes in the three vaccination groups showed no significant difference between groups, with the exception of time point 5 MPV, where the proportion of cattle revaccinated at 4 MPV that seroconverted was greater than in the other two groups ($P < 0.05$).

The NSP test results were negative from time zero to 5 MPV (Figure 10). Antibody titres to NSP were observed in cattle revaccinated at 4 and 6 MPV from 6 MPV onwards (at 6, 8 and 12 MPV). For cattle revaccinated at 4 MPV, 17% of the cattle revaccinated had titres to NSP antibodies at 6 MPV, dropping to 4.3% and 3.8% respectively at 8 and 12 MPV. Of the cattle revaccinated at 6 MPV, only 7% had antibody to NSP at 6 MPV. The NSP test was positive in the unvaccinated control group in 12.5% of the cattle at 6 MPV only.

Table 6. Percentage of cattle showing antibody titres ≥1.6 to SAT 1, 2 and 3 foot-and-mouth disease virus after vaccination with bivalent (SAT 1 & SAT 2) vaccine in animals vaccinated once, animals revaccinated at 4 or 6 months, and unvaccinated control animals.

Group	Serotype	0 (n)	0 % (95% C.I.)	1 MPV[1] (n)	1 MPV % (95% C.I.)	4 MPV (n)	4 MPV % (95% C.I.)	5 MPV (n)	5 MPV % (95% C.I.)	6 MPV (n)	6 MPV % (95% C.I.)	8 MPV (n)	8 MPV % (95% C.I.)	10 MPV (n)	10 MPV % (95% C.I.)	12 MPV (n)	12 MPV % (95% C.I.)
Vaccinated T0	SAT 1	73	0.0 (0;4.9)	40	62.5 (45.8;77.2)	25	4.0 (0.1;20.4)	31	0.0 (0;11.2)	9	66.7 (29.9;92.5)	21	42.8 (21.8;65.9)	22	63.6 (40.6;82.8)	21	28.6 (11.2;52.2)
	SAT 2		0.0 (0;4.9)		62.5 (45.8;77.2)		8.0 (0.9;26.0)		0.0 (0;11.2)		55.6 (21.2;86.3)		57.1 (34.0;78.2)		81.8 (59.7;94.8)		52.4 (29.7;74.3)
	SAT 3		0.0 (0;4.9)		32.5 (18.5;49.1)		0.0 (0;13.7)		0.0 (0;11.2)		55.6 (21.2;86.3)		47.6 (25.7;70.2)		68.2 (45.1;86.1)		42.8 (21.8;65.9)
	Combined		0.0 (0;4.9)[a]		77.5 (61.5;89.2)[a]		12.0 (2.5;31.2)[a]		0.0 (0;11.2)[a]		88.9 (51.7;99.7)[ab]		76.2 (52.8;91.7)[a]		90.9 (70.8;98.9)[a]		61.9 (38.4;81.9)[a]
Vaccinated T0+4	SAT 1	39	0.0 (0;9.0)	33	66.7 (48.2;82.0)	36	2.8 (0.1;14.5)	23	4.3 (0.1;21.9)	30	83.3 (65.3;94.4)	23	56.5 (34.5;76.8)	22	68.2 (45.1;86.1)	26	38.5 (20.2;59.4)
	SAT 2		0.0 (0;9.0)		60.6 (42.1;77.1)		5.6 (0.6;18.7)		21.7 (7.5;43.7)		40.0 (22.7;59.4)		26.1 (10.2;48.4)		86.4 (65.1;97.1)		34.6 (17.2;55.6)
	SAT 3		0.0 (0;9.0)		21.2 (8.9;38.9)		0.0 (0;9.7)		0.0 (0;14.8)		56.7 (37.4;74.5)		39.1 (19.7;61.4)		72.7 (49.7;89.2)		30.8 (14.3;51.7)
	Combined		0.0 (0;9.0)[a]		81.8 (64.5;93.0)[a]		5.6 (0.6;18.7)[a]		21.7 (7.5;43.7)[b]		90.0 (73.4;97.8)[b]		60.9 (38.5;80.3)[a]		90.9 (70.8;98.9)[a]		65.4 (44.3;82.7)[a]
Vaccinated T0+6	SAT 1	63	0.0 (0;5.6)	49	46.9 (32.5;61.7)	35	0.0 (0;10.0)	47	0.0 (0;7.5)	60	58.3 (44.8;70.9)	40	52.5 (36.1;68.5)	37	59.5 (42.1;75.2)	35	48.6 (31.3;66.0)
	SAT 2		0.0 (0;5.6)		57.1 (42.2;71.2)		8.6 (1.8;23.0)		4.3 (0.5;14.5)		35.0 (23.1;48.4)		65 (48.3;79.4)		83.7 (67.9;93.8)		20.0 (8.4;36.9)
	SAT 3		0.0 (0;5.6)		16.3 (7.3;29.6)		0.0 (0;10.0)		0.0 (0;7.5)		48.3 (35.2;61.6)		37.5 (22.7;54.2)		64.9 (47.5;79.8)		20.0 (8.4;36.9)
	Combined		0.0 (0;5.6)[a]		63.3 (48.3;76.6)[a]		8.6 (1.8;23.0)[a]		4.3 (0.5;14.5)[a]		80.0 (67.7;89.2)[ab]		80.0 (64.4;90.9)[a]		89.2 (75;96.9)[a]		54.3 (36.6;71.2)[a]
Control	SAT 1	42	0.0 (0;8.4)	23	0.0 (0;14.8)	16	0.0 (0;20.5)	22	0.0 (0;15.4)	16	43.7 (19.7;70.1)	10	50.0 (18.7;81.3)	11	63.6 (30.7;89.1)	12	16.7 (2.1;48.4)
	SAT 2		0.0 (0;8.4)		17.4 (4.9;38.8)		6.3 (0.2;30.2)		0.0 (0;15.4)		25.0 (7.3;52.4)		40.0 (12.2;73.8)		45.5 (16.7;76.6)		25.0 (5.5;57.2)
	SAT 3		0.0 (0;8.4)		4.3 (0.1;21.9)		0.0 (0;20.5)		0.0 (0;15.4)		18.7 (4.0;45.6)		50.0 (18.7;81.2)		45.5 (16.7;76.6)		50.0 (21.1;78.9)
	Combined		0.0 (0;8.4)[a]		21.7 (7.5;43.7)[b]		6.3 (0.2;30.2)[a]		0.0 (0;15.4)[a]		62.5 (35.4;84.8)[a]		90.0 (55.5;99.7)[a]		72.7 (39.0;93.9)[a]		66.7 (34.9;90.1)[a]

Within each time point, percentages with no superscripts in common differ significantly (*P* < 0.05).

[1] MPV = months post-vaccination

[2] Combined = antibody titre ≥1.6 $\log_{10}$ to any of the three serotypes

[3](n) = number of animals tested

Table 7. Percentage of cattle showing antibody titres ≥2.0 to SAT 1, 2 and 3 foot-and-mouth disease virus after vaccination with bivalent (SAT 1 & SAT 2) vaccine in animals vaccinated once, animals revaccinated at 4 or 6 months, and unvaccinated control animals.

Group	Serotype	Time post-vaccination															
		0		1 MPV[1]		4 MPV		5 MPV		6 MPV		8 MPV		10 MPV		12 MPV	
		(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)
Vaccinated T0	SAT 1	73	0.0 (0;4.9)	40	25.0 (12.7;41.2)	25	0.0 (0;13.7)	31	0.0 (0;11.2)	9	33.3 (7.5;70.1)	21	19 (5.4;41.9)	22	31.8 (13.8;54.9)	21	14.3 (3.0;36.3)
	SAT 2		0.0 (0;4.9)		30.0 (16.6;46.5)		4.0 (0.1;20.3)		0.0 (0;11.2)		11.1 (0.3;48.2)		23.8 (8.2;47.2)		40.9 (20.7;63.6)		4.8 (0.1;23.8)
	SAT 3		0.0 (0;4.9)		7.5 (15.7;20.4)		0.0 (0;13.7)		0.0 (0;11.2)		33.3 (7.5;70.1)		14.3 (3.0;36.3)		31.8 (13.8;54.9)		9.5 (1.2;30.4)
	Combined		0.0 (0;4.9)[a]		37.5 (22.7;54.2)[a]		4.0 (0.1;20.3)[a]		0.0 (0;11.2)[a]		55.6 (21.2;86.3)[a]		42.9 (21.8;66.9)[a]		63.6 (40.6;82.8)[a]		23.8 (8.2;47.2)[a]
Vaccinated T0+4	SAT 1	39	0.0 (0;9.0)	33	33.3 (17.9;51.8)	36	0.0 (0;9.7)	23	0.0 (0;14.8)	30	33.3 (17.3;52.8)	23	21.7 (7.5;43.7)	22	31.8 (13.8;54.9)	26	7.7 (0.9;25.1)
	SAT 2		0.0 (0;9.0)		36.4 (20.4;54.9)		5.6 (0.7;18.7)		4.3 (0.1;21.9)		20.0 (7.7;38.6)		13.0 (2.7;33.6)		45.5 (24.3;67.8)		11.5 (2.4;30.1)
	SAT 3		0.0 (0;9.0)		9.1 (19.2;24.3)		0.0 (0;9.7)		0.0 (0;14.8)		13.3 (3.7;30.7)		13.0 (2.7;33.6)		22.7 (7.8;45.4)		0.0 (0;13.2)
	Combined		0.0 (0;9.0)[a]		48.5 (30.8;66.5)[a]		5.6 (0.7;18.7)[a]		4.3 (0.1;21.9)[a]		53.3 (34.3;71.6)[a]		34.8 (16.4;57.3)[a]		50.0 (28.2;71.8)[a]		15.4 (4.3;34.9)[a]
Vaccinated T0+6	SAT 1	63	0.0 (0;5.7)	49	24.5 (13.3;38.8)	35	0.0 (0;10.0)	47	0.0 (0;7.5)	60	23.3 (13.4;36.0)	40	20.0 (9.1;35.6)	37	35.1 (20.2;52.5)	35	8.6 (1.8;23.1)
	SAT 2		0.0 (0;5.7)		38.8 (25.2;53.8)		2.8 (0.1;14.9)		0.0 (0;7.5)		13.3 (5.9;24.9)		35.0 (20.6;51.7)		43.2 (27.1;60.5)		2.8 (0.1;14.9)
	SAT 3		0.0 (0;5.7)		6.1 (1.3;16.9)		0.0 (0;10.0)		0.0 (0;7.5)		10.0 (3.7;20.5)		7.5 (1.5;20.4)		24.3 (11.7;41.2)		0.0 (0;10.0)
	Combined		0.0 (0;5.7)[a]		44.9 (30.7;59.8)[a]		2.9 (0.1;14.9)[a]		0.0 (0;7.5)[a]		33.3 (21.7;46.7)[a]		42.5 (27.0;59.1)[a]		56.8 (39.5;72.9)[a]		11.4 (3.2;26.7)[a]
Control	SAT 1	42	0.0 (0;8.4)	23	0.0 (0;14.8)	16	0.0 (0;20.6)	22	0.0 (0;15.4)	16	25.0 (7.3;52.4)	10	10.0 (0.3;44.5)	11	27.3 (6.0;60.9)	12	0.0 (0;26.5)
	SAT 2		0.0 (0;8.4)		8.7 (1.1;28.0)		6.3 (0.2;30.2)		0.0 (0;15.4)		6.3 (0.2;30.2)		30.0 (6.7;65.2)		27.3 (6.0;60.9)		0.0 (0;26.5)
	SAT 3		0.0 (0;8.4)		0.0 (0;14.8)		0.0 (0;20.6)		0.0 (0;15.4)		12.5 (1.5;38.3)		10.0 (0.3;44.5)		9.1 (0.2;41.3)		0.0 (0;26.5)
	Combined		0.0 (0;8.4)[a]		8.7 (1.1;28.0)[b]		6.3 (0.2;30.2)[a]		0.0 (0;15.4)[a]		31.3 (11.0;58.7)[a]		30.0 (6.6;65.2)[a]		27.3 (6.0;60.9)[a]		0.0 (0;26.5)[a]

Within each time point, percentages with no superscripts in common differ significantly (P < 0.05).

[1] MPV = months post-vaccination

[2] Combined = antibody titres ≥1.6 $\log_{10}$ to any of the three serotypes

[3](n) = number of animals tested

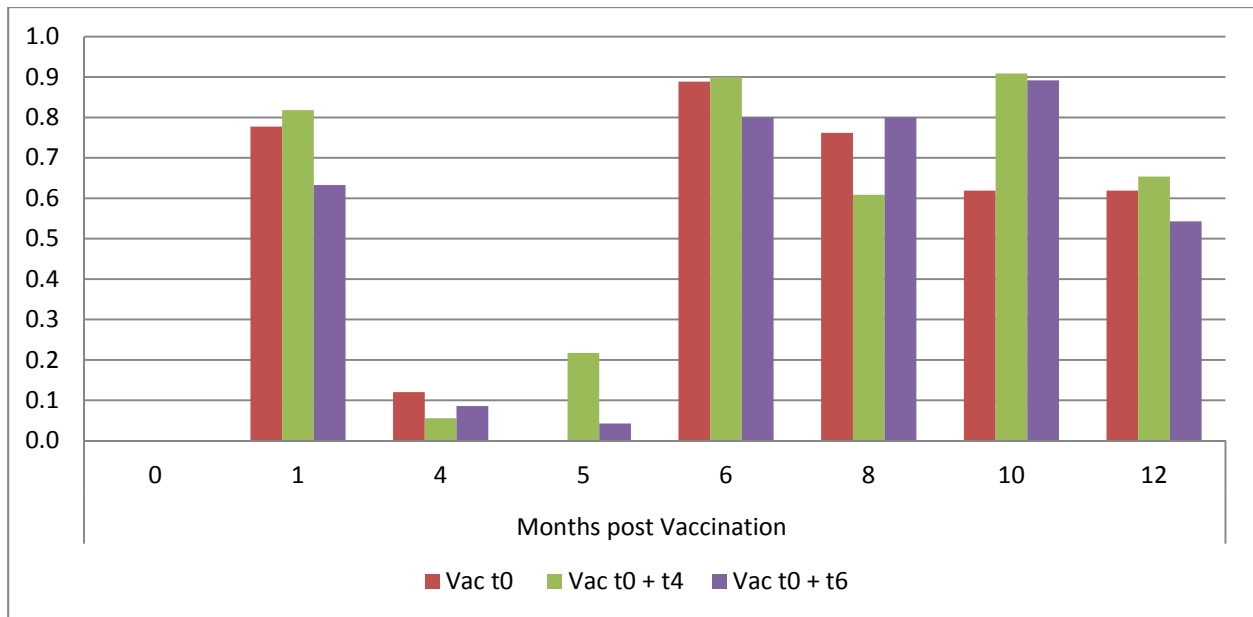Zacarias Elias Massicame. Dissertation for MSc (Veterinary Science)

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Figure 9. Proportion of cattle with antibody titres ≥1.6 for any of the SAT serotypes in the three vaccination groups.

Vac t0 = vaccinated once
Vac t0 + t4 = revaccinated at 4 MPV
Vac t0 + t6 = revaccinated at 6 MPV
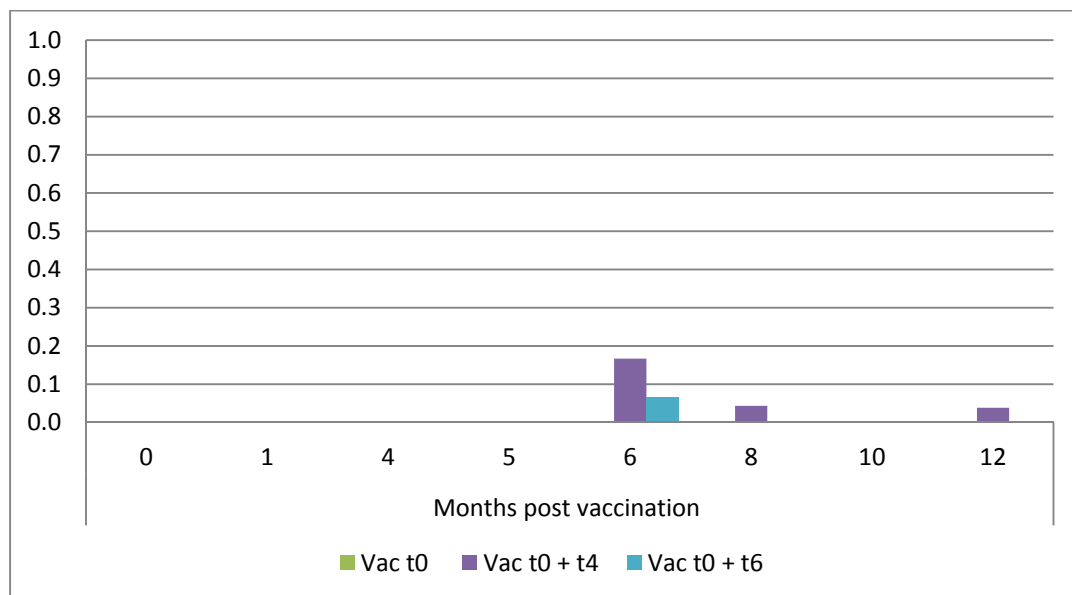
Figure 10. Proportion of vaccinated animals with antibodies to non-structural proteins for different vaccination groups.

4.3. Results by study area

The proportion of vaccinated animals that had antibody titres ≥1.6 to any SAT serotype in each study area at one month after initial vaccination was 82% in Massingir district and 50% in Mabalane district. These proportions dropped to 11% in Massingir and 8% in Mabalane district at 4 MPV (Table 8). At 5 MPV a low proportion of animals (1.7% in Massingir and 14% in Mabalane) had antibody titres ≥1.6. From 6 to 10 MPV the proportion of vaccinated animals with titres ≥1.6 to any of SAT serotypes was high (greater than 65%), then dropped to between 44% and 64% at 12 MPV in Mabalane and Massingir respectively. Protective levels of antibodies (titres ≥2.0) were found in 52% of cattle vaccinated in Massingir and 16% of cattle vaccinated in Mabalane at 1 MPV, then dropped at 4 and 5 MPV to 11% and 1.7% respectively in Massingir and 3% and zero in Mabalane. From 6 to 10 MPV the proportion of animals with antibody titres ≥2.0 rose and remained between 35% and 54% in Massingir and 32% and 58% in Mabalane, then dropped to 17% in Massingir and 13% in Mabalane at 12 MPV.

The serological response to SAT 1 and SAT 2 in each of the study areas in vaccinated groups when combined (Table 8) showed a similar pattern, although the proportion of cattle that were seropositive (≥1.6) was greater for Massingir than Mabalane at 1, 4, 6, 10 and 12 MPV ($P < 0.05$) and greater for Mabalane than Massingir at 5 and 8 MPV ($P < 0.05$). The proportion of animals with protective levels to SAT 1 and SAT 2 in each study area when the vaccinated groups were combined (Table 9) was greater for cattle from Massingir at 1, 4, 5, 6 and 12 MPV ($P < 0.05$) and greater for Mabalane than Massingir at 8 and 10 MPV ($P < 0.05$).

The proportion of cattle in the unvaccinated control group that had antibody titres ≥1.6 to any of the SAT serotypes (Table 10) at 1 MPV in Massingir was 31% and Mabalane 10%. These proportions dropped to zero in Massingir and to 10% and zero in Mabalane respectively at 4 MPV and 5 MPV, then rose to 78% in Mabalane and 43% in Mabalane at 6 MPV. From 6 MPV until the end of the study the proportion of animals with antibody titres ≥1.6 remained between 71% and 87% in Massingir, while in Mabalane the proportion rose from 53% to 100% at 8 MPV then dropped to 75% and zero at 10 and 12 MPV respectively. Antibody titres ≥2.0 (Table 11) were observed in 15% of the cattle in Massingir and zero percent in Mabalane at 1 MPV, then in 44% of cattle in Massingir and 14% in Mabalane at 6 MPV. Between 12% and 14% of animals in Massingir had antibodies to any of the SAT serotypes at 8 and 10 MPV, while in Mabalane 100% of the animals at 8 MPV and 85% at 10 MPV

respectively had protective titres to any of the SAT serotypes. At 12 MPV none of animals in either of the study areas in this group had protective levels of antibodies.

The serological response to SAT 1 and SAT 2 in the unvaccinated control group in the two study areas (Table 10) showed significant differences (titres ≥1.6), with a higher proportion of cattle from Massingir than cattle from Mabalane showing titres at one, 6, and 12 MPV ($P <$ 0.05) and a higher proportion in Mabalane than Massingir showing titres at 4, 8 and 10 MPV ($P < 0.05$).

Table 8. Percentage of cattle showing antibody titres ≥1.6 to SAT 1, 2 and 3 foot-and-mouth disease virus after vaccination with bivalent (SAT 1 & SAT 2) vaccine in the combined vaccination groups from LNP (Massingir) and outside LNP (Mabalane).

Group	Serotype		Time post-vaccination														
			0		1 MPV[1]		4 MPV		5 MPV		6 MPV		8 MPV		10 MPV		12 MPV
		(n)[3]	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)
Vaccinated Massingir	SAT 1		0.0 (0;3.6)		64.8 (53.8;74.7)		31.7 (0.4;11.0)		0.0 (0;6.3)		64.6 (51.8;76.1)		40 (28.0;52.9)		72.1 (59.2;82.8)		39.4 (27.6;52.2)
	SAT 2	99	0.0 (0;3.6)	88	70.5 (59.8;79.7)	63	9.5 (3.6;19.6)	57	1.7 (0;9.4)	65	38.2 (26.7;51.3)	65	41.5 (29.4;54.4)	61	81.9 (70.0;90.6)	66	40.9 (28.9;53.7)
	SAT 3		0.0 (0;3.6)		27.3 (18.3;37.8)		0.0 (0;5.7)		0.0 (0;6.3)		52.3 (39.5;64.8)		47.7 (35.1;60.4)		28.7 (53.9;78.7)		34.8 (23.5;47.6)
	Combined[2]		0.0 (0;3.6)a		81.8 (72.2;89.2)a		11.1 (4.6;21.6)a		1.7 (0;9.4)b		84.4 (73.5;92.4)a		67.7 (54.9;78.8)b		90.2 (79.8;96.3)a		63.6 (50.9;75.1)a
Vaccinated Mabalane	SAT 1		0.0 (0;4.7)		38.2 (22.2;56.4)		3.2 (0;10.6)		2.27 (0;12.0)		70.6 (52.5;84.9)		89.5 (66.8;98.7)		35 (15.4;59.2)		43.7 (19.7;70.1)
	SAT 2	76	0.0 (0;4.7)	34	32.4 (17.4;50.5)	33	3.0 (0.1;15.7)	44	13.6 (5.2;27.3)	34	38.4 (22.2;56.4)	19	89.5 (66.9;98.7)	20	90 (68.3;98.8)	16	0.0 (0;20.6)
	SAT 3		0.0 (0;4.7)		11.8 (3.3;27.4)		0.0 (0;15.7)		0.0 (0;8.0)		50.0 (32.4;67.6)		15.8 (3.4;39.6)		70 (45.7;88.1)		6.3 (0.1;30.2)
	Combined[2]		0.0 (0;4.7)a		50 (32.4;67.6)b		8.3 (0.1;15.7)b		13.6 (5.2;27.3)a		82.4 (65.5;93.2)b		94.7 (73.9;99.9)a		90 (68.3;98.8)b		43.7 (19.7;70.1)b

Within each time point, percentages with no superscripts in common differ significantly (*P* < 0.05).

[1] MPV = months post-vaccination

[2] Combined = antibody titre ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested

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Table 9. Percentage of cattle showing antibody titres ≥2.0 to SAT 1, 2 and 3 foot-and-mouth disease virus after vaccination with bivalent (SAT 1 & SAT 2) vaccine in the combined vaccinated groups from LNP (Massingir) and outside LNP (Mabalane).

Group	Serotype	0 (n)[3]	0 % (95% C.I.)	1 MPV[1] (n)	1 MPV % (95% C.I.)	4 MPV (n)	4 MPV % (95% C.I.)	5 MPV (n)	5 MPV % (95% C.I.)	6 MPV (n)	6 MPV % (95% C.I.)	8 MPV (n)	8 MPV % (95% C.I.)	10 MPV (n)	10 MPV % (95% C.I.)	12 MPV (n)	12 MPV % (95% C.I.)
Vaccinated Massingir	SAT 1	99	0.0 (0;3.6)	88	34.1 (24.3;44.9)	63	0.0 (0;5.7)	57	0.0 (0;6.3)	65	29.2 (18.6;41.8)	65	20 (11.1;31.8)	61	37.7 (25.6;51.0)	66	9.1 (3.4;18.7)
	SAT 2		0.0 (0;3.6)		43.2 (32.6;54.2)		4.7 (0.9;13.3)		1.8 (0;9.4)		13.8 (6.5;24.7)		16.9 (8.8;28.3)		39.3 (27.1;52.7)		7.6 (2.5;16.8)
	SAT 3		0.0 (0;3.6)		9.1 (4.0;17.1)		0.0 (0;5.7)		0.0 (0;6.3)		16.9 (8.8;28.3)		13.8 (6.5;24.7)		24.6 (14.5;37.3)		3.03 (0.4;10.5)
	Combined[2]		0.0 (0;3.6)a		52.3 (41.4;63.0)a		4.7 (0.9;13.3)a		1.8 (0;9.4)a		46.1 (33.7;58.9)a		35.4 (23.9;48.2)b		54.1 (40.8;66.9)b		16.7 (8.6;27.8)a
Vaccinated Mabalane	SAT 1	76	0.0 (0;4.7)	34	8.8 (1.8;23.7)	33	0.0 (0;10.6)	44	0.0 (0;8.00)	34	23.5 (10.7;41.2)	19	21.1 (6.1;45.6)	20	20.0 (5.7;43.7)	16	12.5 (1.6;38.3)
	SAT 2		0.0 (0;4.7)		14.7 (4.9;31.1)		3.0 (0.1;15.7)		0.0 (0;8.0)		17.6 (6.8;34.5)		57.9 (33.5;79.7)		55.0 (31.5;76.9)		0.0 (0;20.6)
	SAT 3		0.0 (0;4.7)		2.9 (0.1;15.3)		0.0 (0;10.6)		0.0 (0;8.0)		5.9 (0.7;19.7)		0.0 (0;17.6)		30.0 (11.9;54.3)		0.0 (0;20.6)
	Combined[2]		0.0 (0;4.7)a		15.9 (8.7;37.9)b		3.0 (0.1;15.7)b		0.0 (0;8.0)b		32.4 (17.4;50.5)b		57.9 (33.5;79.7)a		56.9 (40.8;84.6)a		12.5 (1.6;38.3)b

Time post-vaccination

Within each time point, percentages with no superscripts in common differ significantly (*P* < 0.05).

[1] MPV = months post-vaccination

[2] Combined = antibody titre ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested

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Table 10. Percentage of cattle showing antibody titres ≥1.6 to SAT 1, 2 and 3 foot-and-mouth disease virus in unvaccinated groups combined inside LNP (Massingir) and outside LNP (Mabalane).

Group	Serotype	Time post-vaccination															
		0		1 MPV[1]		4 MPV		5 MPV		6 MPV		8 MPV		10 MPV		12 MPV	
		(n)[3]	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)	(n)	% (95% C.I.)
Control	SAT 1	22	0.0 (0;15.4)	13	0.0 (0;24.7)	6	0.0 (0;45.9)	10	0.0 (0;30.8)	9	55.6 (21.2;86.3)	8	37.5 (8.5;75.5)	7	71.4 (29.0;96.3)	11	18.2 (2.3;51.8)
Massingir	SAT 2		0.0 (0;15.4)		30.8 (9.1;61.4)		0.0 (0;45.9)		0.0 (0;30.8)		11.1 (0.3;48.2)		25 (3.2;65.1)		28.6 (3.6;70.9)		27.3 (6.0;60.9)
	SAT 3		0.0 (0;15.4)		0.0 (0;24.7)		0.0 (0;45.9)		0.0 (0;30.8)		22.2 (2.8;60.0)		50 (15.7;84.2)		28.6 (3.6;70.9)		54.5 (23.4;83.3)
	Combined[2]		0.0 (0;15.4)[a]		30.8 (9.1;61.4)[a]		0.0 (0;45.9)[b]		0.0 (0;30.8)[a]		77.8 (39.9;97.2)[a]		87.5 (47.3;99.7)[b]		71.4 (29.0;96.3)[b]		72.7 (39.0;93.9)[a]
Control	SAT 1	20	0.0 (0;16.8)	10	0.0 (0;30.8)	10	0.0 (0;30.8)	12	0.0 (0;26.5)	7	28.6 (3.7;70.9)	2	100 (15.8;100)	4	50 (6.7;93.2)	1	0.0 (0;97.5)
Mabalane	SAT 2		0.0 (0;16.8)		0.0 (0;30.8)		10.0 (0.2;44.5)		0.0 (0;26.5)		42.8 (15.8;100)		100.0 (15.8;100)		75.0 (19.4;99.4)		0.0 (0;97.5)
	SAT 3		0.0 (0;16.8)		10.0 (0.2;44.5)		0.0 (0;30.8)		0.0 (0;26.5)		14.3 (1.3;98.7)		50 (1.3;98.7)		75.0 (19.4;99.4)		0.0 (0;97.5)
	Combined[2]		0.0 (0;16.8)[a]		10.0 (0.2;44.5)[b]		10.0 (0.2;44.5)[a]		0.0 (0;26.5)[a]		42.8 (15.8;100)[b]		100 (15.8;100)[a]		75.0 (19.4;99.4)[a]		0.0 (0;97.5)[b]

Within each time point, percentages with no superscripts in common differ significantly ($P < 0.05$).

[1] MPV = months post-vaccination

[2] Combined = antibody titres ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested

Table 11. Percentage of cattle showing antibody titre ≥2.0 to SAT 1, 2 and 3 foot-and-mouth disease virus in unvaccinated groups combined in LNP (Massingir) and outside LNP (Mabalane).

Group	Serotype	0 (n)[3]	0 % (95% C.I.)	1 MPV[1] (n)	1 MPV % (95% C.I.)	4 MPV (n)	4 MPV % (95% C.I.)	5 MPV (n)	5 MPV % (95% C.I.)	6 MPV (n)	6 MPV % (95% C.I.)	8 MPV (n)	8 MPV % (95% C.I.)	10 MPV (n)	10 MPV % (95% C.I.)	12 MPV (n)	12 MPV % (95% C.I.)
Control	SAT 1	22	0.0 (0;15.4)	13	0.0 (0;24.7)	6	0.0 (0;45.9)	10	0.0 (0;30.8)	9	33.3 (7.5;70.1)	8	0.0 (0;36.9)	7	14.3 (0.4;57.9)	11	0.0 (0;28.5)
Massingir	SAT 2		0.0 (0;15.4)		15.4 (1.9;45.4)		0.0 (0;45.9)		0.0 (0;30.8)		0.0 (0;33.6)		12.5 (0.3;52.6)		14.3 (0.4;57.9)		0.0 (0;28.5)
	SAT 3		0.0 (0;15.4)		0.0 (0;24.7)		0.0 (0;45.9)		0.0 (0;30.8)		11.1 (0.3;48.2)		0.0 (0;36.9)		0.0 (0;40.9)		0.0 (0;28.5)
	Combined[2]		0.0 (0;15.4)[a]		15.4 (1.9;45.4)[a]		0.0 (0;45.9)[b]		0.0 (0;30.8)[a]		44.4 (13.7;78.8)[a]		12.5 (0.3;52.6)[b]		14.3 (0.4;57.9)[b]		0.0 (0;28.5)[a]
Control	SAT 1	20	0.0 (0;16.8)	10	0.0 (0;30.8)	10	0.0 (0;30.8)	12	0.0 (0;26.5)	7	14.3 (0.4;57.9)	2	50 (1.2;98.7)	4	50 (6.7;93.2)	1	0.0 (0;97.5)
Mabalane	SAT 2		0.0 (0;16.8)		0.0 (0;30.8)		10.0 (0.2;44.5)		0.0 (0;26.5)		143 (0.4;57.9)		100.0 (15.8;100)		50.0 (6.7;93.2)		0.0 (0;97.5)
	SAT 3		0.0 (0;16.8)		0.0 (0;30.8)		0.0 (0;30.8)		0.0 (0;26.5)		14.3 (0.4;57.9)		50 (1.2;98.7)		25.0 (0.6;80.6)		0.0 (0;97.5)
	Combined[2]		0.0 (0;16.8)[a]		0.0 (0;30.8)[b]		10.0 (0.2;44.5)[a]		0.0 (0;26.5)[a]		14.3 (0.4;57.9)[b]		100 (15.8;100)[a]		50.0 (6.7;93.2)[a]		0.0 (0;97.5)[a]

Within each time point, percentages with no superscripts in common differ significantly ($P < 0.05$).

[1] MPV = months post-vaccination

[2] Combined = antibody titres ≥1.6 $\log_{10}$ to any of the three serotypes

[3] (n) = number of animals tested

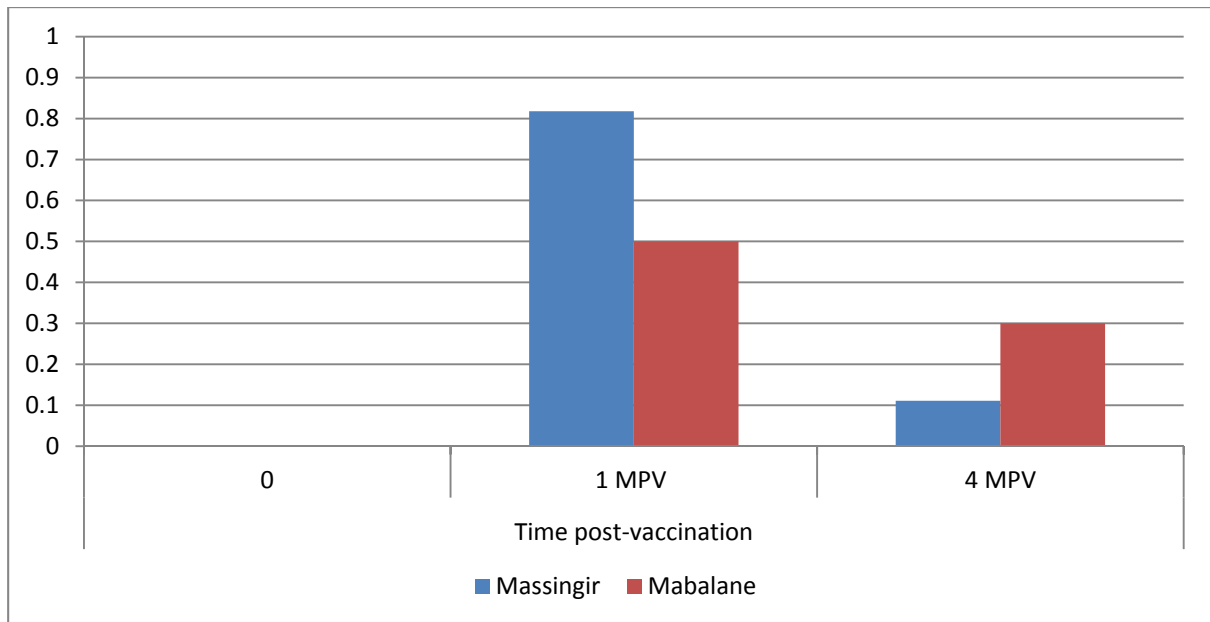Zacarias Elias Massicame. Dissertation for MSc (Veterinary Science)

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Figure 11. Proportion of vaccinated cattle with antibody titres ≥1.6 for any of the SAT serotypes in each study area for the first 4 months after initial vaccination.
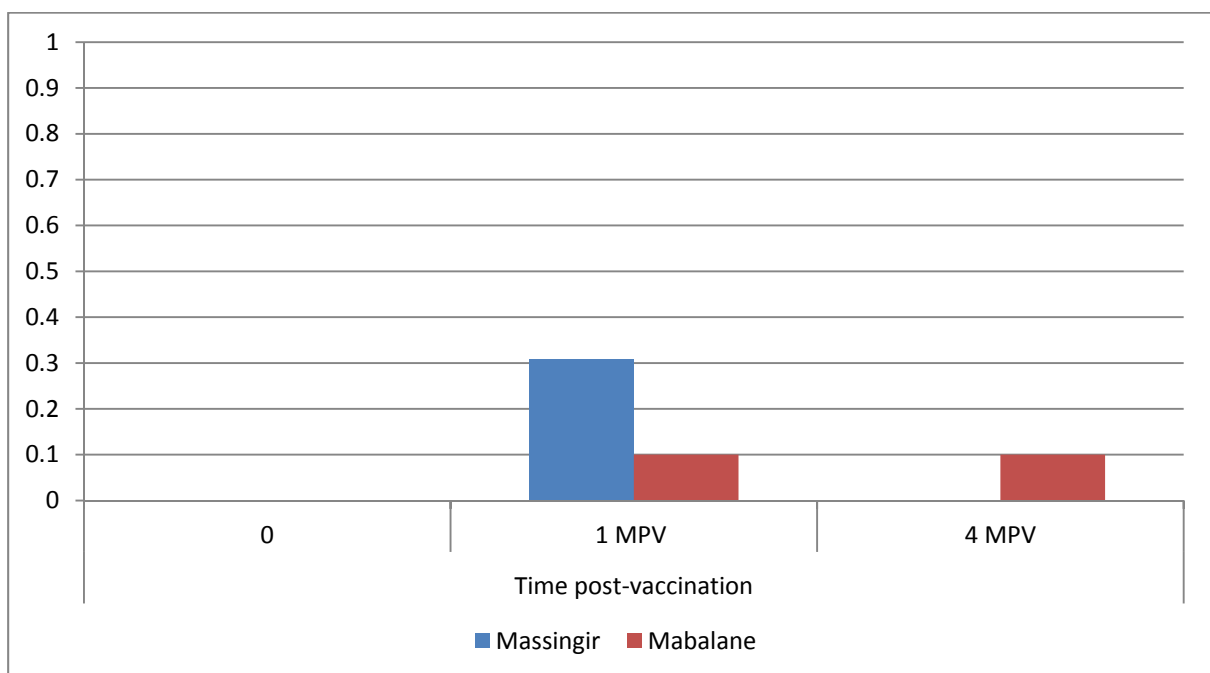

Figure 12. Proportion of unvaccinated control cattle with antibody titres ≥1.6 for any of the SAT serotypes combined in each study area for the first 4 months after initial vaccination.

5. DISCUSSION

The discussion focuses on two main issues: firstly the immunity induced by vaccination, i.e. the level of antibodies produced and the duration of this serological response, and secondly the efficacy of the vaccine in the different study areas, i.e. the ability of the vaccine to induce protective levels of antibody titres against the viral strains likely to be encountered in the study area. Both issues were considered important in assessment of the efficacy of the bivalent FMD vaccine (SAT 1 and 2) used in Mozambique to induce a protective level of antibodies against field challenge of SAT 1 and 2.

This study was based on the vaccination protocol adopted by the Mozambican Veterinary Authority i.e. a single primary vaccination followed by six-monthly revaccination. The results are discussed in the light of the intended objective of the vaccination programme in the country compared to results of other similar studies in the literature.

According to Smitsaart et al. (1998) and Roeder et al. (2007) percentages above 70% of animals with protective levels of antibody (titres ≥2) provide good herd immunity. The results obtained in this study were generally far below the recommended value (27% for SAT 1 and 35% for SAT 2 at 1 MPV) and immunity decreased significantly to zero percent for SAT 1 and 4.2% for SAT 2 at 4 months after initial vaccination.

5.1. Onset and duration of antibody response after primary vaccination

McCullough et al. (1992) described the relationship between neutralizing antibodies against FMDV as measured by diagnostic tests and the protection *in vivo* against FMDV infection and noted that the protection against FMD depends upon the humoral response although the T-cell response also seems to be important.

Foot-and-mouth disease vaccines elicit production of antibodies against FMDV, enabling the destruction of the virus by phagocytic cells. Serological assays are the techniques used to measure *in vitro* the level of the humoral immune response and are not capable of determining protection *in vivo* because they cannot predict the response of animals to challenge as other mechanisms of defence are involved (i.e. cellular). McCullough et al. (1992) observed a correlation between the titres observed in a serological assay with resistance of animals to infection. Our discussion and interpretation of the results of this study will be based on the assumption that the serological response observed indicates whether vaccinated animals are likely to be protected or not.

Cloete et al. (2008) described the ideal vaccines as being capable of stimulating a fast, potent and long-lasting immunity after a single vaccination. However, it is commonly understood that aqueous FMD vaccines are unable to provide immunity that lasts more than six months after vaccination. Thus, ruminants need to be re-vaccinated frequently (two to three times per year) to ensure maintenance of protective levels of neutralizing antibodies (Cox et al. 2003).

The results of our study indicate that the vaccine was able to induce antibody titres ≥1.6 against the two SAT serotypes after a single primary vaccination dose in 72.9% of cattle vaccinated. This was observed at one month after vaccination. These results are in agreement with Doel (2003), who reported that vaccinated animals respond quickly to the first dose and produce peak antibody titres between 14 and 28 days after a single vaccination and Cox et al (2003), who reported that the immune response of sheep after an initial dose elicits production of antibody at titres that can be observed as early as 7 days post vaccination and the antibody titres peak in most animals within 28 days after vaccination. In our study the animals were tested at one month after initial vaccination and the proportion of cattle that seroconverted was high.

However, the proportion of vaccinated animals with a protective level of antibodies (titres ≥2.0) to any of the SAT serotypes at one month after initial vaccination was low (43.4%) when compared to the recommended herd immunity of 70% or above according to Smitsaart et al. (1998) and Roeder et al. (2007). The low proportion of animals with protective levels of antibodies corresponds to the results obtained by Doel, (2003) who reported that in general an initial vaccination with aqueous vaccines does not induce a potent and durable immunity. Because of this, boosters are required to induce and maintain a high level of protection, as recommended by the vaccine manufacturer.

During the study period no outbreaks of FMD were reported in the country. In the study areas, no clinical cases of FMD were observed during the study period in the animals included in the study. The animals used for this study had no residual antibody titres at commencement of the study, therefore the low percentage of animals with protective levels of antibody titres observed suggests a primary reaction to the vaccine rather than an anamnestic effect due to previous vaccination or infection.

The serological response observed was weak in the majority of animals, with titres ≤2. The duration of the antibody titres elicited by the bivalent FMDV vaccine used was shown to be short (less than 4 months) in vast majority of animals. This observation corresponds to the

results obtained by Hunter (1996) and Cloete et al. (2008), who reported a quick fall in antibody titres induced by aqueous FMD vaccines within 2 and 4 months after vaccination. However, our findings are in contrast with previous findings of Nicholls et al. (1983), who reported that Nigerian strains of SAT serotypes of FMDV used as vaccine antigen were able to induce high titres of antibody against FMD and maintain them for more than 6 months. This was probably because the animals studied were exposed to infection with SAT 1, which left residual antibody for more than a year in some animals.

In our study the reagents used in the ELISA were heterologous to the vaccine strains, since for commercial reasons it was not possible to use the homologous strains. This may have affected the titres to some extent and resulted in slightly lower proportions of animals testing seropositive than would have been the case if homologous reagents had been used.

The seropositivity to NSPs observed in this study was at a low level and is unlikely to indicate natural infection, but rather the fact that the vaccine used was not completely purified and therefore likely contained traces of NSP.

5.2. Antibody titres in the control group

Foot-and-mouth disease virus is highly contagious and causes high morbidity (approximately 100%) in susceptible populations (Thomson 1994) and low morbidity (0.2%) in indigenous cattle in southern Africa (Baipoledi et al., 2004). Cattle in the study areas are at permanent risk of infection with SAT serotypes due to carrier buffaloes, and for that reason sera from unvaccinated cattle (control Group) were tested to monitor natural infection. Sobrino et al. (2001) and Lombard et al. (2007) indicated that natural infection with FMDV induces the production of high levels of antibody at titres ≥2.2 that last longer (between 1 and 3 years) after exposure.

In this study we observed that 22% of animals from the unvaccinated control group had antibody titres ≥1.6 at 1 MPV while 8.7% of cattle in the same group had antibody titres ≥2.0. The animals with antibody titres ≥2.0 were from different study sites within LNP (Massingir). The serological response in the unvaccinated control group show significant differences from that in the vaccinated cattle, with a higher proportion of the cattle that were vaccinated showing a serological response at one 4, 5, 6 and 10 MPV and a higher proportion of the unvaccinated control group showing a response at 8 and 12 MPV. Animals in the unvaccinated group with antibody titres against NSP were observed only at 6 MPV in 13% of the animals (2 cattle); these cattle were from two study sites in Massingir district (one from

Chimange and another from Bingo). Taking into consideration the fact that none of the animals had antibody titres to any SAT viruses on LPBE at time zero and were seronegative on the NSP test with the exception of the 6 MPV sampling, this suggests that the seropositive cases observed in the unvaccinated control group might have been caused by unintended vaccination of these animals and not by natural infection.

5.3. Differences in serological responses between vaccinated groups

The results indicated that in cattle revaccinated either at 4 or at 6 MPV the revaccination improved the immune response by increasing the proportion of animals showing antibody titres ≥1.6 from 81.8% at 1 MPV to 90.0% at 6 MPV in cattle revaccinated at 4 MPV. This proportion remained high for approximately 8 months. When cattle were revaccinated at 6 MPV the proportion was increased from 63.2% at 1 MPV to 80.0% at 8 MPV. Our findings are in agreement with the findings of Nicholls et al. (1983) and Cloete et al. (2008) who reported that revaccination of animals improves the immune response. Nicholls et al. (1983) investigated a suitable vaccination regimen as one of the factors affecting the efficacy of FMD vaccine. Cattle were grouped in three vaccination schedules: cattle vaccinated only once at time zero, another group vaccinated at time zero and 21 days and then revaccinated at 4 months and finally the last group, vaccinated at time zero and revaccinated at 4 months after primary inoculation. The findings demonstrated that all the vaccination regimens induced an immune response in the majority of vaccinated animals shortly after vaccination (21 days) but the average titres fell to non-protective levels (between 1.32 and 1.77 $\log_{10}$) by 4 months after the first application.

We also found that the proportion of revaccinated animals with protective levels of antibody titres to any of the SAT serotype was somewhat improved compared with the proportion of animals with antibody titres at one month after initial vaccination with a single primary dose. The proportion of animals revaccinated at 4 MPV with antibody titres ≥2.0 at one month after initial vaccination was 48% while the proportion at 6 and 8 MPV (2 and 4 months after revaccination) was 53% and 35% respectively. The proportion of animals revaccinated at 6 MPV with antibody titres ≥2.0 at 1 MPV was 45% while the proportion at 8 and 10 MPV was 43% and 56% respectively.

The vaccine manufacturer recommends that for the FMD vaccine to provide adequate protection it should be applied as follows: the first dose should be applied in a course of two applications separated by an interval of 4 weeks between the first application and the

second, followed by booster every 4-6 months to maintain the levels of immunity. Nicholls et al. (1983) recommend the same schedule for the Nigerian vaccine.

The results in the group of cattle revaccinated at 6 MPV show that a considerable proportion (80%) of animals were seropositive at 6 months after initial vaccination, the time at which these animals were revaccinated. Although the proportion of seropositive animals revaccinated at 6 MPV was low at 5 MPV, a month after the 4 MPV revaccination, as also observed for cattle revaccinated at 4 MPV, the probable reason for the high proportion of seropositive cattle at 6MPV in the group revaccinated at 6 MPV might have been an accidental revaccination of some of these animals at 4 MPV. Similarly, an unexpectedly high proportion of animals that were supposedly only vaccinated once (Vacc t0 group) were seropositive at 6 MPV, suggesting that many of them were also accidentally revaccinated. The poor handling facilities used were unable to contain the animals properly and some escaped and had to be returned to the crush several times, possibly resulting in incorrect identification of some animals and/or samples. This problem affected the ability to carry out the work correctly.

5.4. Differences between serological reactions to the various serotypes

Foot-and-mouth disease virus has high rate of genetic diversity as a result of frequent mutations (Saíz et al., 2002; Doel, 2003; Vosloo et al., 2005). This variation is reflected in the immunogenicity of different strains and in the immune response induced by FMDV (Shael et al., 2007). Patil et al. (2002) reported that some strains are more immunogenic than others and because of that different strains tend to induce production of variable levels of antibody titres. Nicholls et al. (1983) reported that the SAT 1 and 2 serotypes of FMDV isolated in Nigeria induced a satisfactory immune response; however, the level of immunity induced by each serotype differed in each vaccination protocol.

Our study results show that there were differences in the proportions of animals seroconverting to SAT 1 when compared to SAT 2, with the serological response to SAT 1 somewhat more prolonged than that to SAT 2 after initial vaccination. The seroconversion to SAT1 after revaccination was also more rapid than to SAT 2. These findings correspond to those obtained by Patil et al. (2002), who found differences in immunogenicity between serotypes.

It was observed that in revaccinated groups (revaccinated either at 4 or 6 MPV) the percentage of cattle that seroconverted to each of the SAT serotypes was higher than the

percentage of cattle that had seroconverted at one month after initial vaccination. Nicholls et al. (1983) found that revaccination of animals at 120 and 180 days after primary vaccination improved the immunity response. Cox et al. (2003) reported that booster and revaccination are used to increase and maintain antibody titres at protective levels for long periods of time. However, while revaccination induced an increase in the proportion of animals with protective levels to any of the serotypes the proportion for each serotype did not increase significantly over that obtained at 1 MPV.

5.5. Difference between study areas

Generally, there was no difference in immune responses between cattle vaccinated in LNP and outside LNP that could be related to proximity or distance from wildlife. None of the cattle included in the study had antibodies to any of the SAT serotypes at the beginning of the study. This may demonstrate that cattle in LNP were not exposed to FMDV despite living close to carrier species (buffalo) and susceptible wildlife species, particularly impalas.

In both areas the livestock rearing system is free range, where cattle are corralled at night only during the crop season, November to March, and graze freely for the rest of the time. This period is a dry season and grass and water are scarce, thus domestic animals and wildlife are likely to share water points, which might result in disease transmission, spreading infection between wildlife and domestic animals (Bengis et al., 1986). However, this does not appear to have occurred during our study period.

In both study areas the vaccine demonstrated the ability to induce immune responses in a high percentage of animals after vaccination. No difference was observed between the study areas in the percentage of seroconverted cattle when the results obtained for each serotype were compared using same vaccination protocol or regimen. However, there was a statistically significant difference between the two study areas in the proportion of animals that seroconverted to any of the serotypes at 1 MPV, that favoured cattle from inside LNP (Massingir). The reason for this may have been related to poor maintenance of the cold chain for conservation of vaccines in the areas outside LNP during the first vaccination. This was because the vaccine for these study areas was supplied by the provincial veterinary officers, since the stock from the Headquarters was finished by the time we moved from Massingir to Mabalane. It is likely that a problem in maintaining the cold chain occurred during the transportation of the vaccine from Maputo to Xai-Xai and then a week later to the field, resulting in a lower proportion of animals seroconverting in that area.

5.6. Cross reaction between serotypes

Sera were tested for antibodies to SAT 3 despite the fact that a SAT 1 and SAT 2 bivalent vaccine was being used. Wildlife in the LNP can carry SAT 3 viruses and potentially infect livestock. However, since SAT 3 has never been isolated in Mozambique, the government services do not perceive that as a risk. Surprisingly, we found significant proportions of animals with antibodies to SAT 3.

Foot-and-mouth disease virus is classified into seven serotypes on the basis of its ability to induce cross-protection against reference sera (Sobrino et al. 2001; Sáiz et al. 2002). Due to the antigen being closely related amongst the SAT serotypes, serological tests containing viruses of different serotypes tend to induce variable levels of cross protection reactions amongst FMDV serotypes (McCullough et al., 1992; Sobrino et al. 2001).

Cottral (1972) assessed the level of cross protection reactions of different viruses of each serotype of FMDV using the virus neutralization test and reported that within the SAT serotypes the SAT 3 viruses showed a high cross protection reaction rate with all the serotypes, particularly between SAT 3 and SAT 1, whereas viruses of the SAT 2 serotype showed lower cross reactivity.

Laboratory observation at OVI indicates that cross reactions between viruses of the SAT serotypes is a common finding (L. Heath, 2011, personal communication). Data from the 2010/2011 outbreak of SAT 1 in KwaZulu-Natal province in the Republic of South Africa in unvaccinated cattle showed a high proportion (77,6%) of animals that were SAT 1 positive were also positive to SAT 3, whereas the proportion of SAT 2 positive animals that were also positive to SAT 3 was low. The same findings were obtained with the same samples tested at World Reference Centre for FMD, Institute of Animal Health in Pirbright (WRL). These findings correspond to those reported by Cottral (1972).

Our results show that the average proportion of SAT 1 positive animals that were also positive to SAT 3 was 49.9%, while the average proportion of SAT 2 positive animals that were also SAT 3 positive was 54.8%. The presence of cross reactions amongst SAT serotypes may explain in particular the serological response observed in the test for SAT 3, since it was not incorporated in the vaccine formulation. Infection by a wild SAT 3 virus seems very unlikely due to a very low prevalence of NSP titres.

5.7. Limitations of the study

The study was affected by different factors mostly associated with its duration. This contributed to high attrition of study subjects as monitored across the study period. Most cattle owners had difficulties in bringing their cattle to the crush pen on the indicated dates. This was associated with the rearing system practised in the region (cattle grazing freely, especially during dry season, May to November).

The total number of animals participating in the study was gradually reduced during the study period. In addition, factors such as loss of ear tags led to deficient identification during revaccination and poor handling facilities might also have contributed to making the results of this study less reliable from 4 months after the first vaccinations.

The required minimum sample size to meet the selection criteria was unavailable in one village in each study area. This led to inclusion of other villages in the same study area to attempt to obtain the necessary sample size. Despite the inclusion of additional villages we failed to obtain all the animals required for the study. Because of this, a strictly random method for selection of animals was not able to be applied.

Deficient communication is a common problem affecting rural villages in the study area. It was difficult to change sampling dates and communicate with the farmers while in the field. The duration of sampling in each sampling village was limited to planned days to avoid compromising other sampling points.

Seropositive results to SAT 3 were also a problem that affected the study because it was difficult to interpret them.

There appeared to have been a cold chain problem in Mabalane with the initial vaccination, reducing the proportion of animals which seroconverted.

Finally, the fact that heterologous reagents had to be used in the ELISAs may have reduced the number of animals testing seropositive.

6. CONCLUSIONS

The research findings indicate that, although the vaccine is able to induce production of antibodies against SAT 1 and SAT 2 FMDV in a high proportion of cattle within one month after a single primary vaccination, these antibodies are short lived and appear to have largely disappeared by 4 months post vaccination. This suggests that a protocol of a single primary vaccination is inadequate in naïve animals, even if revaccination takes place four months later.

Revaccination improved the immune response for a longer period, resulting in detectible titres in the majority of animals for 6-8 months after revaccination. This can be used in disease control programmes to ensure some protection of cattle, particularly when it is applied immediately before identified high risk periods of occurrence of FMD outbreaks. However, it seems unlikely that six-monthly revaccination is sufficient to maintain adequate levels of protective immunity.

Proximity of the susceptible livestock to wildlife did not appear to influence the immune response in cattle within LNP, suggesting that transmission of FMDV from buffalo to cattle may not necessarily occur very frequently. Indeed, buffalo populations in Limpopo National Park remain fairly low at this stage; however, buffalo and cattle are reported to come into contact with each other at watering points during the dry season.

6.1. Recommendations

Controlling FMD using the present vaccination protocol is a challenge for the Mozambican Veterinary Authority. Veterinary services need to ensure the minimum level of herd immunity required to protect the animals, particularly during an FMD outbreak. Thus the country should regularly conduct pre- and post-vaccination monitoring. Monitoring the vaccination programme to ensure that a high proportion of the target population is vaccinated, ensuring that revaccinations are regularly carried out, and assessment of the level of herd immunity should be priorities.

6.2. Future areas of study

This study has not explicitly assessed the ability of the vaccine to provide protection for vaccinated cattle against circulating viruses. Therefore, further study should be considered

to gather more information related to the influence of vaccination and vaccination regimens on the level and duration of antibodies induced. Additionally, further studies should consider gathering information related to the ability of the current FMD vaccines to provide protection against the majority of the SAT 1 and SAT 2 FMDV circulating in the country.

Knowledge of how the disease is transmitted needs to be improved and factors leading to infection being transmitted from wildlife to livestock need to be studied, particularly in LNP, where livestock susceptible to wildlife diseases are kept close to wildlife, sharing watering points and grazing areas.

It is suggested that a study should assess the pattern of the wildlife and livestock interface in LNP to determine the epidemiological factors that contribute to preventing transmission of FMDV from wildlife to cattle in this area. This might lead to adoption of effective and efficient control strategies in FMD high risk areas.

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