

A BOVINE SEROLOGICAL SURVEY OF FOOT-AND-MOUTH DISEASE IN THE
NORTHERN COMMUNAL AREA OF NAMIBIA

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL WORD
AHT	ANIMAL HEALTH TECHNICIAN
CBPP	CONTAGIOUS BOVINE PLEUROPNEUMONIA
C-ELISA	COMPETITIVE ELISA
CFT	COMPLIMENT FIXATION TEST
CPE	CYTOPATHIC EFFECT IN CELL CULTURE
DVS	DIRECTORATE OF VETERINARY SERVICES
FMD	FOOT AND MOUTH DISEASE VIRUS
FPA	FLUORESCENCE POLARISATION ASSAY
I-ELISA	INDIRECT ELISA
IU	INFECTIOUS UNITS
LPS	LIPOPOLYSACCHARID
M	POPULATION SIZE
MAB	MONOCLONAL ANTIBODY
MRT	MILK RING TEST
N	SAMPLE SIZE
N\$	NAMIBIAN DOLLARS
NSP-ELISA	
°C	DEGREE CELSIUS

OIE	WORLD ORGANIZATION FOR ANIMAL HEALTH
PCR	POLYMERASE CHAIN REACTION
RNA	RIBONUCLEIC ACID
SADC	SOUTHERN AFRICA DEVELOPMENT COMMUNITY

CHAPTER 1

SUMMARY

Foot-and-Mouth Disease (FMD) is a disease of cloven-hoofed animals well-known not only for significant production losses but also restricted market access for livestock and livestock products from affected areas. It severely limits market opportunities for poor farmers and nations wishing to access more lucrative markets, both regionally and internationally. In the Northern Communal Area (NCA) of Namibia, FMD has not been detected in Kunene, Omusati, Oshana, Oshikoto and Ohangwena regions for the past 45 years or more, while outbreaks occurred in Eastern Kavango towards the end of 2008 as well as beginning of 2009 and there have been frequent outbreaks in the Zambezi region. Although, FMD has not been detected in five of the six regions of the Namibian NCA, no studies have been carried out to substantiate the FMD status in this highly potential livestock production area as required by the World Organisation for Animal Health (OIE).

It is based on this fact that a study was carried out in the above mentioned regions to determine whether there is Foot-and-Mouth Disease Virus (FMDV) circulating in the cattle population of the NCA Namibia. A set of 4214 serum samples were taken from non-vaccinated animals against FMD in Area A, which included Kunene north, Omusati south, Oshikoto(NCA) and Oshana. Meanwhile, 5228 serum samples were taken from Area B, which consisted of Omusati north, Ohangwena and Kavango region where cattle had been vaccinated against FMD for the past 15 years or more. Samples were primarily screened for FMDV using the Ceditest® NSP ELISA and positive samples were subsequently tested with the Viral Neutralization Test (VNT). Herds containing animals that were positive on both tests were followed up and retested with the Ceditest® NSP ELISA and examined clinically for FMD signs. The initial results of the Ceditest® NSP ELISA in Area A and B revealed an apparent prevalence of 0.24% and 0.27% with corresponding 95% confidence intervals between 0.13, 0.44 and 0.16, 0.45 respectively. The follow up results found no clinical evidence of FMD and all subsequent Ceditest® NSP ELISA results were negative. The results indicate that the initial Ceditest® NSP ELISA positive cattle were false positive. Thus the overall

outcome indicates that there is little chance of FMD virus circulation in the NCA Namibia. The survey also highlighted the need for taking into account sociological and cultural practices when designing a survey of communal cattle in order to get a representative sample of the cattle population.

The study also recommended that Directorates of Veterinary Services (DVS) Namibia should maintain and continuously review FMD surveillance activities in the NCA, especially along the Angola – Namibia border and continuously harmonise these activities with Angolan counterparts.

A more comprehensive study should be carried out to address the insufficient knowledge on sociological factors, which may influence sampling strategies in communal farming areas.

CHAPTER 2

2. INTRODUCTION

Namibia is situated in south western Africa and shares borders with Angola in the north, Zambia in the north-east, Botswana in the east, South Africa in the south and the Atlantic Ocean coast on the western side (Figure 1). Namibia is a vast country with a land mass of 824,116 square kilometers and a total human population of about 2.1 million (NPC 2001).



Figure 1: Namibian map showing the national borders

The livestock sector of Namibia accounts for about 90% of the overall agricultural production, thereby constituting the backbone of the agricultural sector. The country exports over 80% of its livestock, and livestock products to South Africa, Scandinavian countries and the European Union. The livestock industry currently generates about 800 million Namibian dollars per annum. The commercial sector contributes approximately 81% of agricultural production, and the Northern Communal Area only contributes 19% of the agricultural output, despite 60% of the cattle population being in this area. Apart from the cash value of the livestock industry, Namibian agriculture is the largest employer and supports, directly or indirectly, 70% of the population (IFAD 1997). Animal products, live animals, and crop exports constitute roughly 5% of total Namibian exports.

Agriculture is practiced on 700,000 km² of land that can be divided into two main distinct sectors; capital intensive and subsistence-based. The first is characterized by a relatively well developed commercial sector and is export oriented to attain a high profit margin. The second is more labour intensive, uses lower technology and has a low profit margin. The NCA agricultural practices are mixtures of transhumance and sedentary agro pastoral systems. However, the transhumance system of farming is generally collapsing because of the increasing human and livestock population density. For instance, the cattle population has been growing rapidly in communal areas north of the veterinary cordon fence (VCF) from about 620, 000 in 1990 to 1.03 million in 2005 as per Annual report 2005 of the Directorates of Veterinary Services (Figure 2). The small stock population has remained steady at around 1 million, as this species can be traded throughout the country and internationally, provided the movement requirements from the north to south of the VCF are met. There is also popular demand for small stock for traditional events and during festive seasons, further impacting on their numbers. The dramatic increase in cattle population in the NCA of Namibia is an outcome of improved institutional services delivery such as veterinary and extension services and the low off-take to the formal markets.

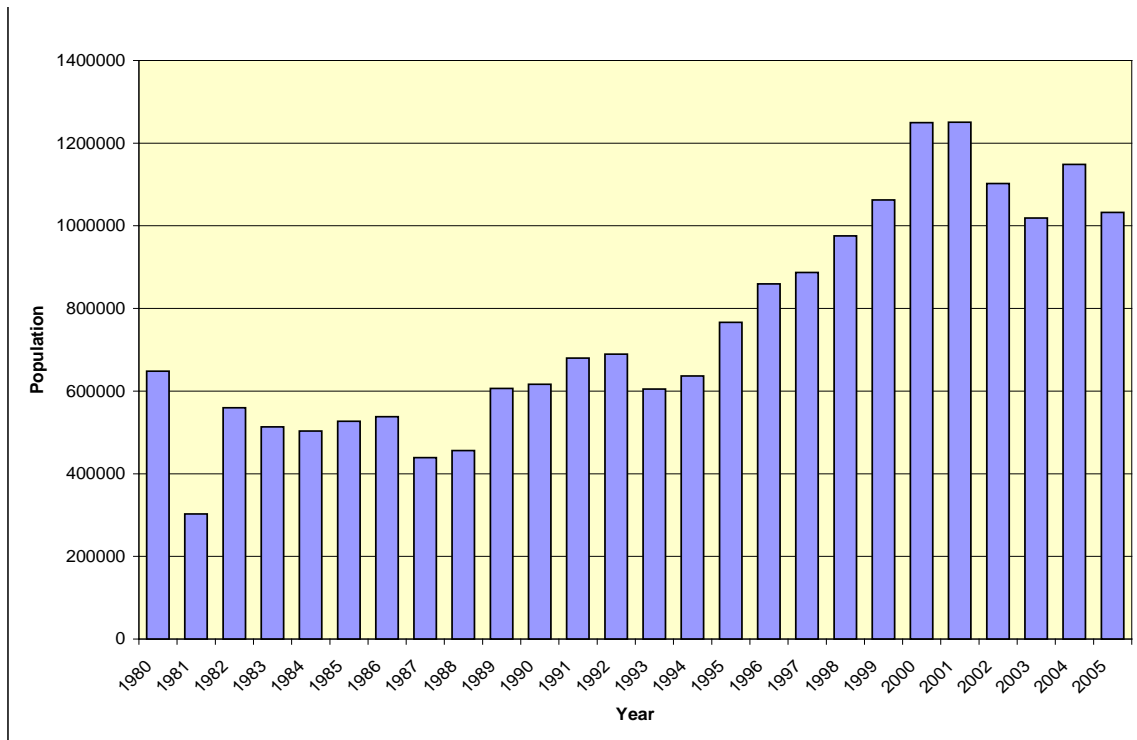


Figure 2: Change in cattle numbers in the Northern Communal Areas of Namibia (1980 – 2005)

From the government perspective, the major challenges affecting the marketing of livestock and livestock products in the NCA of Namibia are perceived to revolve around the presence of the VCF, which divides the country into essentially three FMD control zones. The zones south of the VCF are recognized by the OIE as FMD free and thus are able to access local and international markets freely. Areas north of the VCF however, are regarded to be under the threat of FMD and the presence of Contagious Bovine Pleuropneumonia (CBPP), further justifying the existence of the fence. Farmers in the NCA incur high transaction costs when marketing their livestock to the Meat Corporation of Namibia (MEATCO), mainly due to transportation cost and 21 days quarantine at an approved facility before slaughter. The average total costs of quarantining and transportation are estimated at about N\$ 275 (Namibian dollars) per animal, of which 68% results from weight loss suffered when animals walk long distances and insufficient feed, 26% transport cost and 6% labor to look after the animals during the quarantine period (Vigne 2005). MEATCO is the sole institutional

buyer of livestock in the NCA region and has experienced a number of viability problems in its operations due to the low throughput, poor quality animals and lack of consistency in the supply of slaughter stock at Oshakati and Katima Mulilo abattoirs respectively. Both abattoirs are managed by MEATCO on behalf of the government with the purpose of job creation, increase output, revenue and poverty alleviation.

The overall national objectives, goals and aspirations are epitomized in the national vision document referred to as Vision 2030. Namibia Vision twenty thirty sets the goals of achieving development status for the country by the year 2030. Accordingly, Namibia's Vision 2030, as interpreted for action by successive periodic National Development Plans, domesticates this global vision for a better world. National Development Plan 4 is specifically anchored on the 1st Millennium Development Goal which speaks of reduction of poverty and hunger through "increased and sustained economic growth, creation of wealth and employment; and even distribution of that wealth (NPC 2013). Among the strategies that are highlighted under agriculture is the need to remove the VCF, which may result in the integration of the Namibian livestock markets as a measure for value addition to animal products. Although the NCA had no outbreaks of FMD since 1970 there were no studies carried out to determine the FMD status in order to declare the area free of FMD using the guidelines set by the OIE. This situation poses a huge challenge to the government of the day which strives to create an improved animal disease status that will open up new marketing opportunities and improve the living standards of the people in these areas.

2.1 Objectives of the Study

Based on the potential for existing and new market opportunities, the Government and the Meat Industry of Namibia decided to fund this study with the primary objective being to establish whether the Namibian cattle population in the NCA of the buffer zone (now the protection zone) is FMD virus free.

CHAPTER 3

3. LITERATURE REVIEW

3.1 Introduction

Understanding the people and customs in communal societies is very important if their livestock are to be adequately surveyed. This knowledge assists in identifying what motivates these peoples to participate in surveys and the way in which they will participate. Unlike commercial farms there is no fixed abode or physical address that can be linked to a sampling frame. Some of these farmers are nomadic and herds of various owners are intermingled, which means a Western/OIE approach to sampling may not be fully applicable.

3.2 People in the NCA of Namibia and their customs.

The NCA Namibia is comprised of six regions namely Kunene, Omusati, Oshana, Ohangwena, Oshikoto and Kavango respectively. The Namibian map showing the regions and surveyed area are displayed in (Figure 3). In the NCA most people perceive animals to be more important than crops, because they are used widely for household purposes, are available throughout the year, survive drought better than crops and are thus a more reliable source of income and security. Livestock therefore form an important component of their lifestyle both sociologically and economically.

People in these communal land shares in many ways the same customs and traditions in terms of sociological and cultural background. In the northern communal areas of Namibia, livestock play multiple roles in the sustenance of the people through the provision of draught power, manure, milk, hide, meat, and household monetary income from sales, as a source of storing wealth, socio-cultural support and food security (Bishi & Kamwi 2008). Weddings are extremely important social events in Namibia, bringing family and friends together to sing, dance and feast. Many Owambo couples, for example, say their vows in a church ceremony accompanied by identically-dressed bridesmaids and groomsmen, then exit to a crowd of guests shouting praises, dancing

and waving horsetail or cattle tail whisks. Funerals are equally important events that bring together family and friends in mourning. Both events combine old traditional and new modern elements. The number of animals given as gifts for wedding and slaughter at funerals is correlated to family and friends affordability. The number of slaughtered animals could be as high as fifty per occasion. They also have a very high social value, wealth and status symbolism as well as in the fulfilment of religious, cultural and traditional practices (Malan 1995).

Generally, livestock are kept in a kraal during the night, fairly close or adjacent to the homestead as a method of preventing their livestock from being stolen and eaten by predators. Cattle grazing starts in the early morning for about two hours before the manual milking begins. Once the milking is done they are released again onto extensive grazing till late in the afternoon. Stock owners are naturally reticent about answering questions about how many livestock they owned as a westerner would be about questions regarding his/her bank balance (Kruger 1997).

3.2.1 Kunene region

The Kunene region occupies the northwestern corner of Namibia, bounded by the North Central region of Omusati to the east, Atlantic Ocean to the west, Damaraland to the south and Kunene River to the north as illustrated in (Figure 3). Kunene is a region which is inhabited mainly by Herero speaking people, most notably the Ovahimba, Tjimba and the Herero proper (who also comprise various subgroups), who are currently settled in the northern central areas of Namibia, while the groups left in the northwest are the Himba and the Tjimba. They share a similar basic culture and tradition and their language belongs to the Bantu group of languages.

The Ovahimba people used to migrate from one place to another (nomadic life style) in search of better grazing and water for their livestock. However, this practice is becoming limited and impossible to continue due to the increase density of human and animal populations across the region. In the light of the concept that animals are wealth preserving, the health of livestock is very important to the Kaokoland pastoralists and they tend to participate enthusiastically in official vaccination campaigns and have in

recent years started to buy their own medication such as vaccines and stock remedies for prevention and treatment of other diseases.

3.2.2 North Central regions (Omusati, Oshana, Ohangwena & Oshikoto)

These four regions are in the centre of the NCA bordering with Angola on the north eastern side and with the VCF on the south and west respectively. The inhabitants of these regions are mainly the Ovambo speaking peoples. The above mentioned regions tend to be more fertile than other regions in Namibia, due to relatively good rainfalls. The Ovambo people have remained mainly crop and cattle farmers, making these regions the largest population group in Namibia. The Ovambo people belong to the southwestern Bantu group. They are culturally, closely related to the matrilineal agriculturists of central Africa and migrated as a single group in a westerly and later south-westerly direction until they eventually reached the Kavango River in the 16th century (Malan 1995). The present group divisions of the Ovambo are Kwanyama, Ndonga, Kwambi, Ngandjera, Mbalantu, Kwaluudhi, Eunda and Nkolonkadhi. According to Malan (1995), each of the eight tribes has its own dialect and there is no lingua-franca for the whole territory, despite frequent references to the existence of a language called Oshiwambo. The pronunciation and accent of these languages differs but in principle they will be able to speak with each other. In comparison to the other regions, animals are alternatively kept on extensive grazing at cattle posts called “Ohambo”, either permanently or only during the dry season and may be brought back to the homestead to consume the post harvest sorghum, maize and Pearl millet locally referred to as mahangu. In the early 1990 towards the years 2000, farmers in these four regions were afraid to participate in official vaccination campaigns because they believed that if their animals are vaccinated they will die.

3.2.3 Kavango region

Kavango region is also known as Okavango which is located in the North Eastern part of Namibia and shares common borders with neighboring Angola & Botswana (Collins 2014). To the North East, the region borders the Zambezi (Formerly the Caprivi region, to the West it borders Ohangwena and part of the Oshikoto region.

Today the Kavango consist of five individual tribes, namely the Kwangali, Mbunza, Shambyu, Gciriku and Mbukushu, each inhabiting an area of its own along the southern bank. The Kwangali and Mbunza tribes have similar social practices, such as preparing young boys for manhood and young girls to take care of a household. The two tribes speak the same language, namely Rukwangali. The split between the Shambyu and Gciriku tribes occurred when they were settled on the southern bank of the Okavango River 47 km east of Rundu, opposite Rundjarara. According to Travel News Namibia, the languages spoken by these tribes, Rushambyu and Rugciriku are very similar. The Mbukushu, who speak Thimbukushu and live in the eastern part of Kavango, differ socially and ethnologically from the other four tribes.

The traditional economy in Kavango is based on a combination of horticulture (pearl millet, sorghum and maize) and animal husbandry (cattle and goats). Because of its rather higher rainfall than most other parts of Namibia, this region has definite agricultural potential including irrigation schemes situated close to the water source of the great Okavango River. There is also potential for organised forestry and agro-forestry, which should stimulate furniture making and others, related industries. An important local industry is woodcarving. The woodcarvers of the Kavango region are known for their skills and produce masks, ornaments, bowls and furniture for the tourist and other markets. Many Kavangos make a living on farms, mines or in urban centers (Namibia tourism 2012).

Although, cattle are rarely slaughtered for domestic consumption while oxen are mainly used as draught animal power, transport of water, goods, firewood, sand or clay for house building and Pearl Millet during harvest season (Van Rooyen & Gartside 1999). Farmers are aware of the importance of vaccination in order to avoid losses caused by diseases and other related conditions. When there is a mass vaccination campaign against FMD and CBPP they all tend to bring animals to the crush pens early in the morning and cattle are processed as they arrive (State vet observation).

3.3 Overview of Foot-and-Mouth Disease

Foot-and-Mouth Disease is greatly feared all around the world and is one of the most

contagious diseases of mammals affecting over 70 different species of cloven-hoofed animals (Shimshony 1988, Bengis & Erasmus 1988, Pinto 2004, Arzt *et al.* 2011a, Karesh 2012). It is also one of the most important infectious diseases from both a clinical point of view as well as an economic point of view.

The first written description of FMD probably occurred in 1514, when Fracastorius described a similar disease of cattle in Italy (Fracastorius 1546). Almost 400 years later, in 1897, Loeffler & Frosch (1898) demonstrated that a filterable agent caused FMD. This was the first demonstration that a disease of animals was caused by a filterable agent and ushered in the era of virology.

Epidemics of FMD have resulted in the killing of millions of animals, despite this being a frequently non fatal disease for adult animals (Domingo, *et al.* 1990 and Woodbury 1995). Critics of current policies to cull infected herds argue that the economic implications need to be balanced against the slaughter of many animals. Although the disease typically has a short-term effect on an animal's health, chronic FMD typically reduces milk yields by 80% (Bayissa *et al.* 2011, Barasa *et al.* 2008, Bulman & Terrazas 1976). The main signs are the development of vesicles and erosions in the mucosa of the mouth and skin of interdigital spaces and coronary band (Thompson & Bastos 2004). The recovered animals may become carriers or remain in very poor condition over a long period leading to reduced production, reproduction and severe economic loss to the farming enterprise and the livestock industry. Outbreaks still occur with devastating consequences particularly to poor farmers (Gall & Leboucq 2004, Scudamore & Harris 2002, Yang *et al.* 1999). The main concern of many countries, including Namibia is to prevent the introduction of the virus and to rapidly eradicate it should an outbreak occur.

3.4 Foot-and-Mouth Disease

3.4.1 Aetiology

Foot-and-mouth disease virus belongs to the genus Aphthovirus within the positive strand RNA Viral family Picornaviridae (Belsham 1993 & Thompson 1994). The only

other member of this genus is equine rhinitis virus (King *et al.* 2000). Although often referred to as a single disease, there are seven serotypes of the virus distributed around the globe such as serotypes O, A, C, Asia 1 and South African Territories (SAT1, SAT2 and SAT3). Each one has a wide spectrum of antigenic and epidemiological multiple subtypes (Bachrach 1968). They are indistinguishable clinically, although they have different and extensive geographical distributions. The wide diversity is considered a consequence of the high mutation rate, and recombination (Carrillo *et al.* 2005). FMD virus there may be variations in serotypes, distribution and this can be demonstrated using various serological and molecular biology assays. For instance, the serotype variations experienced in an SAT1 outbreak in West Africa between 1975 and 1981. The serotypes in this outbreak constituted three different evolutionary lineages (I-III), which corresponded to three discrete continental regions, although some of these serotypes could have occurred in another continent due to introductions (Sangare *et al.* 2003).

3.4.2 Epidemiology

Foot-and-mouth disease affects domestic and wild cloven-hoofed animals worldwide except in Greenland, North America, Australia, New Zealand and the smaller Islands of Oceania. The disease is classified by OIE as a listed disease which by definition, means that it has the potential for rapid and extensive spread within and between countries and can cause severe economic impact (OIE 2014a).

The disease has an acute course but African buffalo, cattle, sheep, and goats may become prolonged, symptomless, persistent infected for years, the so-called “carriers” animals by virus replicating sub-clinically in the pharynx (Burrows, 1966; Bengis *et al.*, 1986; Alexandersen *et al.*, 2003). FMD carriers are defined by the World Organisation for Animal Health (Office International des Épizooties: OIE) as persistently infected animals which are recovered, vaccinated or exposed and from which FMDV can be isolated from the oropharynx for more than 4 weeks after acute stages of disease (OIE, 2009 and Salt 1993). About 50% of ruminants are thought to become persistent carriers (Arzt *et al.*, 2011b). Cattle are capable of maintaining the

virus for up to 3.5 years, sheep for at least 9 months, goats for 4 months and African buffalo (*Syncerus caffer*) for 5 years (Condy *et al.*, 1985; Alexandersen *et al.*, 2002; Arzt *et al.*, 2011b). The introduction of the virus or a new serotype to previously free herds, areas or countries is likely to lead to a very rapidly spreading epidemic with high morbidity rates. However, there is much uncertainty about whether these animals transmit FMDV to other animals, and, if they do, which particular factors cause a persistently infected animal to recommence virus shedding to the extent that it can infect another animal (Thomson, 1996). By 4 weeks, recoverable virus has disappeared from all other secretions, excretions and tissues of animals that have passed through the acute stage of the infection.

The epidemiological pattern of the disease tends to be different in temperate and tropical/semitropical parts of the world. In the former, the greater survival of the virus in the environment means that indirect transmission through fomites may be as important as direct contact between infected and susceptible animals. Windborne virus spread is possible under some environmental circumstances. For instance, airborne (up to 60 kilometer (km) overland and 300 km by sea) in temperate zones under proper conditions (these include a high viral load, stable atmospheric conditions, and a susceptible population downwind (Lubroth 2002). On the other hand, in hotter climates indirect means of transmission assume less relative importance than direct means of transmission. It is often the movement of potentially infected animals and livestock trading patterns that provides the key to understanding the epidemiology of FMD in such areas, regions or country including Namibia.

It is a regrettable fact that FMD still persists currently in South America, most African countries, the Middle East, and many parts of south, central and south-east Asia where 75% of the world poor live (Thornton *et al.* 2002). Major re-incursions of the disease have occurred recently in south-east Asia (Taiwan, Japan, South Korea and Indonesia), South America (Argentina, Uruguay and Brazil) and Western Europe (UK, The Netherlands, France and Ireland). In some cases this has involved the transcontinental spread of the pan-Asian topotype O virus from Asia. This occurred in September 2000 in South Africa and in February 2001 in the UK (Sangare *et al.* 2001, Samuel &

Knowles 2001). It's widely distributed in developing countries, in particular Africa, South America, South Asia, South East Asia and East Asia. The lack of infrastructure and human resources renders them particularly vulnerable to the spread and poor control of FMD. The exception has been in north and southern Africa, including Namibia, where considerable success in reducing the prevalence of the disease and in developing FMD free zones has been achieved. In many countries outside these two regions there is little attempt to control the infection while in others the policies and practices applied sometimes ignores important epidemiological principles and are therefore largely futile.

These limitations constitute a major obstacle to international trade in animals and animal products. In terms of International Disease Regulations (Terrestrial Animal Health Code of the OIE) export of meat to FMD-free markets is allowed for countries or for zones /compartments within countries that are internationally recognized as free of Foot-and-Mouth Disease (OIE 2014b). To qualify for freedom from FMD, the country or zone in question must be separated from FMD infected areas by a series of boundaries and must internally conform to certain standards.

3.4.3 Possible mechanism of FMD transmission and the role of wildlife within Southern Africa Development Community (SADC)

The highly contagious nature of FMD is a reflection of the wide range of species which are susceptible, the enormous quantities of virus liberated by infected animals and the range of excretions and secretions which can be infectious. Conditions in the southern African region are favorable for causing FMD outbreaks, primarily associated with SAT 1, SAT2 and SAT3 sero-types of FMDV (Radostitis *et al.* 1994, Thomson 1994). The SAT 1 and SAT 2 are well known to be maintained in buffalo. The two serotypes have also been able to “escape” from sub-Saharan Africa to cause livestock outbreaks in North Africa, the Middle East and Europe without involvement of buffalo or any other wildlife species (Bastos 2003, Dimitriadis & Delimpaltas 1992, Rweyemamu *et al.* 2008).

The most common mechanism by which FMD is spread is the movement of infected animals and subsequent direct transmission of virus to susceptible animals in exhaled

droplets. This is an especially common means of spread among ruminant species including buffalo. The next most common mechanism is by the movement of contaminated animal products such as meat, milk, offal, and many others. FMD virus can also be transmitted mechanically, for example, by contaminated milking machines, by vehicles, especially those used for transporting animals such as trucks, trains, and by people (Donaldson 1987, Sellers 1971). In southern Africa the three SAT serotypes are prevalent (Vosloo & Thomson 2004) and the only recent occurrence of serotype O was caused by an introduction from elsewhere and quickly eradicated.

Serotype SAT3 appears to be mainly confined to buffalo with only a small number of outbreaks reported in domesticated species (Thomson 1995). The primary outbreaks have been as a result of livestock cross border movement and buffalo as well as cattle contact at the wildlife/cattle farming interface. The disease was imported by cross border movement from their northern neighbors into respectively Namibia (1958, 1962, 1968) and Zambia (1976, 1982, 1984), while intercontinental spread has occurred most when the Pan-Asian O lineage caused an outbreak in Kwazulu Natal in September 2000 (Thompson & Bastos 2004). Outbreaks of SAT 2 serotype have occurred in the Middle East and North Africa and the Pan Asia strain of serotype O has resulted in outbreaks in the UK in 2001 are some of the examples (Knowles *et al.*, 2001; Di Nardo *et al.*, 2011; Valdazo-González *et al.*, 2012). The movement of FMDV strains from their endemic areas into other regions is serious due to the potential risk of establishing in new areas previously naïve to those strains. These introductions can have extensive implications in terms of disease spread and severity even if resident FMDV strains are already present, because of poor cross-protection against exotic strains (Vosloo *et al.*, 2010). Host vulnerability to new strains, for instance, was evident in a recent incursion of SAT 2 into Egypt, where mortality rates were as high as 20% in livestock (Ahmed *et al.*, 2012).

Wildlife, especially the African buffalo (*Syncerus caffer*) is a particularly efficient maintenance host and plays a central role in the epidemiology of FMD viruses in southern Africa due to its ability to maintain and transmit FMD virus (Bengis *et al.* 1986). The long-term carriers mostly become sub-clinically infected, maintaining the

disease and posing a threat to other susceptible wildlife and domestic species. Individual animals may maintain the infection for periods of at least 5 years but in most buffalo the rates peak in the 1-3 year age-group (Condy *et al.* 1985). Numerous antigenic and genetic variants are generated in an individual animal during persistence (Vosloo *et al.* 1996). There were only a few SAT2 outbreaks in cattle in Zimbabwe between 1983 and 1991 in which a role of vaccinated carriers cannot be excluded (Thomson 1996, Vosloo *et al.* 1992). Under African circumstances virus transmission can also occur by other means e.g. visitors and vehicles coming from game parks with endemic FMD, connections of the animal caretakers, contaminated materials, improperly inactivated vaccine. Bastos *et al.* (1999) proposed that sexual transmission of the disease from carrier buffalo bulls to domestic cows could also occur, because SAT3 virus was isolated from both semen and from sheath washes from a naturally infected African buffalo. This was considered a persistent infection since the virus genotype had not been currently circulating in the buffalo herd. The virus in the sheath-wash of the buffalo bull presumably originated from a stratified squamous epithelium tissues of the prepuce.

In contrast to cattle, buffalo can maintain the SAT virus types in small isolated populations. It is thought that carrier buffalos infect their calves when they lose the protection of the maternal antibodies and that the infected calves may infect other game through direct contact with kudu and impala. This may lead to reactivation of the carrier status in the herd and further propagation of the disease. The belief in the role of carriers as disseminators of FMD was originally supported by the observation that coughing by persistently infected cattle spread FMDV to pigs (Sutmoller *et al.* 1968). Transmission of SAT-type virus from persistently infected African buffalo to cattle under experimental and natural conditions has been unequivocally demonstrated (Bastos *et al.* 2000 & Dawe *et al.* 1994). Separation of buffalo and livestock by fencing and vaccination of cattle with viruses that are antigenically closely related to those carried by nearby buffalo form an integral part of FMD control in southern African countries including Namibia (Hunter 1998).

Persistently infected buffaloes in the wild constantly generate variants of SAT1 and

SAT2 which explain the wide range of genomic and antigenic variants which occur in SAT1 and SAT2 viruses in southern Africa (Vosloo *et al.* 1992). Circulating neutralizing antibodies develop within four to ten days of infection. Convalescent animals usually have a very long immunity to re-infection (as long as at least five years) with closely related virus of the same serotype, but remain fully susceptible to other serotypes. The genetic analysis of virus strains has proven to be valuable in increasing our understanding in the spread of FMD in Africa. This review shows that there is a difference in FMD occurrence between southern Africa and the rest of the continent; this distinction is most likely based on differences in animal husbandry and trade systems (Teklehiorghis *et al* 2014).

Control of FMD is mainly carried out by controlling its spread from infected to susceptible animals, either by preventing the movement of the virus from the infected animals, animal products, fomites and aerosol, or by reducing the number of susceptible animals by vaccination (Kitching 2005). The degree of protection after vaccination is greatly influenced by the antigenic relationship between the vaccine strain and the challenge strain. Vaccines provide only partial immunity against antigenic variants of the same serotype. Potent vaccines confer immunity as early as four days after injection. However, vaccine immunity is not long lasting and therefore revaccination at regular intervals (e.g. 6-12 months) is required. Manufacturers of commercial FMD vaccines normally recommend a primary immunization regime of an initial dose followed within three to four weeks by a second dose of vaccine (BVI 2014). Boosters should be given every 4-6 months depending on the epizootiological situation of each country and legislation in place. A proportion of vaccinated animals, although protected against the clinical disease, may become sub-clinically infected after natural challenge and excrete virus. It is important to note that animals incubating the disease when vaccinated may still develop the disease, sometimes in a milder form, and that vaccinated, exposed animals may still transmit infection for 7-14 days after vaccination and exposure (Geering & Lubroth 2002).

3.4.4 Pathogenesis and clinical signs

From the studies of Loeffler & Frosch (1897), it was clear that FMD was caused by a very small particle that passed an ultra-filter. However, the way the virus finds its first target cells or tissues and how it is propagated, remained undiscovered for many years. It was clear that the virus could spread from animal to animal or through contact with contaminated persons or objects, and through the air (Fogedby 1963, Röhrer & Olechnowitz 1980). In the 30's it was demonstrated that infected hedgehogs exhaled air that could infect other hedgehogs by inhalation (Gibbs 1931, Edward 1934, Korn 1957).

Virus can gain entry through abrasions in the epithelium, for instance the oral cavity, feet or teats but is very inefficient; requiring almost 10,000 times more virus (Donaldson 1987). It is now generally accepted that the common portal of entry of the virus is by the respiratory tract (McVicar & Suttmoller 1976, Sellers & Parker 1969). Early virus multiplication and histopathological changes in the upper respiratory tract of FMD infected cattle clearly indicated that the primary site of early virus localization and growth is predominantly in the mucous membrane of the nasal passages (Burrows 1972, Garland 1974). Similar observations were also reported by McVicar *et al.* (1971) and McVicar & Suttmoller (1976). The virus multiplies during the pre-viremic state when the classic oral lesions are not yet detectable either macroscopically or microscopically.

Korn (1957) described histopathological changes in the nasal mucosae prior to the development of clinical signs and concluded that the primary sites of virus multiplication were in the nasal passages. Although Korn's hypothesis was altered in the light of later investigations, his idea of air-borne infection still forms the basis of much of the present concepts of the pathogenesis of the disease. Fogedby (1963) reported on air-borne FMD transmission over long distances e.g. from Germany onto Danish islands. Hyslop (1965) detected FMD virus release in the air surrounding diseased cattle and Sellers and Parker (1969) made similar observations in the air surrounding cattle, pigs and sheep even before clinical signs developed.

Most viruses will be trapped in the upper respiratory tract, with subsequent multiplication in the mucosa of the oro-pharynx. After experimental pulmonary

infection of cattle, FMD virus will multiply in lung tissue (Eskildsen 1969) and virus that reaches the alveoli can also pass readily into the blood stream (Sutmoller and McVicar 1981). The virus is then distributed throughout the body, to reach multiplication sites such as the epithelium of the oro-pharynx, oral cavity, heart, feet and the udder. This type of fiery clinical syndrome will take place after contact exposure to infected animals just prior to the development of clinical signs (Graves 1971). When virus is instilled in the mammary glands of susceptible cows, virus appears in the milk in high titers. Virus replication is accompanied by signs of mastitis 2-4 days before other clinical signs developed (Burrows 1968a). Intra-nasal exposure of susceptible cows resulted in the detection of virus in the milk when the cows had generalized lesions (Leeuw *et al.* 1978). Virus probably replicates in the pituitary gland (Scott *et al.* 1965) and the pancreas with selective necrosis of the islets of Langerhans as reported by Manocchio (1974). Virus reaches high titers in the skin of infected cattle even in areas where there are no gross lesions (Gailiunas & Cottral 1966). However, gross lesions are most frequently observed in tissues that are subject to vigorous activity or trauma (Potel 1958, Seibold 1963, Skinner & Knight 1964).

In the 60's prior to the vaccination programs in Argentina, Uruguay and the southern States of Brazil, serious sequels of FMD were seen including survivors with a cardiac-pulmonary syndrome. Also, the 'panting' or 'heat intolerance syndrome' is seen in cattle. The latter may be indicative for FMD virus affecting the pituitary gland (Domanski & Fitko 1959, Scott *et al.* 1965). These animals develop hairy shaggy coats and become very sensitive to warm weather and become very poor producers.

During the 2001 outbreak in Argentina and Uruguay there were several cases of acute cardiac involvement in young animals (calves and piglets), causing mortality, and even of adult cattle with a typical soft, flaccid heart with white or greyish stripes (the so-called "tiger heart") or spots, seen mainly in the left ventricle and interventricular septum. This may be due to the completely naïve immune status of the population as a result of the non-vaccination policy. Strain differences may also play a role. Abortions and mastitis are other common sequels of FMD that affects productivity. When clinical disease develops, the degree of contagiousness peaks just before and during the

beginning of the clinical signs and drops rapidly 4-5 days later even though at that time external lesions might still be very evident (Graves 1971).

For the duration of the viremic phase, and thereafter, dependent on the lesions associated with epithelial involvement, virus is present in secretions and excretions. Virus is excreted from all lesions and externalized as an aerosol in exhaled air (Sellers *et al.* 1971). Unfortunately little information exists on the relative levels of virus from aerosols and secretions for infections of each species with a specific virus strain. Dekker *et al.* (1996) indicated how differences in aerosol excretion between virus strains, after infection of pigs, could be a major factor in the area at risk around infected pig farms. Virus output varied as much as 300-fold, with enormous implications for airborne transmission. However virus strains not adapted to produce high level of aerosol excretion, such as the Pan-Asia types O strain, also appear successful in nature (Donaldson 2001). This raises the importance of the level and duration of virus concentrations in excretions/secretions of various host species. Although pigs are major producers of virus aerosols, cattle produce several magnitudes more virus in the epithelium of the tongue, which often sloughs off and is spat out during clinical disease, as well as in saliva, urine, feces and milk. For example, the 10-30 g of tongue blister material which a cow with FMD can spit out may represent not less than a billion infectious units (IU). These enormous quantities of virus contaminate the environment including boots, clothes, tyres etc and therefore, cattle are probably the main source of environmental contamination. FMDV infection is often airborne through the upper and lower respiratory tract. FMDV can also enter the new host through abrasions of the mouth epithelium of the skin of feet and udder. The peak of infectivity is just prior to or during the development of lesions. Infectivity is much reduced 3-4 days after the lesions develop. Some virus strains are host adapted. Although pigs are major producers of virus aerosols, cattle produce several magnitudes more virus. Cattle are probably the main source of environmental FMD contamination.

The incubation period of an infectious disease is defined as the time interval between exposure to an infective dose and first appearance of clinical signs. The incubation period for FMD is highly variable, and depends on the strain and infecting dose of virus,

the route of transmission, the animal species and the husbandry conditions (Gailiunas & Cottral 1966). In general, the incubation period is about 2-21 days (average 3-8 days). However, the incubation period for farm-to-farm airborne spread ranges from 4-14 days and this is also the normal range for farm-to-farm spread by indirect contact (Sellers & Forman 1973). The incubation period for farm-to-farm spread resulting from direct contact may range from 2-14 days in cattle, depending on the viral strain and dose and the level of susceptibility of the animal. The initial sign is pyrexia up to 41.1°C. Pyrexia is followed by anorexia, reduction in milk production for 2-3 days. Under normal conditions, recovery generally occurs within 8-15 days.

Clinical signs in cattle are mainly salivation, fever, depression, shivering, anorexia and severe lameness caused by the presence of painful sores and blister formation in the skin, coronary bands, and interdigital spaces. Animals are unable to eat or walk properly (lameness), lose condition, the vesicles rupture, leaving large denuded areas which may become secondarily infected but milder. In pigs, sheep and goats the signs are similar but generally mild and can be difficult to distinguish from other common conditions (Donaldson & Sellers 2000, Geering 1967). Lameness is the predominant sign and can also be observed from the distance which may bring to the attention of the farmer/herder of being suspicious. FMD is frequently only a mild disease, with transitory clinical signs which can easily be missed by the herders, animal health technician and veterinarian, or could be confused with other diseases presenting similar lesions (De la Rúa *et al.* 2001, Watson 2002).

Recovered pigs are not carriers but cattle may be carriers for 18-24 months and sheep for 1-2 months. A critical issue in this respect is the occurrence of carrier animals and the risk they pose in transmitting the virus. Asymptomatic persistent infection is a common after-effect following infection of ruminants with FMD virus. Animals in which live FMDV persists beyond and can be recovered 28 days after initial infection are defined as carrier (Sutmoller & Cottral 1967), which may also occur in vaccinated ruminants exposed to live virus (Rossi *et al.* 1988 and Straver *et al.* 1970). Persistent infection can follow either a clinical or a sub-clinical FMDV infection, occurring at the same rate in vaccinated and non-vaccinated animals. There is a small risk that carrier

animals transmit FMDV, but reports thereof are few (Dawe *et al.* 1994, Condy *et al.* 1985). The epidemiological significance of these persistently infected animals, termed carriers, is highly controversial (Alexandersen *et al.* 2003). However, the fear that they may occasionally initiate new outbreaks has led to international trade rules requiring either long waiting periods for carriers to recover from infection or use of methods of carrier elimination before the FMD-free status can be restored to regions that suffer from outbreaks of the disease (OIE 2014). Morbidity in unvaccinated herds can be high, but mortality usually does not exceed 5 per cent. If it occurs during the calving season, calf mortality can be considerable (Seifert 1996). Young calves may even die before the development of clinical signs usually because the virus attacks the heart muscles.

3.4.5 FMD control in southern Africa

Improving the management of FMD in the region is not only depended on the technical progress but on ameliorating the conflict between animal disease management requirements and other equally vital development imperatives *viz.* expanding trans-frontier conservation to promote ecotourism and sustainable utilization of wildlife as well as creating flexible economic opportunity for poor rural communities based on more effective use of their abundant livestock resources (Thompson 2008).

Disease control scenarios have included:

- 1) Zonation and area based disease freedom strategies
- 2) Accepting and managing endemic Foot- and-mouth disease through vaccination
- 3) Compartmentalization
- 4) Commodity based trade options

In Africa systematic vaccination against the SAT viruses is only applied in the Southern zone. This is done in border zones with fenced areas of endemic FMD, e.g. the Kruger National Park in South Africa where the buffalo are permanently infected with SAT-strain viruses (Thomson 1996). In general this policy keeps the rest of South Africa and large areas in surrounding countries free of FMD. In the beginning of 2001 after excessive rainfall and accompanying floods, fences around the Kruger National Park

were flushed away and buffaloes and other game broke out of the restricted zones, causing FMD in surrounding districts. Also, at the end of 2000, South Africa suffered from a limited Type O₁ outbreak that started at a pig farm where swill from a harbor was fed. Initial attempts were made to control the outbreak by stamping-out but when the disease entered an area with community farming, which made control by stamping-out practically impossible, the outbreak was successfully controlled by ring/area vaccination of all susceptible animals. Thus both a restriction of the movement of the virus and a reduction of susceptible animals by vaccination synergistically helps to control FMD (Sutmoller *et al.* 2003). An individual livestock keeper cannot adequately control FMD by his actions alone, but also depends upon a collective effort from their neighbors' and trading partners. The same is true for a country; effective FMD control requires regional and global cooperation (Foreman *et al.* 2009).

The destruction of animals is primarily to halt further spread, as growth and milk production may be permanently affected, even in animals that have recovered. Due to international efforts to eradicate the disease, infection would also lead to trade bans being imposed on affected countries. Critics of current policies to cull infected herds argue that the financial imperative needs to be balanced against the killing of many animals especially when a significant proportion of infected animals, most notably those producing milk, would recover from infection and live normal lives, albeit with reduced milk production. On the ethical side, one must also consider that FMD is a painful disease for the affected animals. The vesicles/blisters are painful in themselves, and restrict both eating and movement. Through ruptured blisters, the animal is at risk from secondary bacterial infections and, in some cases, permanent disability.

3.5 Methods of detecting Foot-and-Mouth Disease

The precise and rapid diagnosis of infection with FMDV is of prime importance for both control and eradication campaigns of FMD (Rémond *et al.* 2002). As part of many disease control, surveillance or eradication programs, entire herds or flocks are tested to ascertain if the specified disease is present or conversely to ensure that the disease is absent. Surveys to substantiate freedom from disease are becoming increasingly

important. This is due to the changes in rules governing international trade in animals and animal products and to an increase in disease eradication and herd-level accreditation schemes (Cameron & Baldock 1998b). Testing entire herds or flocks is a very expensive exercise and veterinarians, trading partners and stakeholders have to accept the results of testing only a portion of the animal population. To provide the necessary assurances, these surveys must have a sound theoretical basis. Regardless of this limitation, sampling can provide valid insight into the communal herd health status of the population due to the fact that it is rare for only one animal in a communal herd to have the disease of interest (Martin *et al.* 1987).

Rapid and accurate diagnosis is needed for effective control and eradication of FMD. Laboratory-based methods such as virus isolation (VI), antigen detection ELISA and real-time Polymerase Chain Reaction RT-PCR specific real-time reverse transcription polymerase chain reaction (rRT-PCR) can provide an objective result within a few hours of sample receipt (Ferris & Dawson 1988). The ability to diagnose FMD rapidly and accurately is a prerequisite for efficient animal health control as well as for the support of export-oriented agricultural economies in large parts of the world. Foot-and-Mouth Disease cannot be differentiated clinically from other vesicular diseases, such as swine vesicular disease, vesicular stomatitis and vesicular exanthema (Grubman & Baxt 2004). Laboratory diagnosis of any suspected FMD case is therefore a matter of urgency.

3.5.1 Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a rapid diagnostic method for FMD based on amplification of specific sequences of the viral genome by reverse transcription polymerase chain reaction (RT-PCR), which can be applied to different kinds of biological samples, such as fluids and tissues (Vangrysperre & De Clercq 1996). Increasingly, highly sensitive Polymerase Chain Reaction (RT-PCR) based systems are used on initial field material not only to determine whether or not FMDV is present but also to type and assign the virus present in the materials submitted to viral lineages.

Assay has been used for FMDV and a number of pan-serotype specific real-time reverse

transcription polymerase chain reaction (rRT-PCR) assays have been developed capable of detecting all seven serotypes of FMDV (Reid *et al.* 2002, Callahan *et al.* 2002, Moonen *et al.* 2003, Rasmussen *et al.* 2003, Moniwa *et al.* 2007). In addition to improved sensitivity, the benefits of real-time PCR assays over conventional endpoint detection methods include their large dynamic range, a minimised risk of cross contamination, an ability to be scaled up for high throughput applications and the potential for accurate target quantification (Schweiger *et al.* 2000)

3.5.2 Need for a Standardised and Validated Serological Test

Although methods based on virus isolation or the demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid or culture products is sufficient to make a diagnosis, in general, the ELISA, using type-specific serological reagents is the preferred procedure for the detection of FMD viral antigen and identification of viral serotype in the early stages of FMD diagnosis. Also because it is more specific, sensitive and efficient, and it is not impacted by pro- or anti-complement factors the ELISA has access to better development and even replaced complement fixation (CF) in most laboratories in the early investigation of FMD (Ferris & Dawson 1988). Serological tests are performed in support of four main purposes namely:

- 1) to certify individual animals for export and import (international trade).
- 2) to confirm suspected cases of FMD.
- 3) to substantiate the absence of infection.
- 4) to demonstrate efficacy of vaccine.

To demonstrate freedom from infection, several approaches are required according to whether the population has been vaccinated or not and if vaccination has been used, whether this has been applied as an emergency application or as part of an ongoing surveillance programme of vaccination. Serology is a useful tool to help diagnose FMD and to certify freedom from infection. This is complicated by the administration of FMD vaccines, which elicit antibodies that may cross-react in tests designed to detect

evidence of past infection (Clavijo *et al.* 2004). FMD vaccines can prevent illness and reduce viral replication and excretion (Barnett *et al.* 2002). However, FMD vaccination does not necessarily prevent FMDV infection and some degree of virus replication; such animals can become viral carriers and may be considered a threat to other animals. Consequently, a constraint to the use of vaccination is the need for a reliable method to demonstrate that vaccinated animals have not been sub-clinically infected with FMDV and are not still carrying the virus (Council Directive 2003/85/EC, Thierman 2005).

3.5.3 Serological tests for FMD antibodies are of two types and are as follows:

Those that detect antibodies to viral structural protein (SP) and those that detect antibodies to viral non-structural protein (NSPs). Serological tests could have different interpretations of test results which will be appropriate according to the above mentioned purposes and the validation of the selected procedure must take account of the purpose. For example, test cut-offs may be set at a different threshold for herd-based serosurveillance than is appropriate for certifying freedom from infection for individual animals for the purposes of international trade.

The SP tests are serotype-specific and detect antibodies elicited by vaccination and infection; examples are the virus neutralisation test (VNT), the solid-phase competition ELISA (SPCE) and the liquid phase blocking ELISA (LPBE). These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. They are the prescribed tests for trade and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field.

3.5.4 Compliment Fixation Test (CFT)

The Compliment fixation test has been the traditional test for diagnosis, but has been replaced in many laboratories by the enzyme-linked immunosorbent assay (ELISA), as this is more specific and sensitive. The test has been used extensively to distinguish different strains of FMDV (Ivanov & Tekerlekov 1989, Rweyemamu *et al.* 1978).

Although CFT was a relatively fast test it needed high virus load and the results were sometimes affected by pro or anti-complimentary activities of the samples (Ferris & Dawson 1988). The test is recommended by OIE as an alternative test for international trade (OIE 2009).

3.5.5 ELISA

The ELISAs are blocking or competition-based assays that use serotype-specific polyclonal antibodies (PAbs) or monoclonal antibodies (MAbs) are quicker to perform and are not dependent on tissue culture systems and the use of live viruses. It can be performed with inactivated antigens thus requiring less restrictive biocontaminant facilities (OIE 2009). Although ELISA is a more accurate test than CFT, low titre false-positive reactions can be expected in a small proportion of the sera (Grubman & Baxton 2004). An approach combining screening by ELISA and confirming the positives by the VNT minimises the occurrence of false-positive results. Reference sera to standardized FMD SP serological tests for some serotypes and subtypes are available from the Reference Laboratory at Pirbright.

The detection of antibody to the NSPs of FMDV can be used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated. Therefore the tests can be used to confirm suspected cases of FMD and to detect viral activity or to substantiate freedom from infection on a population basis. For certifying animals for trade, the tests have the advantage over SP methods that the serotype of virus does not have to be known. However, there is experimental evidence that some cattle, vaccinated and subsequently challenged with live virus and confirmed persistently infected, may not be detected in some anti-NSP tests, causing false-negative results.

3.5.6 Liquid phase blocking ELISA

The liquid phase blocking ELISA (LPBE) was developed by Hamblin *et al.* (1986). It uses bovine convalescent sera for characterization of field isolates with comparable results with conventional VNT (Kitching *et al.* 1988, Lunt *et al.* 1994). The LPBE is a

preferred test in large scale serology for FMD and has the benefits of being adaptable to the serum of all FMD-susceptible species. This test although highly sensitive, is less specific and therefore has a tendency to produce false positive results with potentially serious repercussions. A solid phase ELISA has been developed which has high specificity. The virus neutralisation test (VNT) and liquid phase blocking ELISA (LPBE) 7-9 are currently the recommended tests by the OIE. However, these tests require each serotype to be tested separately, are time consuming to perform, require virus containment facilities and cannot differentiate vaccinated from convalescing animals.

3.5.7 Ceditest® FMDV-NS

The Ceditest® FMDV-NS is a commercially available kit produced by Cedi Diagnostics B.V. Ceditest® FMDV-NS kit. It is a blocking ELISA that detects antibodies against the non-structural 3ABC protein of FMDV of all seven serotypes and it may be used to detect infection of vaccinated animals (Sorensen *et al.* 2005). Furthermore, it is independent of the serotype that causes the infection and independent of the fact that the animal is vaccinated or not. In addition, as a blocking ELISA it can be used for all species without the need for a species specific antigen. However, this test has not been validated for use in wildlife species. The monoclonal antibody (MAb) is based on European viruses and the test is not well suited to SAT type viruses (Sorensen *et al.* 1998). The test can therefore be expected to perform sub-optimally for African viruses and exposed animals may test negative. The test is assumed to have a Sensitivity of 97.2% and Specificity of 98.1% (Paton *et al.* 2006).

The theoretical background to the test system is as follows: Animals infected with FMDV produce antibodies to both structural proteins (SP) and non-structural proteins (NSP) of the virus. The tests for SP antibodies include the SP-ELISA and the Virus Neutralization Test (VNT). The SP tests are serotype specific and for optimal sensitivity should utilize an antigen or virus closely related to the field strain against which antibodies are being sought. Tests for NSP antibodies include NSP I-ELISA 3ABC and the Electro-immunotransfer blotting technique (EITB) as per recommendation in the

Terrestrial Manual of the OIE (2006) or equivalent validated tests. FMDV non-structural proteins (NSP) are only produced when the virus replicates in permissive cells. Modern FMDV vaccines produced by reputable manufacturers are highly purified and inactivated. They cannot replicate, and have, to date, shown no evidence of containing co-purified 3ABC NSP. Therefore seroconversion of susceptible animals to 3ABC is considered indicative of FMDV infection.

It should be noted that antibody to 3ABC appears later in infection than antibody to the structural proteins and that the duration of the response has not been elucidated. Due to low sensitivity of this assay, especially early in infection, the assay is recommended only for screening herds of animals, not on an individual animal basis. Even though from its beginning, 3ABC is proven to be best in performance in many studies, no single NSP can differentiate infected from vaccinated animals with complete confidence.

3.5.8 Viral Neutralization test

Antibody to FMD virus can be detected in the FMD viral neutralisation test (VNT) by the ability of the serum to prevent CPE when mixed with virus of known titre and added to cultures of susceptible cells. The titre of the serum is defined as that dilution of serum that is able to neutralize 100 TCID₅₀ of virus in 50% of individual inoculated cell monolayers, i.e., the 50% end point titre. (Golding *et al.* 1976). The VNT is now largely used as a confirmation test for sera found positive by ELISA and for import/export certification when importing countries specify the use of the VNT.

The Viral Neutralization Test is serotype specific and highly sensitive provided the virus or antigen used in the test is closely matched with the strain circulating in the field (Golding *et al.* 1976). The VNT requires cell culture facilities, the use of live virus and takes 2–3 days to provide results. Traditional antibody assays, such as the virus neutralisation test, rely on cells as an indicator of viral activity. Cells may not only become contaminated but also vary in sensitivity towards the agents being tested. In addition, a wide range of antibodies are involved in the immune response, not just those that neutralize. Therefore, non-sterile assays that can use either live or inactivated

antigens, measure all antibodies binding to a virus, and use an enzyme reaction as an indicator, are often preferred. This is the prescribed test for international trade (gold standard). The quantitative VN micro test for FMD antibody is performed with IB-RS-2, BHK-21, lamb or pig kidney cells in flat bottomed tissue-culture grade microlitre plate's appendix B. The VNT is routinely used at Botswana Veterinary Institute (BVI) as a confirmatory test for FMD diagnostic and samples from Namibia are normally sent there for confirmation either in an event of outbreak or for surveillance.

3.6 History of Foot-and-Mouth Disease and control measures in Namibia

Namibia has a history of Foot-and-Mouth Disease (FMD) occurrence, prevention and control measures that dates far back into the 1930s, Figure 3. FMD is a notifiable disease in Namibia under the Animal Health Act 1 of 2011, previously the Animal Disease and Parasites Act 13 of (1956). This entails the compulsory notification of a suspected FMD outbreak by the owner of such animals, prevention of access to such animals, the control of the movement of animals infected and the keeping of required registers in respect of number of animals, proper identification, departure and arrival registers. A national control programme has been developed with the aims to:

- 1) safeguard the Namibia livestock industry against the introduction of the disease
- 2) diagnose outbreaks as early as possible
- 3) prevent the spread and achieve eradication by an immediate high level control activity
- 4) create a reasonable immune cattle population in areas most likely to be infected from neighboring territories by means of bi-annual and tri-annual prophylactic vaccinations.

The dramatic outbreak of Foot-and-Mouth Disease that began in 1961 led to erection of the Namibian Veterinary Cordon Fence (Schneider 1994). At that stage, no FMD control infrastructure existed in Namibia, and the situation that developed can only be characterized as a nightmare. The original outbreak started on a farm in the Windhoek district in July 1961. Clinical symptoms were seen in domestic livestock and game. The origin was suspected to have been infected small stock imported illegally from a

neighboring country. The disease spread rapidly across nearby farms, with wild antelope apparently playing a key role in its spread. By the end of the year, farms in most of the commercial farming areas and communal areas north of Windhoek and Rehoboth were infected.

Since August 1961, a network of fences was erected including the present-day Veterinary Cordon Fence. The construction took many years to complete and the last strand of wire was put in place in 1980. The VCF runs from near Palgrave Point in the west, along the southern border of the Etosha National Park and curves around to the Gam area (20th parallel) in the east. It is a double fence, consisting of a high game proof fence on the northern side, separated by a 10-metre space or passage from a stock proof fence on the southern side. All told, the VCF is approximately 1251 km long. The effect was to divide the country into compartments with a good infrastructure for animal movement control (i.e. the honeycomb of farm fences in the central areas) from areas where movement control was not possible, and would prevent large-scale game migrations. Where possible, existing farm fences were raised in height to become, game-proof fences and elsewhere, new fences were constructed. The idea was that if FMD broke out in one of the compartments, its border fences would be elevated to full cordon status, and all thoroughfares would be blocked thus sealing off the disease. Strategic vaccination, working from the outside of the heavily infected central area towards the centre, was carried out. The 1961 outbreak had a profound effect on the rendering of veterinary services in Namibia, and thus bears describing (Schneider 1994).

A total ban on all livestock exports from Namibia was imposed with disastrous consequences for the livestock industry. A quarantine area with a diameter of 80 km around all foci was created, in which livestock were inspected at 3-day intervals. A further control area (80 km around the quarantine area) was instituted, with inspections at weekly intervals. Roadblocks were implemented on roads leading from all infected farms to enforce an embargo on livestock and animal product movement. By December 1962, the outbreak had come to an end and Veterinary Services had been restructured and was better able to cope with FMD. However, during 1962, another outbreak of

FMD occurred near Ondangwa, originating from animals illegally imported from Angola. In May 1964, FMD flared up for the last time in the commercial farming area. It was reported on four farms in the Kalkfeld area. The virus type was found to be closely related to that which caused the 1961 outbreak. It is thought that the virus had lain dormant in a game animal for 13 months before being transmitted to domestic animals.

In August 2000 the first outbreak of FMD (SAT1) since 1994 occurred in the Kasika area of the Katima Mulilo district. The usual movement control measures plus ring vaccination with trivalent SAT1, 2 and 3 vaccines around the focus and vaccination in the rest of the district was instituted. There was no secondary spread detected after a thorough surveillance was done in the region. A total of 138542 ($\pm 95\%$) head of cattle were vaccinated in the first round and repeated in a second round. Because this area lies in the FMD infected, zone exports from the OIE-recognized FMD-free zone were not affected, again illustrating the importance of these zones in control of transboundary animal diseases. There is thus empirical evidence that the integrity of these zones has been successfully maintained. The records of primary FMD outbreaks and their possible sources are summarized in the table below:

Table 1: Time line of FMD outbreaks 1934-2013

Year	Area/District	Type of FMDV	Probable Source	Control Measures
1934	Gobabis	?	Botswana	Stamping out and movement control Stock free zones
1945	Kavango	?	Botswana	Artificial Infection Stock free zones
1946	Ovambo-Ombalantu	?	Angola	Disinfection 17 cattle destroyed
1949	Kavango	SAT 1	Angola	Stock free zones Cordons Artificial Infection
1956	Eastern Caprivi	SAT 2	Zambia	Artificial Infection

1958	Ovambo/Kaokoland	Valleé A	Angola	Stock free zones Artificial infection Fencing
1960	Eastern Caprivi	SAT 2	Zambia	Stock free zones Artificial infection
1961	Central Districts	SAT 1	Botswana	Game & stock-proof fencing, Cords Vaccination Artificial infection
1962	Ovambo (north central Namibia)	A	Angola	Vaccination Cords
1964	Kalkfield	SAT1	Namibia	Vaccination Cords
1967	Ovambo	A	Angola	Vaccination
1968	Kavango	SAT 2	Angola	Vaccination
1969	Ovambo	SAT 2	Angola	Vaccination
1970	Ovambo	SAT 2	Angola	Vaccination
1971	Eastern Caprivi	SAT 2	Zambia	Vaccination
1975	Eastern Caprivi	SAT 2	Zambia	Vaccination
1978	Eastern Caprivi	SAT 2	Botswana	Cordon Vaccination
1980	Eastern Caprivi	SAT 1	Zambia	
1989	Eastern Caprivi	SAT 2	Botswana- Buffalo	Movement control, vaccination: bivalent SAT1 & 2
1991	Eastern Zambezi	SAT 2	Botswana- Buffalo	Movement control, ring vaccination
1992	Kavango-Nanazi	SAT 2	Undetermined- ?Angola Buffalo	Movement control, ring vaccination then mass vaccination of whole of Kavango (103500 cattle)
1994	Eastern Zambezi- Kasika	SAT 3	Buffalo	Movement control, ring vaccination SAT 1, 2 & 3.
2000	Eastern Caprivi- Kasika	SAT1	Buffalo	Ring vaccination, trivalent SAT1, 2 & 3.
2007	Eastern Zambezi- Nankuntwe, Muzi	SAT 2	Spread from Zambia	Movement control, ring vaccination SAT 1, 2 & 3.
2008	Kamutjonga	SAT 2	Spread from Botswana	Movement control, ring vaccination SAT 1, 2 & 3.
2010	Impalila Island	SAT1	Buffalo/Cattle contact	Movement control, ring vaccination SAT 1, 2 & 3.
2011	Masikili	SAT1	Buffalo/Cattle	Movement control, ring

			contact	vaccination SAT 1, 2 & 3.
2013	Ivilivinzi	SAT1	Buffalo/Cattle contact	Movement control, ring vaccination SAT 1, 2 & 3.

According to Table 1 above the last FMD outbreak occurred in protection zone (eastern part of Kavango east) in 2008. This outbreak was due to SAT 2 strain of the FMD virus. The last outbreak of FMD in the rest of the protection zone was in 1970, which is forty four (44) years ago.

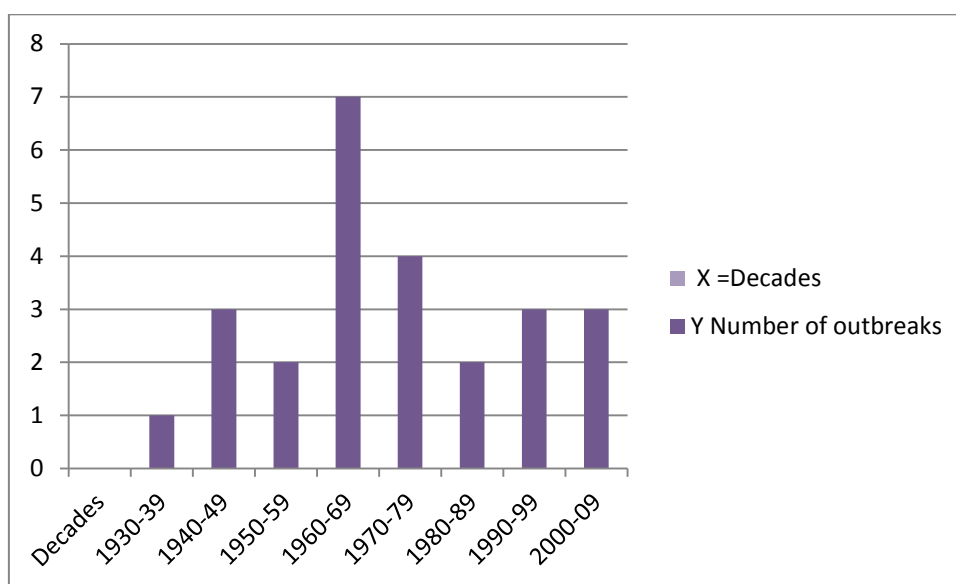


Figure 3: Temporal Distribution of FMD Outbreaks by Decade

Foot and mouth disease in the infected Zone (Zambezi Region)

The Zambezi Region is the only FMD-infected region of Namibia. Four (4) FMD outbreaks occurred in Zambezi Region during the period 2007 to 2013. Before 2007, the last outbreak in Zambezi region had occurred in 2000, at Kasika in the Kabbe Constituency involving a SAT-1 virus attributed to contact with buffaloes (Bishi & Kamwi 2008). Historically, outbreaks occurred in the region in 1956, 1960, 1971, 1975 and 1978 (SAT-2), 1980 (SAT-1), 1989 and 1991 (SAT-2, believed to have originated from buffaloes in Botswana) and 1994 (SAT-3, attributed to contact with buffaloes)

(Bishi & Kamwi 2008). Infection in the earlier outbreaks was believed to have come from Zambia or Botswana, with the source species not identified (Bishi & Kamwi 2008).

The 2007 outbreak, caused by a SAT-2 virus, was reported on 15 November 2007 to OIE after pre-confirmation on 14 November. The report gives the start date as 7 November 2007 in Nankuntwe, in Kabbe Constituency, on the border with Zambia. It spread to 14 crush pens in Kabbe Constituency and eight (8) in the neighboring constituency of Katima Rural. The outbreak was finally reported to be resolved on 1 December 2008.

Subsequent outbreaks occurred in April 2010, November 2011 and August 2013, all involving SAT-1 viruses and all probably having originated from buffaloes. The 2010 outbreak occurred in cattle on Impalila Island in the far eastern tip of the region and was attributed to cattle and buffaloes being trapped on the island by rising flood waters. The outbreak did not spread beyond the original focus and was declared resolved on 6 August 2010.

The next outbreak was reported to have started on 26 November 2011 at Masikili on the southern border of the Kabbe Constituency. The infection was attributed to contact with buffaloes, probably from the Chobe Reserve in Botswana. Spread was restricted to three neighboring crush pens, Ihaha to the east in Kabbe Constituency and Ngoma and Ikumwe to the west, the latter situated in Katima Rural Constituency on the main road from Ngoma to Katima Mulilo. It was declared resolved on 20 March 2012.

The last outbreak prior to this report is reported to have started on 4 August 2013 in Ivilivinzi village, on the south-eastern border of Kabbe Constituency, with a case recorded in Ikumwe (Katima Rural) on 8 August 2013, but no further cases were discovered and the event was retrospectively declared resolved on 17 February 2014 after the last laboratory results were received.

3.6.1 Zoning and how it works.

Zoning is defined as procedures implemented by a Member Country under the provisions of Chapter 4.3 and Article 4.3.1 of the OIE terrestrial code with a view to defining subpopulations of distinct health status within its territory for the purpose of disease control and/or international trade (OIE 2014). While zoning applies to an animal subpopulation defined primarily on a geographical basis (using natural, artificial or legal boundaries).

Compartmentalization is a procedure implemented by a Member Country also under the provisions of this Chapter 4.3 with a view to defining subpopulations of distinct health status within its territory for the purpose of disease control and/or international trade. Compartmentalization applies to an animal subpopulation defined primarily by management and husbandry practices related to biosecurity. In practice, spatial considerations and good management including Biosecurity plans play very important roles in the application of both zoning and compartmentalization.

A biosecurity plan means a plan that identifies potential pathways for the introduction and spread of disease in a zone or compartment, and describes the measures which are being or will be applied to mitigate the disease risks, if applicable, in accordance with the recommendations in the Terrestrial Code.

Commodity means live animals, products of animal origin, animal genetic material, biological products and pathological material.

Table 2, represents a hypothetical country (square in shape) which has a free zone within its borders. The free zone must, in terms of OIE regulations at the time of the study be separated from the infected zone by a buffer zone (where vaccination is practiced) and a surveillance zone (where vaccination is not practiced).

Table 2: Hypothetical country to illustrate zoning (square in shape) which has a free zone within its borders

Infected zone	Presence of free-roaming buffalo, periodic FMD outbreaks, regular vaccinations of livestock & high frequency surveillance by veterinary staff.
Buffer zone	No free-roaming buffalo, but sporadic outbreaks possible due to proximity to infected zone: Livestock vaccinated regularly, high frequency surveillance.
Surveillance zone	No free-roaming buffalo, danger of FMD outbreaks very remote, no FMD vaccinations, medium frequency surveillance, and stock movement control.
Free zone	Possibility of FMD outbreaks non-existent, no free-roaming buffalo, no FMD vaccinations, low frequency surveillance, stock movement control and livestock traceability system in place.

Table 3: The Namibian context of Foot-and-Mouth Disease zoning until the end of year 2009.

Infected Zone	<p>Caprivi east of the Kavango River.</p> <p>There are free-roaming buffalo here, and in neighboring countries; FMD outbreaks occur sporadically in cattle and cattle are vaccinated against FMD twice or even three times a year. Cattle herds are inspected on a monthly basis by veterinary staff. It is separated from the Buffer Zone by the Kavango River, which acts as a natural barrier.</p>
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Buffer Zone	<p>The rest of Namibia north of the present Veterinary Cordon Fence, including the former Kaokoland (Kunene region). The Owambo-speaking regions (Oshikoto, Oshana, Omusati, Ohangwena) and the Kavango region. Annual FMD vaccination is carried out in the largest part of these regions except in Kunene, and Omusati areas, FMD outbreaks are rare except recent outbreak in Kavango at Mukwe constituency. Herds are inspected on a monthly and weekly basis in this region. Animals are quarantined for 21 days before slaughter, deboned thereafter and kept for another 21 days in refrigeration before marketing takes place. It is separated from the Surveillance Zone by the Veterinary Cordon Fence. This study focused on this specific zone (buffer) and was split into two main areas (A and B).</p>
Surveillance Zone	<p>For the most part (except where it encloses communal areas), this is an area approximately two farms wide immediately to the south of the Cordon Fence where monthly herd inspections are carried out. No FMD vaccinations are carried out, and it is separated from the Free Zone by the southern borders (fences) of the farms/communal areas concerned.</p>
Free Zone	<p>The rest of the country south of the Surveillance Zone is regarded as a free zone. The last FMD outbreak in this area was in 1964; no FMD vaccinations are carried out, and herd inspections are mostly scheduled on a six-monthly basis.</p>

Table 3 and Figure 4, represents the Namibian context of FMD control zone until the end of year 2009.

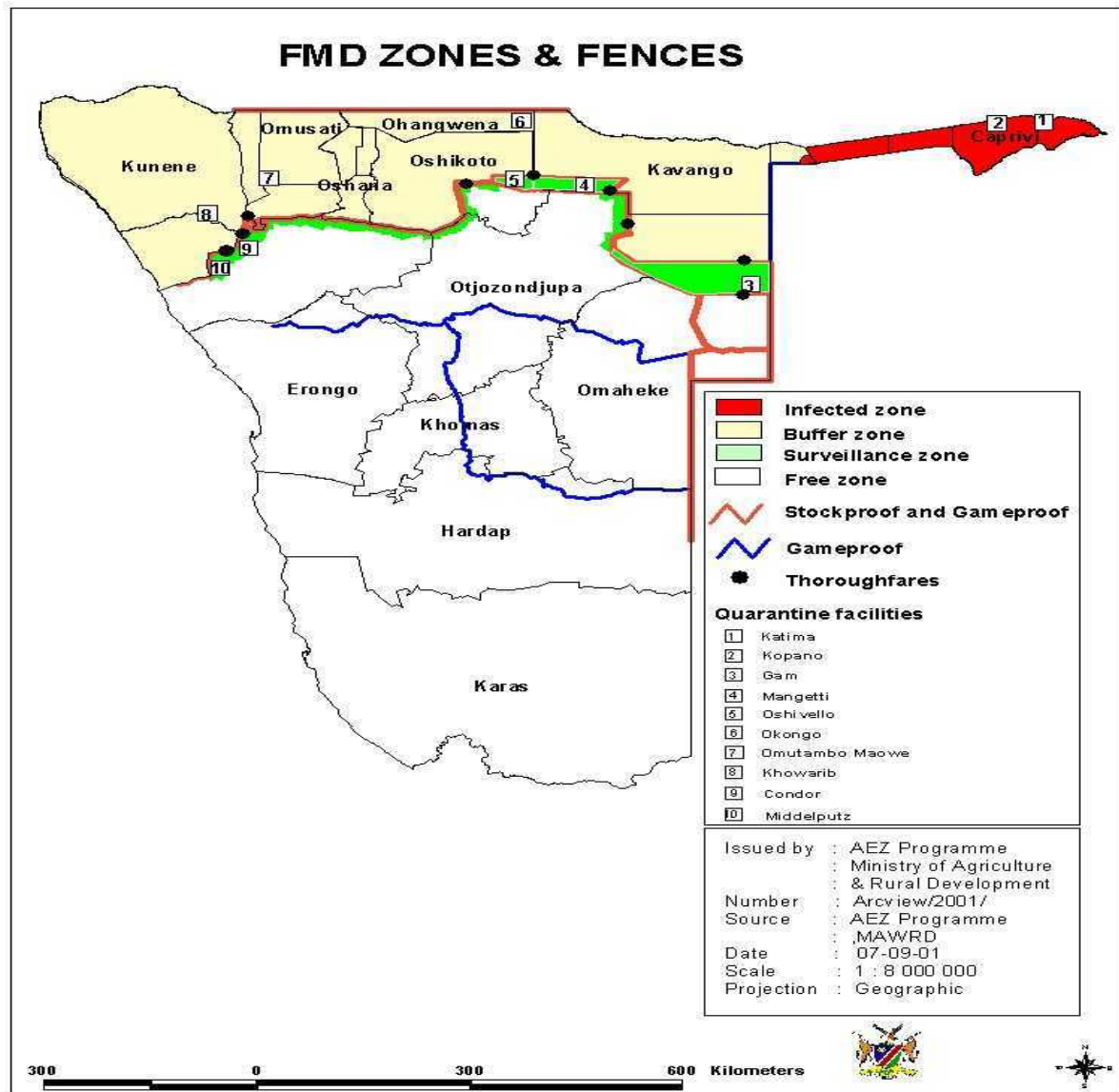


Figure 4: Namibia Map of Foot-and-mouth Disease control used until end of year 2009

Thanks to these control measures Namibia still enjoy export of beef to lucrative markets and the strategy is to make sure that the whole country can export to profitable markets such as China, Hong Kong, Dubai and the United States of America. The zones are separated from one another either by natural boundaries which is in this case is the Kavango and Kunene rivers or artificial barriers which is commonly the fence as

indicated in the maps.

The literature review has highlighted the importance of maintaining disease freedom in Namibia and the significance of the zone system with respect to effective disease control measures, to safeguard the existing lucrative market and the potential of new market opportunities. Livestock trades play a very big role in the national economy as well as accrued benefit to the farmers and job creation. Hence the objective of this study was to survey the Namibian cattle population in the NCA Namibia for FMD virus to establish if this area of the country is free of FMD. This would then justify opening the area to trade in animals and animal products.

CHAPTER 4

4. MATERIALS AND METHODS

4.1 Study site and population

The survey was conducted in the Northern Communal Areas (NCA) of Namibia for the period between May and November 2007. The area of interest was the FMD buffer zone (currently the FMD protection zone) that comprises of six regions namely Kunene, Omusati, Oshana, Oshikoto, Ohangwena and Kavango as shown in (Figure 3).

This was a multistage survey designed to detect Foot-and-Mouth Disease Virus, which was also extended to determine the prevalence of Brucellosis in cattle. The total cattle population was estimated to be around 1 090 978, as per 2006 census figures provided by the Directorates of Veterinary Services (DVS) and shown in Table 4 and 5.

Table 4: Census figures and estimated number of samples required in Area A (not vaccinated against FMD) of the Namibian NCA in May – August 2007

Parameters	Kunene north	Omusati south	Oshikoto (NCA)	Oshana	Grand Total
No. of Cattle (N)	217879	116147	218882	108175	661083
No. of CP per region	140 (19%)	131 (18%)	347 (47%)	116 (16%)	734 (100%)
Estimated No. of CP sampled (stage 1)	49	47	122	42	260
Estimated No. of cattle sampled per region (stage 2)	735	705	1830	630	3900

Table 5: Census figures and estimated number of samples required in Area B (vaccinated against FMD) of the Namibian NCA in May – August 2007

Parameters	Omusati north	Ohangwena	Kavango	Grand total
No. of cattle(N)	130527	171385	127983	429895
No. of CP per region	159 (20%)	198 (25%)	432 (55%)	789 (100%)
Estimated No. of CP sampled (stage 1)	54	68	148	270
Estimated No. of cattle sampled per region (stage 2)	1080	1360	2960	5400

The census figures were collected in the NCA in 2006 by para-veterinary staff referred to as Animal Health Technicians (AHT) in DVS during the annual vaccination campaign against FMD and Contagious Bovine Pleuropneumonia (CBPP) in the study area. A three stage sampling strategy was used, in which crush-pens (CP) were the primary sampling unit, herds the secondary sampling unit and individual animal, the tertiary sampling unit. Furthermore, in Area B animals were stratified by age to maximize the detection rate.

4.2 Definitions of a Crush-Pen and a Herd

For the purpose of this study, the term “Crush-pen” was defined as an official cattle handling facility mainly constructed and maintained and used by DVS officials for animal inspections, vaccinations and demonstration of other veterinary procedures such as branding, dehorning and castration. It is either made of steel or wooden poles. They are widely distributed in farming areas in such a way that every cattle farmer is within an average distance of 5 km from one crush-pen to another. The density of crush-pens also depends on the cattle population density in a particular area. The number of cattle

belonging to a crush-pen ranges from 150 to 2000 cattle.

For the purposes of disease surveillance, a crush-pen in Namibia is considered as an epidemiological unit which could be equivalent to a “dip tank” in Zimbabwe or “parcel of land” in South Africa (Hesterberg 2007). Within a crush-pen there may be one or more herds of cattle. For the purposes of this study, a cattle “herd” is defined as any cluster or aggregate of cattle belonging to the same kraal or cattle post in a communal area, which is equivalent to a farm in a commercial area. A herd may be owned by more than one farmer or family.

4.3 Definition of study areas

For the purpose of this study, the six regions were divided into two main areas; Area A and B depending on the FMD vaccination status. Furthermore, the Omusati region was subdivided into Omusati north (bordering Angola) and Omusati south which is more towards Oshana region. Area A, included Kunene north, Omusati south, Oshana, and Oshikoto(NCA) regions and Area B, is comprised of Omusati north, Ohangwena and Kavango region (Figure 5).

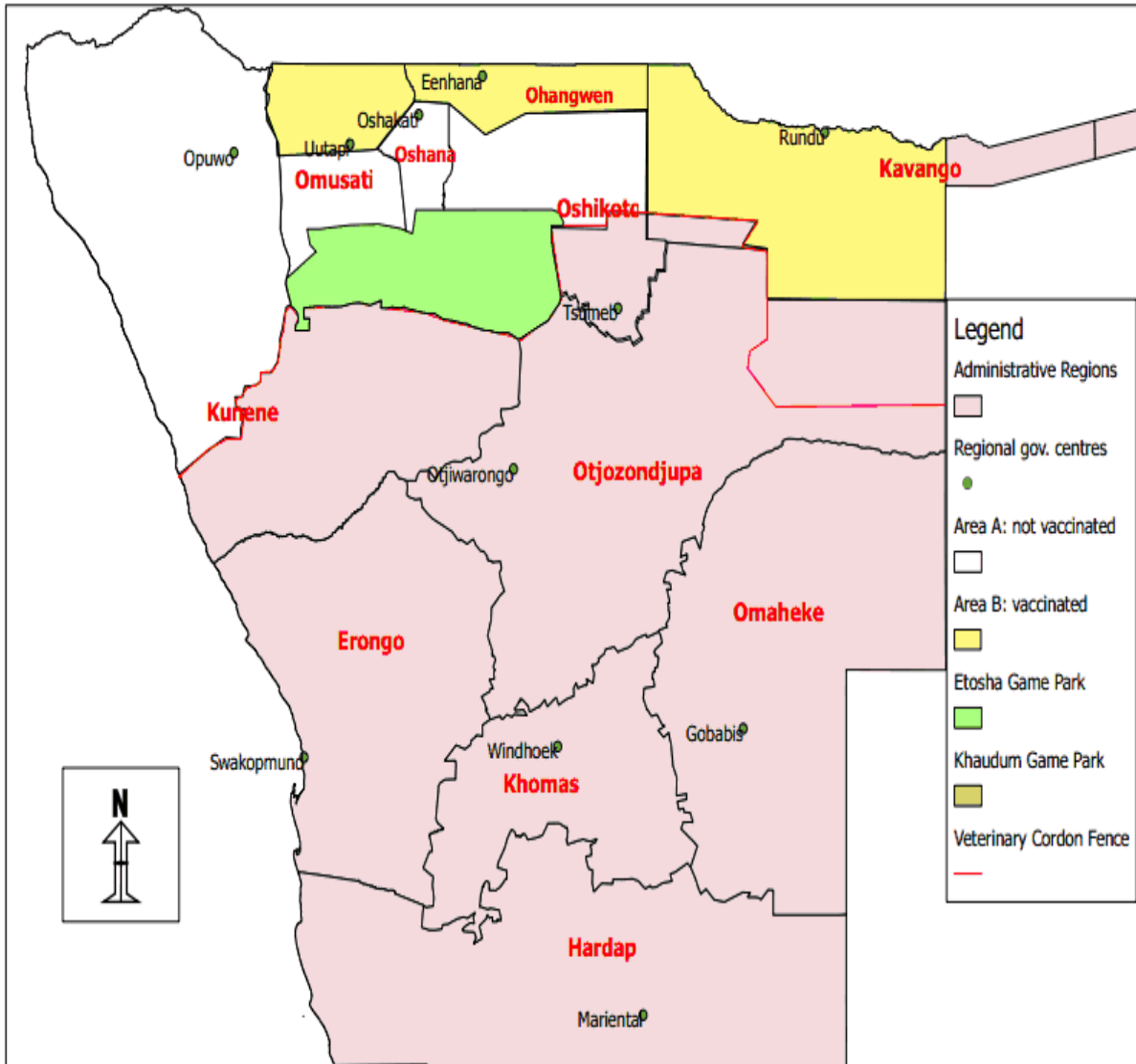


Figure 5: Map of Namibia showing the regions and surveyed areas (Area A & B).

It was assumed that 70% of the cattle population had never been vaccinated against FMD and the remaining 30% had not been vaccinated for the past 8 years. Meanwhile, in Area B cattle had been vaccinated against FMD within the last 10 years and the area included Ohangwena, Kavango and Omusati north.

4.4 Identification of cattle

All cattle were tagged as soon as they were sampled as per Figure 6. Each ear tag used had a unique codification as a means of individual identification and was applied either

to the left or right ear using an applicator. The tag was intended to become part and parcel of individual identification and was recorded on a National database for livestock identification system of Namibia. Cattle were selected because it is known that they are the most prominent livestock found in these areas and for being a highly susceptible species to FMD and would thus serve as a good indicator of the presence of FMD virus circulation.



Fig 6: Ear tags used for individual identification of sampled cattle in both areas.

The target group included all types of breeds found in the area such as Sanga, Nguni, Brahman and Crossbreeds over six month of age of both sexes. Importantly, the two areas in the survey are contiguous and practically there are no natural or artificial barriers to prevent animal movement from one area to another. Thus it is possible for cattle to migrate from one area to another with or without change of ownership and that some animals living along the border of Namibia–Angola could graze or drink water across the border and vice versa. Strategies were being put in place by Veterinary Services Namibia to control more effectively animal movements through an acceptable movement permit system including border control (Hendrix 2001). This permit system is supported by the compulsory requirement that all animals must have an official hot

iron brand mark at 6 month of age as stipulated in the Stock Brand Act of 1953 and its amendment.

4.5 Sampling

4.5.1 Sampling frame

The sampling frame consisted of a list of crush-pens as the initial sampling unit, a list of cattle herds as a secondary sampling unit and individual animals within the herd as a tertiary sampling unit, which also took into consideration the age category in Area B. The number of sampling units as well as the number of animals sampled per CP was calculated by using the software programme Survey Tool Box (FreeCalc). In brief, this is an epidemiological probability calculator designed to assist with the planning and analysis of surveys to demonstrate freedom from disease, or surveys to detect disease (Cameron & Baldock 1998a). The program enables the accurate calculation of survey sample-size requirements, and the precise analysis of survey results. As a result, survey costs can be minimised, and survey outcome will reliably provide the required level of proof. The component FreeCalc uses a trial-and-error searching algorithm to calculate the exact sample size. Using an iterative procedure, it calculates the number of animals that must be tested in order to provide evidence, at the specified level of confidence, that the disease is not present. It also reports the cut point number of reactors, or the maximum numbers of test-positive results that can be observed and still conclude the population is free from disease (i.e. false positives). At the heart of FreeCalc Epitools is a probability formula which adjusts for imperfect tests and finite population size, which was derived in the following way:

Perfect test, infinite population.

Over multiple trials, the probabilities of a positive or a negative at each trial are multiplied to give the general formula for the binomial distribution Equation 1 (Cochran 1977).

$$P(T^+ = x) = \binom{n}{x} p^x (1 - p)^{(n-x)}. \quad \text{Equation 1}$$

Where p is the disease prevalence

If a perfect test is used, a survey to substantiate freedom from diseases requires that no diseased animals are found. When $x = 0$ this formula simplifies to:

$$P(T^+ = 0) = (1 - p)^n. \quad \text{Equation 2}$$

Imperfect test, infinite population.

When an imperfect test is used, the situation becomes more complex. The probability of getting a positive test result $P(T^+)$ when testing a single animal depends on the true disease status of that animal. If it is disease positive, $P(T^+)$ is equal to Se ; if it is disease negative, $P(T^-)$ is equal to $1 - Sp$. The overall probabilities $P(T^+)$ and $P(T^-)$ are given by: $P(T^+) = pSe + (1 - p)(1 - Sp)$ and $P(T^-) = p(1 - Se) + (1 - p)Sp$. **Equation 3**

The probability of observing x reactors when testing n animals from an infinite population is given by the binomial distribution Equation 2 with the positive and negative probabilities substituted from Equation 3.

$$P(T^+ = x) = \binom{n}{x} [pSe + (1 - p)(1 - Sp)]^x [p(1 - Se) + (1 - p)Sp]^{n-x}. \quad \text{Equation 4}$$

Perfect test, finite population.

In the case of finite population sizes, trials are not independent. When the first animal is drawn, the probability of drawing a D^+ is d/N , but with each D^+ drawn, d is decreased by one. N decreases with every animal drawn. When a perfect test is used, the probability that T^+ and D^+ will equal x is given by the hypergeometric distribution:

$$P(T^+ = x) = \frac{\binom{d}{x} \binom{N-d}{n-x}}{\binom{N}{n}}. \quad \text{Equation 5}$$

When aiming to substantiate freedom from disease x is equal to zero, and the formula simplifies to (Cannon and Roe, 1982):

$$P(T^+ = 0) = \frac{(N-d)!(N-n)!}{(N-d-n)!N!}. \quad \text{Equation 6}$$

Unfortunately, factorial formulae involving numbers are awkward to calculate. Hand-held calculators can usually calculate factorial no higher than about 70, and personal computers use logarithmic approximations to exceed factorials of about 170! A convenient approximation to this formula, (Cannon & Roe 1982) is:

$$P(T^+ = 0) = \left(1 - \frac{d}{N - \frac{n-1}{2}}\right)^n. \quad \text{Equation 7}$$

Imperfect test, finite population.

To overcome the limitations of the above formulae, the hypergeometric distribution Eq. (5) above was modified for imperfect tests (Cameron & Baldock 1998a). The number of D^+ in the sample has a hypergeometric distribution. Given y D^+ in the sample, the number of true positives is $\text{Bin}(y, \text{Se})$, and the number of false positives is $\text{Bin}(n-y, 1-\text{Sp})$. We will have x T^+ if we have j true positive and $x-j$ false positives. By considering the possible values of y and j , we can write down the final formula as:

$$P(T^+ = x) = \sum_{y=0}^d \frac{\binom{d}{y} \binom{N-d}{n-y}}{\binom{N}{n}} \sum_{j=0}^{\min(x,y)} \binom{y}{j} \text{Se}^j (1 - \text{Se})^{y-j} \binom{n-y}{x-j} (1 - \text{Sp})^{x-j} \text{Sp}^{n-x-y+j}. \quad \text{Equation 8}$$

Equation 8 was used for the calculation to calculate the sample design using FreeCalc.

4.6 Sampling procedures

The animal health technicians are staff members of the Directorates of Veterinary Services under the supervision of the author. Among their routine activities, is the annual programme to visit various communities at least twice a year. This opportunity was used by the author to sensitise farmers about this specific survey. The survey was carried out under the guidance of the author during the annual vaccination campaign in 2007. On the sampling day, farmers were gathered once again to explain to them the

procedures and to get their verbal consent to bleed their animals. A list of CPs for each region was created in Excel by the author and each CP was allocated a reference number. The CPs was proportionally weighed so that more CPs was selected from regions where there was more CP (Table 4). The required number of CP for each area was then randomly selected using random number selection. The sampling was subdivided into various stages as indicated earlier and detailed below:

4.6.1 Stage 1- Sampling Area A

Area A consisted of 734 CP of which 260 were selected. The calculated number of 257 CP was calculated using the software programme FreeCalc Epitools using Equation 8. Three CP were added to the calculated sample size to make provision for unforeseen incidents such as low turn up of animals, failures to get farmers consent for the animals to be sampled, samples getting spoiled, depleted or lost.

This was done by entering the number of CP and assuming that if FMD is present in the area at least 1% of the CP or more will be infected. It was assumed for the calculation that herd sensitivity was equal to 99%. The sensitivity of 99% was based on the assumption that cattle in Area A have never been vaccinated against FMD making them more prone to infection than the vaccinated group in Area B. In other words if the disease occurred in a herd, it is most likely that the majority of animals would show signs and the herd would be easily diagnosed as having FMD. The herd specificity was assumed to be 100% based on the assumption that if a positive herd was found, the diagnosis of FMD would be confirmed 100% of the time using a combination of clinical and serological diagnostic tools.

4.6.2 Stage 1: Sampling Area B

Area B consisted of 789 CP of which 270 were selected. The calculated number of 259 CP was also calculated using the software programme FreeCalc Epitools using Equation 8 (Cameron & Baldock 1998b). Ten CP were added to the calculated sample size to accommodate any unforeseen incidents for the reason mentioned earlier in stage 1 sampling Area A and to account for the fact that some CP were not functional in this

area. It was also assumed that, if FMD is present in the vaccinated area (Area B), at least 1 % of CP will be infected. A herd sensitivity of 95% and a herd specificity of 100% were used for calculation. A lower herd sensitivity of 95% was used to calculate the sample size for area B because it was assumed that if animals are vaccinated they are less likely to show clinical disease (Robiolo *et al.* 2006). Hence lower herd sensitivity can be anticipated but an animal that shows clinical signs was unlikely to be misdiagnosed.

4.6.3 Stage 2 and 3: Sampling Area A

The second stage was to determine the number of animals that were sampled at each crush-pen by using Survey Toolbox, FreeCalc sample size (Equation 8). The Cedi-NSP-ELISA sensitivity of 97.2 and specificity of 98.1 and assumed prevalence of 30% was used for these calculations. A required sample size of 14 was obtained with a cut point number of reactors equal to one per CP. The 14 samples required were rounded to 15 to cater for any type of incidental losses of samples. Blood samples were then randomly taken from each of the 260 CP chosen in the previous stage. A total of 3900 serum samples were taken from area A (Table 3). An initial sampling protocol to acquire the required number of blood samples was designed for this area as follows:

First of all, the team leader had to randomly select 3 herds, by assigning all the herds presented for vaccination at the crush-pen with a number corresponding to a herd owners name written onto a piece of paper. The papers would be folded and thrown into a hat for random selection. Secondly, the team leader would then ask different farmers or a staff member to withdraw one of the papers without looking into the hat. The first 3 selected numbers/names were the herds that were supposed to be sampled. Thirdly, once the cattle were lined up in the crush-pen, the team leader proceeded to count and assign a number to each animal. The first five papers drawn from the hat represented the cattle sampled.

4.6.4 Stage 2 and 3: Sampling Area B

Area B, which has undergone FMD vaccination in the last 10 years, included Omusati

north (border with Angola), Ohangwena and Kavango region. Calculations were made to determine the number of animals sampled per crush-pens by using Survey Toolbox, FreeCalc sample size the same way as in Area A. The Cedi- NSP- ELISA sensitivity of 97.2, specificity of 98.1, and assumed prevalence of 25% were used for the sample size calculation. A required sample size of 17 was obtained with a cut point number of reactors equal to one. The 17 samples obtained were rounded to 20 to cater for any type of incidental losses of samples. Blood samples per crush-pen were randomly taken from each of the 260 crush-pens chosen in the previous stage which bring the total serum samples taken to 5400 (Table 5).






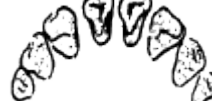

An initial sampling protocol to obtain the required number of blood samples in Area B was designed as follows:


Initially, the team leader randomly selected 5 herds by assigning all the herds presented for vaccination a written number or owner name on a piece of paper. The papers would be folded and placed in a hat for random selection. Secondly, the team leader requested one of the farmers or a staff member to blindly pick one of the papers at a time. The first 5 selected numbers/names were then used for sampling. In this area, animals were stratified by age into 3 risk groups based on the possible chance of being infected as indicated below:

Group 1: 6 to 11 months: Assumed to be unvaccinated (10 samples)

Group 2: 12 to 24 months: Assumed to be vaccinated once or twice. (5 samples)

Group 3: ≥ 24 month upward: Assumed to be vaccinated more than twice (5 samples)

Illustration	Age	Number of teeth present
	At birth to 1 month	Two or more of the temporary incisor teeth present. Within first month, entire 8 temporary incisors appear.
	2 years	As a long-yearling, the central pair of temporary incisor teeth or pinchers is replaced by the permanent pinchers. At 2 years, the central permanent incisors attain full development.
	2 1/2 years	Permanent first intermediates, one on each side of the pinchers, are cut. Usually these are fully developed at 3 years.
	3 1/2 years	The second intermediates or laterals are cut. They are on a level with the first intermediates and begin to worn at 4 years.
	4 1/2 years	The corner teeth are replaced. At 5 years the animal usually has the full complement of incisors with the corners fully developed.
	5-6 years	The permanent pinchers are leveled, both pairs of intermediates are partially leveled, and the corner incisors show wear.
	7-10 years	At 7 or 8 years the pinchers show noticeable wear; at 8 or 9 years the middle pairs show noticeable wear; and at 10 years, the corner teeth show noticeable wear.

	<p>12 years</p>	<p>After the animal passed the 6 th years, the arch gradually loses its rounded contour and becomes nearly straight by the 12 th years. In the meantime, the teeth gradually become triangular in shape, distinctly separated, and show progressive wearing to stubs. These conditions become more marked with increasing age.</p>
<p>Figure 7: Handy guide to determining the age of cattle by looking at the teeth. Source: Source RF Johnson. The Stockman Handbook by Ensminger, 2nd ed., page 539.</p>		

The aging of cattle was done by the AHT by examining the teeth and classified into age group based on the guide above (Figure 7).

4.7 Pilot visit to the study area

A pilot visit took place in the second week after the sampling had started with the aim to assess whether sampling procedures were working as planned. This is discussed further in the results.

4.8 Sample collection and handling procedures

Blood serum samples without heparin were collected by using a needle, vacutainer tubes as a container and needle adapter. The blood was withdrawn from the jugular or tail vein. The sample tubes were labeled immediately upon withdrawal of blood and the details filled onto a sample form. This exercise was performed with extreme care to ensure that the number on the tubes corresponded with the individual ear tag number. The label information had the ear tag number, dates of sampling, sex, breed and age of animals. Where samples could not reach the laboratory within 3 hours, the blood in vacutainer tubes was stored at room temperature for at least 10 hours (overnight) to allow separation of the blood clot and serum. The blood tubes were then placed in a tube holder before putting it in a cool box with adequate dry ice for transportation to the

state veterinary office or the regional laboratory. The regional laboratory is in Ondangwa town in Oshana region and the state veterinary office used were Outapi in Omusati region, Opuwo in Kunene region and Rundu in the Kavango region. The serum was separated before being sent to the Central Veterinary Laboratory in Windhoek for testing and to Botswana Veterinary Institute (BVI) when required.

4.9 Laboratory Test

4.9.1 Foot-and-Mouth Disease Serology

All serum samples were first screened with the Ceditest® FMDV-NS kit Cedi Diagnostics BV, Lelystad, The Netherlands (2006) to detect antibodies directed against the non-structural 3ABC protein of FMDV. For detecting antibodies against non-structural proteins, a blocking ELISA assay was carried out as per standard protocol procedures described by Sorensen *et al.*, (1998) see Appendix A.

The percentage inhibition (PI) of the controls and the test sera were calculated according to the formula and by Interpreting of the PI as indicated below:

The OD450 values of all samples are expressed as PI relative to the mean OD450 of the negative control (ODmax).

$PI = 100 - OD450 \text{ test sample} * 100 / OD450max.$

PI=<50% Negative no antibodies against the NS protein of FMDV.

PI= \geq 50% Positive there is antibodies against the NS protein of FMDV.

All positives samples to the Ceditest® NSP ELISA were subjected to the VNT to minimise the occurrence of false-positive results. VNT was performed according to the standard procedures in the OIE's Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE 2007) at BVI. It was assumed that the test has a specificity of 95% and a sensitivity of 97%. A reactor was considered negative if the titer was less than 0.9 (Titer cut off<0.9) and positive if higher than 0.9.

If a sample reacted on the VNT, a field follow up was made to do clinical examination of all animals in the herd from which the reactor animals originated. All cattle in a herd were then retested to double confirm that these animals are really reactors.

4.10 Processing and data analysis

Raw data was stored in Microsoft Excel where descriptive statistics were performed. The geographical distributions of the results were plotted using Quantum GIS Version 0.6 of 2002. In brief, Quantum GIS (QGIS) is a cross-platform free and open source desktop geographic information systems (GIS) application that provides data viewing, editing, and analysis capabilities.

CHAPTER 5

5. FINDINGS OF THE PILOT VISIT

5.1 Introduction

As a result of a pilot visit to the study site it became apparent that the original sampling strategy given above was not well directed to take into account the cultural practices in the various regions. It was therefore decided to try and incorporate cultural practices into the sampling strategy to get a representative sample of the cattle population. The first portion of the results explains the cultural aspects that were revealed and later incorporated into the final sampling strategy.

5.2 Area A (non vaccinated)

5.2.1 Kunene north region

When the study was designed using OIE guidelines it was envisage that all farmers would gather their animals at the CP early morning for vaccination, however this was not the case in this region since herd owners only took the cattle to the CP at intervals when it was their turn for the herd to be vaccinated. This meant there was not a pool of cattle from which herds could be selected and subsequently bled. This is to be expected given the cultural background of this people (see literature review), which will be discussed further later. Based on this finding the sampling protocol was modified taking into account the cultural practice. All the vaccination team leaders received a modified sampling protocol to carry out in this region. The sampling below involved selection of sampling units at equal intervals with the first selected randomly (Thrusfield 2005).

First of all, to come up with the sampling interval for the selection of the three required herds, a random number was randomly selected between 1 and 5 by simply placing numbered equal sized pieces of paper in a hat and without looking picking one of the five. The selected piece of paper gave the first herd to be sampled on arrival. Thereafter the sampling interval was applied to select the other two remaining herds upon their

arrival. For example, if number 4 was picked up, it meant that the first five animals would be sampled from the 4th herd to arrive at the crush pen and also meant that every fourth herd would be sampled until the required number of herds was obtained. In unpredictable circumstances where the targeted herd according to the intervals was not presented for vaccination, the next herd available was taken as replacement.

Secondly, once the cattle were lined up in the crush pens, the team leader proceeded to count and assign a number to each individual animal. Again using the "numbered papers in a hat system" five animals was randomly selected from the selected herd. While the process was not ideal it resulted in a more representative sample given the uncertainty of which farmers would end up bringing their cattle to the crush pens.

5.2.2 Omusati south/north, Oshana, Ohangwena and Oshikoto (NCA) region

In these regions all animal' owners gathered their cattle early in the morning at the crush-pens but without allowing animals to mix with other herds (Figure 8). This practice was according to the initial planned sample design and no modification was required for these regions.



Figure 8: Picture of a herd owner preventing her cattle not to mix with others herds in Omusati region

5.3 Area B (vaccinated)

5.3.1 Kavango, Omusati north and Ohangwena regions

Area B composed the following peoples such as the Mbalantu, Kwaludhi, Kwanyama and Rukavango in the Kavango region. Omusati south composed mainly of the Mbalantu speaking people and Ohangwena by Kwanyama.

When there is a vaccination campaign people in the region tend to bring animals to the crush pens early in the morning and cattle are processed as they arrive. However, they will not also allow them to mix with other herds Figure 9.



Figure 9: Picture showing the farmers with their herds without allowing them to mix with others herds in Ohangwena region

The sampling protocol described in the materials and methods was applied in this region since the farmers gather the animals at the crush pen early in the morning, except that step 3 was modified to cater for each age category.

CHAPTER 6

6. RESULTS

6.1 Area A (Not vaccinated against Foot-and-Mouth Disease)

Table 6: Results of the Ceditest® NSP ELISA for FMD in cattle with 95% confidence intervals (CI) in Area A during May - August 2007 for the different regions

Regions	Number of cattle tested	Cedi (+)	Apparent Prevalence (%)	95% CI
Kunene north	645	4	0.62	0.24,1.58
Omusati south	703	0	0	0.0,0.54
Oshana	657	4	0.61	0.24,1.55
Oshikoto	2209	2	< 0.01	0,0004,0.33
Total (Overall)	4214	10	0.24	0.13,0.44

Table 6 shows the results of the Ceditest® NSP ELISA for FMD in cattle with 95% CI in Area A during May - August 2007 for the different region. Out of the 4214 samples collected and tested in the four regions 10 reactors to the Ceditest® NSP ELISA with an average apparent prevalence of 0.24 and a 95% confidence interval of 0.13,0.44. Kunene north and Oshana each recorded 4 reactors, Oshikoto 2 and none were detected in Omusati south.

Table 7: Results of the Ceditest® NSP ELISA for FMD in cattle with 95% CI in Area A during May - August 2007 displayed according to crush pen

Regions	Crush Pen	Number Tested	Cedi (+)	Apparent Prevalence	95% CI
Kunene north	Omuramba	15	1	6.67	1.19,29.82
	Omakange	15	1	6.67	1.19,29.82
	Otjikukutu	15	1	6.67	1.19,29.82
	Otjiyawa	15	1	6.67	1.19,29.82

Oshana	Ondjondjo	20	1	5.0	0.89,23.61
	Oikango.G	20	1	5.0	0.89,23.61
	Omatando	20	1	5.0	0.89,23.61
	Onakamwandi	20	1	5.0	0.89,23.61
Oshikoto	Nyungu # 2	14	1	7.14	1.27,31.47
	Ohayiyanda	15	1	6.67	1.19,9.82

The apparent crush-pen level seroprevalence are shown in Table 7. Of the 260 crush-pens tested, only 10 CP had one sample reactor with a seroprevalence between 5.0 and 7.14 and a 95% confidence interval between 0.89, 23.61 and 1.27, 31.47 respectively. The number of reactors found at each crush pen was consistent with the number of cut point reactors anticipated by the software program FreeCalc.

Table 8: Viral Neutralisation test results in Area A during May - August 2007

Region	Crush Pen	SAT1 Titre	SAT2 Titre	SAT3 Titre	Positive/ Negative
Kunene north	Otjijawa	<0.15	<0.15	<0.15	Negative
	Otjikukutu	<0.15	<0.15	<0.15	Negative
	Omuramba	<0.15	<0.15	<0.15	Negative
	Omakange #2	<0.15	0.3	0.45	Negative
Oshana	Ondjondjo	<0.15	<0.15	<0.15	Negative
	Omatando	<0.15	<0.15	<0.15	Negative
	Oikango.Gabes	<0.15	0.3	0.45	Negative
	Onakamwandi	<0.15	<0.15	0.6	Negative
Oshikoto(NCA)	Nyungu # 2	<0.15	<0.15	no sample depletion	Negative
	Ohayiyanda	1.65	0.45	1.95	Positive SAT 1 & 3

The results of the VNT are shown in Table 8 and their spatial distribution is illustrated in Figure 10. Only one sample from the Ohayiyanda crush pen tested positive on the

VNT, the other 9 Ceditest® NSP ELISA previously positive reactors came out negative.

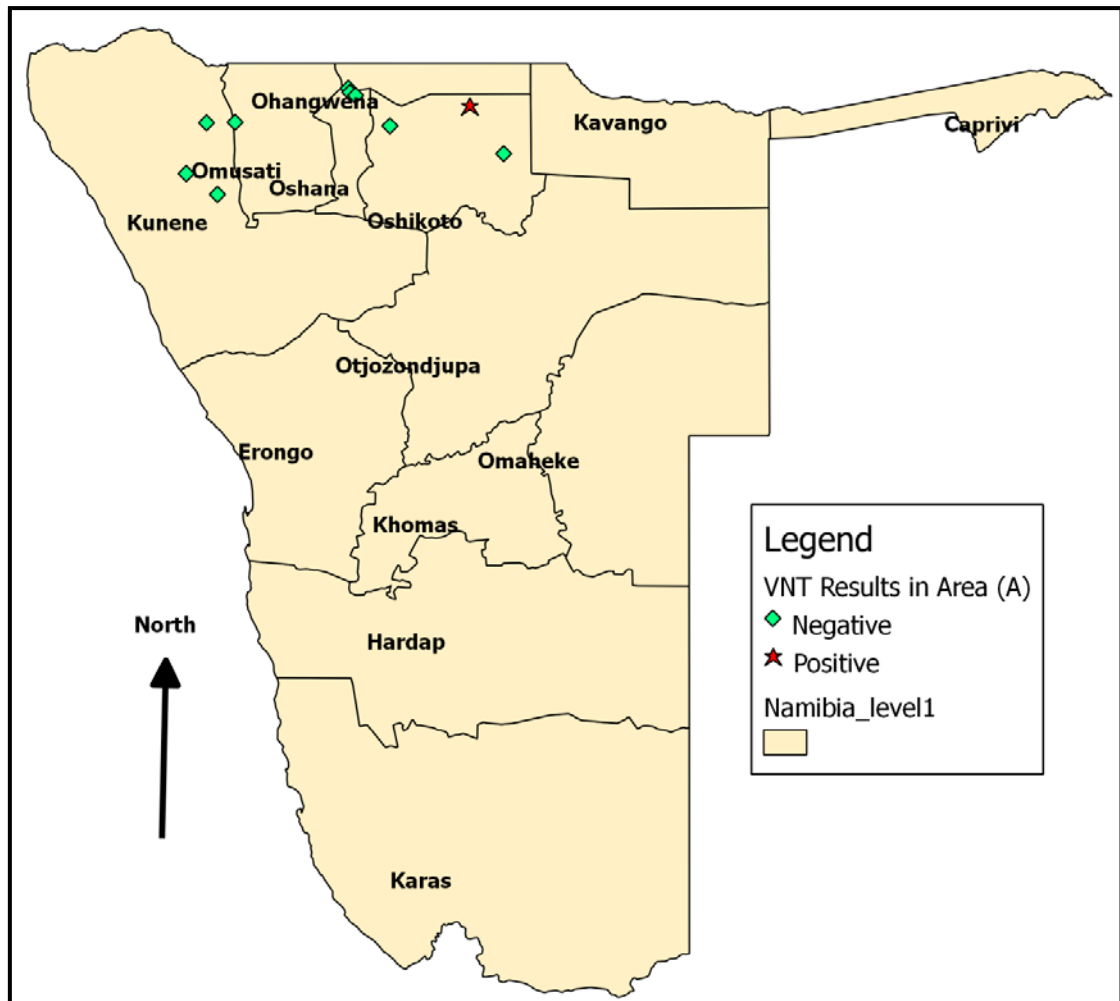


Figure 10: Spatial distribution of Viral Neutralisation test results in Area A

The Ohayianda sample was positive for SAT1 & 3. The herd from which these animals came from was not subsequently followed up as per initial plan due to logistical problems.

6.2 Area B (vaccinated against Foot and Mouth Disease in the last 10 years)

Table 9: Results of the Ceditest® NSP ELISA for FMD in cattle with 95% CI in Area B during May - August 2007 shown by regions

Regions	Number Tested	Ceditest® NSP ELISA (+)	Apparent Prevalence	95% Confidence Interval
Omusati north	1076	3	0.29	0.0009, 0.82
Ohangwena	1297	5	0.39	0.16,0.9
Kavango	2855	6	0.21	0.1,0.46
Total(overall)	5228	14	0.27	0.16,0.45

Table 10 shows the results of the Ceditest® NSP ELISA for FMD in cattle with 95% CI in Area B during May - August 2007. Out of the 5228 samples collected and tested in the three regions, 14 reacted positive to the Ceditest® NSP ELISA with an average apparent prevalence of 0.27% and a 95% CI of 0.16, 0.45. The Kavango region recorded the highest number of seropositive reactors (6), meanwhile Ohangwena region recorded 5 reactors and Omusati north had 3 reactors. These 14 sera were then tested further with the Virus Neutralisation Test and the results are provided in Table 12.

Table 10: Results of the Ceditest® NSP ELISA for FMD in cattle with 95% CI in Area B during May - August 2007 shown per crush pen

Regions	Crush Pen	Number Tested	Ceditest® NSP ELISA (+)	Apparent Prevalence (%)	95% CI
Omusati north	Okafitu K	20	1	5.0	0.89,23.61
	Omunyele	20	1	5.0	0.89,23.61
	Etapaela	20	1	5.0	0.89,23.61
Ohangwena	Omunekadi	20	1	5.0	0.89,23.61
	Omahenge.A	20	1	5.0	0.89,23.61
	Okaonya	20	1	5.0	0.89,23.61

	Oshamukweni	20	1	5.0	0.89,23.61
	Onamafila	20	1	5.0	0.89,23.61
Kavango	Brio	20	1	5.0	0.89,23.61
	Rughono	18	1	5.56	0.99,25.76
	Manyondo	20	1	5.0	0.89,23.61
	Kayongona	20	1	5.0	0.89,23.61
	Shamangoruwa	20	1	5.0	0.89,23.61
	Kauti	20	1	5.0	0.89,23.61

The apparent crush pen level seroprevalence with 95% CI in Area B are shown in Table 9. Of the 270 crush pens tested, 14 recorded one animal with a positive reaction with seroprevalence between 5.0 and 5.56 and a 95% confidence interval between 0.89, 23.61 and 0.99, 25.76 respectively. Omusati north recorded the lowest crush pen reactions with 3 seropositive and Kavango had the highest with 6 seropositive crush pen, while Ohangwena had 5 positive crush pens as illustrated in Fig 10. The number of positive reactors found at each crush pen was consistent with the cut point reactors anticipated by the software program FreeCalc. Despite this, those samples were also tested with the virus neutralisation test.

The results of the VNT test are shown in Table 11 and the spatial distribution in Figure 11. Four (4) of the fourteen (14) serum samples had positive titres on the VNT, (>0.9 titres cut off point) with a scattered distribution along the border Namibia-Angola..

Table 11: Results of serotype identification with Viral Neutralisation test in Area B

Region	Crush-pen	SAT1 Titre	SAT2 Titre	SAT3 Titre	Positive/ Negative
Omusati north	Omunyele	1.5	0.9	1.2	Positive SAT1,2,3
	Etapayela	<0.15	<0.15	<0.15	Negative
	Okafitu Kasisiya	1.35	0.9	0.3	Positive

					SAT1 & 2
Ohangwena	Okawonya	0.45	0.3	0.45	Negative
	Omahenge A	<0.15	<0.15	0.45	Negative
	Oshamukweni	<0.15	<0.15	<0.15	Negative
	Onamafila	0.6	0.45	0.45	Negative
	Omunyekadi	0.45	0.3	0.9	Positive
Kavango	Manyondo	0.6	0.3	0.75	Negative
	Brio	1.05	0.45	no sample	Positive SAT1
	Kauti	0.3	<0.15	0.75	Negative
	Kayogona	<0.15	<0.15	<0.15	Negative
	Shamangoruwa	<0.15	<0.15	<0.15	Negative
	Rughongo	<0.15	<0.15	<0.15	Negative

NB: Negative Titer cut off<0.9

One animal at Omunyele crush pen had heterologous positive titres for SAT1, 2 & 3 and the animal from the Okafitu Kasisiya crush pen were positive for SAT1& 2 both were from Omusati north. The animals from Brio crush pen was positive to SAT1 in Kavango and the animals from the Omunyekadhi crush pen in Ohangwena region was positive for SAT 3.

Table 12: Results from the herds re-sampled and re-tested with Ceditest® NSP ELISA, in both Area (A & B) in October 2007

Region	Number tested	Results
Ohangwena	24	Negative
Omusati north	26	Negative
Kavango	14	Negative
Total	64	All Negative

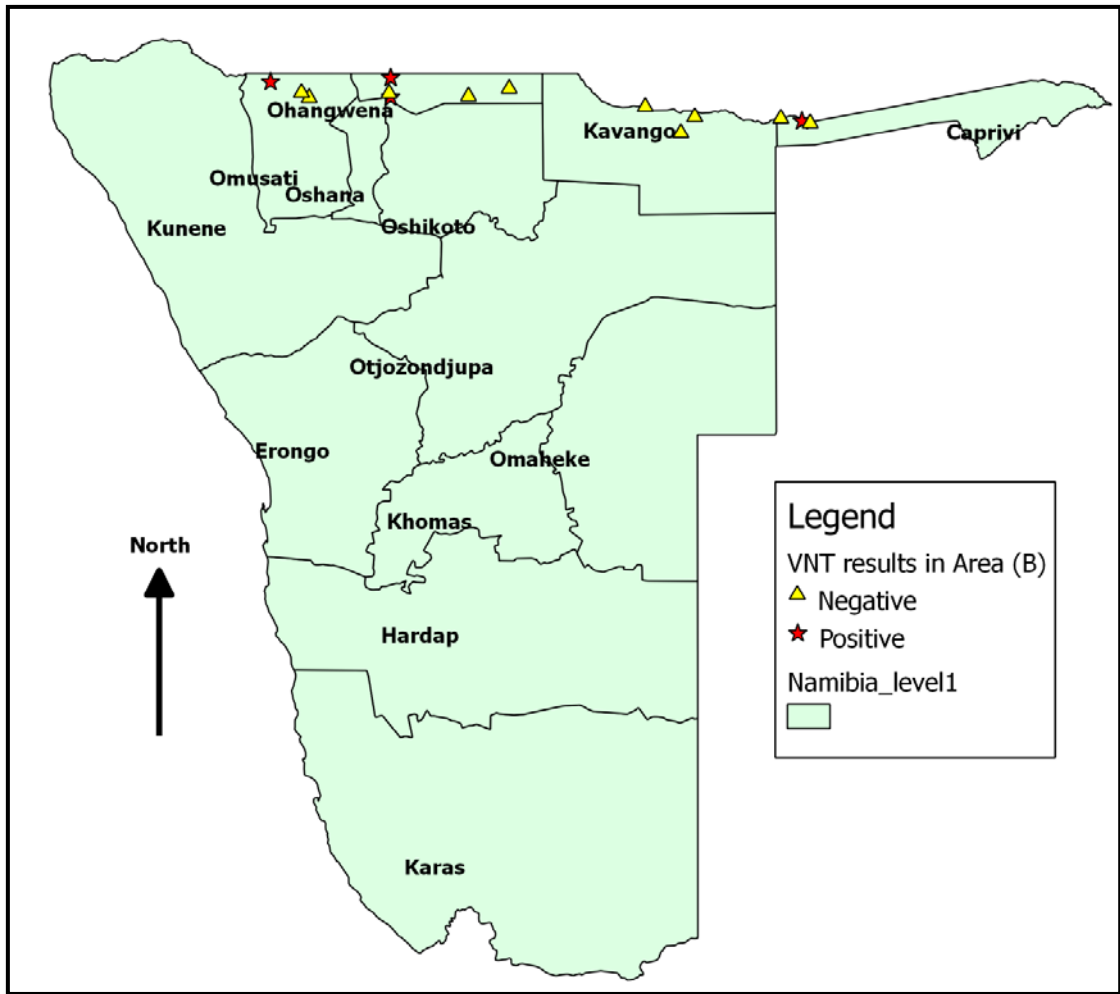


Figure 11: Spatial distribution of Viral Neutralisation test results in Area B

The herds of all 4 positive reactors were followed up and examined for clinical signs and rebled and tested again on the Ceditest® NSP ELISA. These results are shown in Table 12 and no animals were found to show clinical signs or were seropositive on the Cedi test. Results from the herds re-sampled and re-tested with Ceditest® NSP ELISA from area B revealed negative results.

CHAPTER 7

7. DISCUSSION

The study was able to establish that there was no FMD virus circulation in the NCA in both FMD vaccinated and non-vaccinated areas. The study was also able to gather information on sociological factors which may influence an appropriate sampling strategy for future surveys.

The Ceditest® NSP ELISA was used to screen the sera because, as a blocking ELISA it easily detects antibodies against the non-structural 3ABC protein of FMDV. It is independent of the serotype that causes the infection and independent of the fact that the animal is vaccinated or not. In addition, antibodies to the 3ABC protein are considered to be reliable indicators of infection/exposure regardless of vaccination status and of the serotype of FMDV. The disadvantage of Ceditest® NSP ELISA is it cannot distinguish between the seven different serotypes of FMD (Spiegelhalter *et al.*, 2002).

In area A without vaccination, Ceditest® NSP ELISA detected 10 reactor animals which were subsequently tested with the VNT and only one reacted and rest fails to react. Meanwhile in area B, the Ceditest® NSP ELISA detected 14 reactors animals and subsequently 4 reacted further to the VNT. There was no significant difference in apparent prevalence between the non-vaccinated and vaccinated populations. The vaccinated population was perceived to be at higher risk of FMD infection due to its proximity with herds of lower FMD status in neighboring Angola and the FMD infected zone of Namibia.

The serum samples from reactor animals to Ceditest® NSP ELISA were subjects to the VNT as an approach to minimise the occurrence of false reactors (OIE Manual 2009). Of the 24 reactors to the previous test, five (5) reacted further to the VNT of which four of the reactors were from the vaccinated area (Area B) and only one from the non-vaccinated area (Area A). The four reactors from the vaccinated area could be attributed to the fact that the test does not distinguish antibodies induced by natural infection from those induced by vaccination. The serum from the five animals that reacted to VNT had

antibodies to all serotypes of the SAT viruses. Ohayiyanda crush-pen, as part of Area A in Oshikoto (NCA), had one animal positive to SAT1& 3. In Omusati north all three SAT serotypes (SAT1, 2 & 3) were detected in one animal at Omunyele crush pen and SAT 1 & 2 were detected at Okafitu Kasisiya whilst at Brio crush pen in Kavango, antibodies to SAT1 and SAT3 virus were detected in one animal. Meanwhile, Omunyekadi crush pen in Ohangwena region had one reactor to SAT3. It is not clear why such results were obtained as no vaccination with SAT1 and 3 were used in Oshikoto, Omusati or in Ohangwena region that may have caused animal's to sero convert. On the other hand, in Kavango a trivalent SAT1, 2 & 3 vaccine is used and this could explain the results in this region. A possible explanation for the multi antibodies (SAT1, 2 & 3) detected in Omusati could be that these animals originated from Angola having crossed the border to take advantage of the vaccination campaign in Namibia. It is known that vaccinations against FMD are carried out in Angola albeit with a low coverage at this stage due to lack of access roads, mined grazing areas as a result of civil war, which limit access to most of the farmers in the northern province of Kunene. The farmers did not have herd health records except the vaccination card thus the age of animals had to be estimated by us and the technician using dentition techniques so a detailed history of animals was uncertain Figure 6.

All the samples in reactor herds fail to react to a second Ceditest® NSP ELISA, which supported the assumption that they were actually false positives. This could be attributed to cross reactions and vaccine impurities since the current vaccine being used in the Southern African Development Community (SADC) is not purified and hence could potentially induced antibodies to NSP proteins. These factors need to be taken into account in the vaccinated areas since these animals have been vaccinated several times.

On the other hand in this case the BVI laboratory, where the samples were tested, reported a lower Se 97% and Sp 95% for the VNT than what has been published elsewhere, which is of great concern since the VNT was supposed to be the gold standard test and should be highly specific. This could be also attributed to the fact that the antigen used is not closely matched with the circulating virus, among other factors.

Also the Titer cut off < 0.9 point reactor as determined by the laboratory could have an influence on the results. Intent to contact the lab was futile. Brocchi *et al.*, (2006) and Paton *et al.*, (2006) point out that in most laboratories positive samples are re-tested as a matter of procedure as this has been found to increase the specificity of the test. This strategy is necessary because of the poor repeatability of the VNT and in order to eliminate false positives.

In selecting individual animals sampled in Kunene it was not possible to establish the number of farmer's herds that would have turned up before sampling was started. Some of the reasons for this are cultural beliefs, social activities, such as attending to crops, funerals, receiving government pension, or visiting clinics thus resulting in farmers arriving at the crush pen at different times, making it difficult to draw up a representative sampling frame strategy. The new sampling frame strategy described in this study was not ideal but an improvement on the traditional approach. There was however no guarantee those animals could not have moved between area A and B prior to the sampling. Movements are known to occur due to inheritance, water and grazing availability, sale and exchange of livestock between farmers without pre-testing for disease such as FMD and Brucellosis. Hence, there is no clear demarcation between vaccinated and non-vaccinated areas which may have influenced the results.

Cross-border movement of cattle between Namibia - Angola occurs on a daily basis within a ± 10 km radius agreed on by both governments because of the varied needs of water or grazing on either side. The movements are well known and documented and monitored frequently for early detection of any disease. Veterinary authorities of both countries meet twice a year to discuss and harmonise animal health issues and production activities (inspections, surveillance strategy and vaccinations) along the border especially regarding FMD and Contagious Bovine Pleuropneumonia (CBPP). However, lack of proper infrastructure and adequate personnel at veterinary control points cannot be ignored as this has an impact on effective veterinary services delivery. Zones of common action and intervention have been agreed upon on both sides of the borders including specification of border crossing points for animals and products of animal origin. This confounds the results of this survey. In order to improve the

situation, the Namibian government is working to establish a reliable animal Identification & Traceability System throughout Namibia with an accompanying permit system and electronic recording system which Angolan counterparts also pledge to adopt in the near future. This system coupled with improved staffing levels assures optimum animal health management along the porous Angola-Namibia border and all data gathered during surveillance activities is processed, analyzed and reports generated on a regular and timely basis by the Directorate of Veterinary Services (Namibia) for timely decision making. No report of clinical FMD has been recorded from any of the study areas for four decades now except in Kavango in 1992 and 2008. However both outbreaks were effectively controlled, which further supports this finding of no FMD virus circulating along the border.

In the non-vaccinated area, animals were stratified by age in order to increase the sensitivity of detection, making the survey more reliable in these areas. In this study re-sampling occurred after 110 days from positive herds, thus giving ample time for the possibility of sero-conversion in the intervening period and therefore increasing the possibility of detecting the disease. The exception was at Ohayiyanda crush-pen as the owners translocated to other areas in search of better grazing and could not be traced at the time of follow up. Sampling the entire herd was done in order to rule out the presence of FMD transmission within the herd with positive results. As the results showed no evidence of circulating virus it was found unnecessary to then collect virological samples but a thorough clinical examination was carried out before re-sampling took place. Animals that were initially positive to VNT may have tested negative in the second round due to antibodies waning during the period between the first sampling and the second as was observed by Paton *et al.* (2006).

The findings of this study are important from a trade perspective as they provide evidence that allows Namibian NCA to be declared FMD free area and boost the confidence of international trading partners in the safety of Namibian animals and animal products. This puts the Namibian government as well as the meat industry in a competitive position to maintain current markets and explore access to new markets especially in an emerging Asia and other regions with new trade opportunities.

Compartmentalization for commodity-based trade could be considered after repeated surveys at determined intervals to provide ongoing assurance to trading partners. Furthermore, the requirement for the 21 day quarantine period for cattle to be slaughtered at Oshakati abattoir could be potentially lifted. This quarantine was instituted because of the unknown FMD status in the Namibia NCA as a precautionary measure to mitigate risk.

CHAPTER 8

8. CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

From the survey, it was concluded that:

There is < 5% prevalence of circulating FMD virus in Kunene, Omusati, Oshana, Oshikoto, Ohangwena and Kavango of the Northern communal area of Namibia. These conclusions are supported by ongoing active and passive surveillance programmes which have been unable to detect FMD in the area for more than 20 years.

Movement of cattle across the Angola – Namibia border remains as one of the major challenges and poses a threat to the FMD status in the NCA Namibia. This can be dealt with by maintaining and improving surveillance activities between Namibia and Angola as well as addressing the staffing along the border line.

There is room and scientific justification to explore alternative markets of bone in meat to potential clients in the regions and beyond. The fact that FMDV could not be found circulating in the cattle population of the NCA Namibia provides an argument for opening up the area to increased trade in livestock.

Cultural practices need to be taken into account in the design of future studies to assess disease freedom as these were found to play a role when trying to get a representative sample of the population. This is the first survey in Namibia to take this into account.

The VNT test has not performed as expected since there were too many false positives detected.

8.2 Recommendations

The Directorate of Veterinary Services (DVS) of Namibia should use the information to support the dossier submission to the OIE to declare the NCA Namibia free of FMD and to remove the 21 day quarantine prerequisite for cattle to be slaughtered at the export abattoirs. Alternatively establishment of new Animal Disease Control Zones or Compartments can be considered as a basis for commodity based trade.

DVS should maintain and continuously review FMD surveillance activities in the NCA especially along the Angola – Namibia border and continuously harmonise these activities with Angolan counterparts. This needs to be supported with improved diagnostic service capability and research laboratory capacity.

The Namibian Government should maintain and possibly increase the number of Veterinary technical staff in NCA regions and keep them refreshed and knowledgeable about FMD to enhance the country's capacity to pick up the disease in the unlikely event that it occurs in future.

Regular serosurveillance will be highly recommended in an area exposed to potentially infected populations, proximity of buffalo in (Kavango) and extended porous border between Namibia and Angola. Ongoing surveillance could be randomized or make use of sentinels, or be implemented at abattoirs and cattle sales events.

Individual livestock Identification through NamLiTS should be implemented nationwide and maintained to promote herd health management and in turn support commodity-based trade as a favorable marketing alternative before the NCA Namibia can be declared free of FMD.

Since there is insufficient knowledge on sociological factors which may influence appropriate and more informative sampling strategies, a more comprehensive study must be carried out to address this concern.

The development of a single, quick to use test that covered all 7 serotypes as well as differentiating vaccinated from convalescing animals would be a major advance in the

epidemiological tool kit for FMDV.

8.3 Significance and Outcome of the Survey

As mentioned in the introduction, this survey was funded by the Ministry of Agriculture Water and Forestry Namibia as well as the Meat Industry of Namibia with the primary objective to establish whether the Namibian cattle population in the NCA of the buffer zone (now the protection zone) is FMD virus free and to pave the way for new potential markets in the region and overseas.

The outcome of the survey was presented to the management within the Directorates of veterinary Services and various stakeholders by the author shortly after the survey had been conducted so that the data available could be used in their decision making process. The results of this survey contributed to the decision to waver the 21 day, quarantine period for cattle before slaughter in the NCA Namibia and this regulation was revoked in 2009 making it much easier to export meat from the NCA (Bamhare 2010)

The survey also influenced the decision to introduce a new FMD zoning policy in 2010. This resulted in the incorporation of the surveillance zone into the free zone thus opening up further trade opportunities in the region. Figure 12 and Table 13 give a more detailed overview of the changes that were made after this survey was conducted. These changes were also in alignment with the new OIE requirement for disease zoning.

Table 13: The Namibian context of Foot-and-Mouth Disease zoning as from year 2010 up to date.

Infected Zone	<p>Zambezi east of the Kavango River: There are free-roaming buffalo here, and in neighboring countries; FMD outbreaks occur sporadically in cattle and cattle are vaccinated against FMD twice a year. Cattle herds are inspected on a monthly basis by veterinary staff. It is separated from the Buffer Zone by the Kavango River, which acts as an effective natural barrier.</p>
Protection Zone	<p>Runs from the western boundary of Bwabwata Game Park in the Kavango region to Kunene (north) region. There is a physical barrier which is a game proof double-electrified fence separating livestock from the Game Park, running from the border with Angola on the northern part to the Kavango River in southern part. The protection <i>zone</i> includes the following regions: Kavango, part of Oshikoto region north of the VCF, Ohangwena , Oshana , Omusati, part of Kunene north of the VCF</p> <p>Bi-annual prophylactic FMD vaccination is done only in 2 most eastern constituencies of Kavango region which are Mukwe and Ndiyona. Sero-surveillance is done to demonstrate that there is no serological evidence of FMD infection or virus circulating in the zone.</p> <p>Movement of small stock to the free zone is only allowed after a 3 week quarantine period followed by negative serology in sentinel cattle running together with them during the period. The animals are further quarantined at farm of destination for 90 days in the free zone before they move under a permit system to other areas.</p> <p>Livestock individual identification is being improved to include officially approved ear tags and a database to capture and record to control all animal movements.</p>
Free Zone	<p>The rest of the country south of the Surveillance Zone is regarded as a free zone. The last FMD outbreak in this area was in 1964; no FMD vaccinations are carried out, and herd inspections are mostly scheduled on a six-monthly basis.</p>

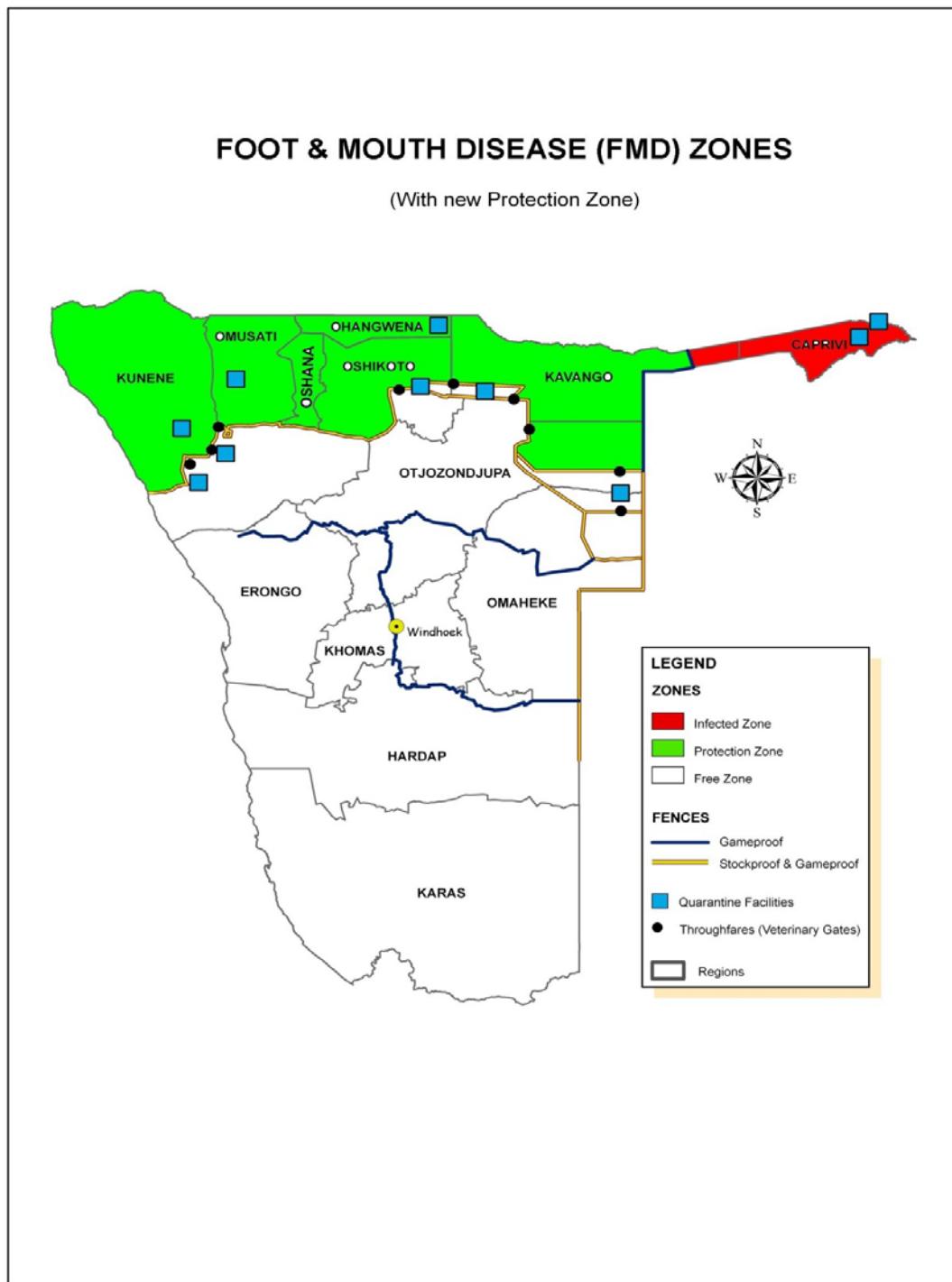


Figure 12: New zoning Map for controlling Foot-and-Mouth Disease in Namibia

The survey highlighted the need for better identification of individual cattle and the Namibian Individual Livestock Identification (NamLiTS) was expanded to all corners of the country in 2012 with the aim to maintain, improve surveillance and promote herd health management.

The survey also highlighted the need for increased diagnostic support required for such surveys and in June 2014, the Namibian Government approved the re-structuring of DVS in order to increase the number of Veterinary technical staff, including veterinarians, in the NCA of Namibia and other parts of the country. In addition a new state of the art Veterinary Laboratory is currently under construction in the Oshana region of the NCA Namibia to increase the diagnostic capability in the region.

In May 2013, a Memorandum of Understanding (MOU) was signed by the two Honorable Ministers of Agriculture of Angola and Namibia respectively. The aim of the MOU is to enhance collaboration in the control, prevention and eradication of CBPP, FMD and other transboundary animal diseases in the trans-frontier areas of the 2 countries; exchange of notes and information on disease surveillance and implement harmonized disease and import control protocols among a number of issues.

The survey has therefore had several major knock on effects in influencing Namibian policy with regards to surveillance in the NCA of Namibia.

REFERENCES

Alexandersen, S., Zhang, Z., Donaldson, A.I. & Garland, A.J.M., 2003, 'The pathogenesis of Foot-and-mouth disease', *AJ. J Comp Pathol* 129 (1), 1-36.

Ahmed, H.A., Salem, S.A.H., Habashi, A.R., Arafa, A.A., Aggour, M.G.A., Salem, G.H., Gaber, A.S., Selem, O., Abdelkadar, S.H., Knowles, N.J., Madi, M., Valdez-Gonzalez, B., Wadsworth, J., Hutchings, G.H., Miolet, V., Hammond, J.M. & King D.P., 2012, 'Emergence of foot-and-mouth disease virus SAT 2 in Egypt during 2012', *Transbound. Emerg. Dis* 59, 476–481.

Animal Diseases and Parasites Act 1956 (Act No. 13 of 1956), Government Gazette of the Republic of Namibia,

Animal Health Act 2011(Act No.1 of 2011), Government Gazette of the Republic of Namibia, No. 4694 of 20 April .

Arzt, J., Baxt, B., Grubman, MJ., Jackson, T., Juleff, N., Rhyan, J., Rieder, E., 2011b, 'The pathogenesis of foot-and-mouth disease II': viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus-host interactions *Transboundary and Emerging Disease* 58, 305–326.

Arzt, J., Juleff, N., Zhang, Z. & Rodriguez, L.L., 2011a, 'The pathogenesis of foot-and-mouth disease I': viral pathways in cattle *Transboundary and Emerging Disease* 58, 291–304.

Bachrach, H.L., 1968, 'Foot-and-mouth disease. Annu', *Rev. Microbiol* 22, 201-244.

Bamhare, C., 'Control of Livestock Movement in Kavango and Caprivi regions, Circular V3/2010, Directorates of Veterinary Services, Windhoek.

Barasa, M., Catley, A., Machuchu, D., Laqua, H., Puot, E., Tap Kot, D. & Ikiror, D., 2008, 'Foot-and-mouth disease vaccination in South Sudan: Benefit–Cost Analysis and Livelihoods Impact', *Transboundary and Emerging Disease* 55, 339–351.

Barnett, P.V. & Carabin, H., 2002, 'A review of emergency Foot-and-mouth disease vaccines, *Vaccine* 20, 1505–1514.

Bastos, A.D., 2003, 'The implications of virus diversity within the SAT2 serotype for control of Foot-and-mouth disease in sub-Saharan Africa', *J. Gen. Virol* 84, 1595–1606.

Bastos, A.D.S., Bertschinger, H.Y.J., Cordel, C., van Vuuren de, W.J., Keet, D., Bengis, R.G., Grobler, D.G., Thomson, G.R., 1999, 'Possibility of sexual transmission of Foot-and-mouth disease from African buffalo to cattle', *Vet. Rec* 145(3), 77-79.

Bastos, A.D.S., Boshoff, C.I., Keet, D.F., Bengis, R.G. & Thomson, G.R., 2000, 'Natural transmission of Foot-and-mouth disease virus between African buffalo (*Syncerus caffer*) and impala (*Aepyceros melampus*) in the Kruger National Park, South Africa', *Epidemiol Infect* 124, 591.

Bayissa, B., Ayelet, G., Kyule, M., Jibril, Y. & Gelaye, E., 2011, 'Study on seroprevalence, risk factors, and economic impact of Foot-and-mouth disease in Borena pastoral and agro-pastoral system, Southern Ethiopia.

Belsham, G.J., 1993, 'Distinctive features of Foot-and-mouth disease virus, a member of the picornavirus family; Aspects of virus protein synthesis, protein processing and structure', *Progress in Biophysics and Molecular Biology* 60, 241–260.

Bengis R.G., Thomson, G.R., Hedger, R.S., Vos, V. De. & Pini, A., 1986, 'Foot-and-mouth disease and the African buffalo (*Syncerus caffer*).I. Carriers as a source of infection for cattle *Onderstepoort Journal of Veterinary Research* 53, 69–73.

Bengis, R.G. & Erasmus, J.M., 1988, 'Wildlife diseases in South Africa': a review *Rev. sci. tech. Off. Int. Epiz* 7, 807–821.

Bishi, A. & Kamwi, J., 2008, 'Veterinary science, transboundary animal diseases and markets: Pathways for policy in Namibia, Transboundary animal disease and market access: Future options for the beef industry in Southern Africa', Working Paper 4-6, and Brighton: Institute of Development Studies.

Bishi, A., 2013, 'Chief Veterinary Officer's Report', State Veterinarians' Annual Meeting, Swakopmund 2-4 October, 5-18.

Brocchi, E., Bergmann, I.E. & Dekker, A., 2006, 'Comparative evaluation of six ELISAs for the detection of antibodies to the non-structural proteins of foot-and-mouth disease virus. *Vaccine* 24, 6966–6979.

Brückner, G.K., Vosloo, W., Du Plessis, B.J.A., Kloeck, P.E.L.G., Connoway, L., Ekron, M.D., Weaver, D.B., Dickason, C.J., Schreuder, F.J., Marais, T. & Mogajane, M.E., 2003, 'Foot-and-mouth disease: the experience in South Africa.

Bulman, G. & Terrazas, M.I., 1976, 'Consideraciones sobre el efecto de la fiebre aftosa en la producción lacteal de un tambo modelo en Cochabamba, Bolivia', *Revista de Medicina Veterinaria, Argentina* 57(1), 1-2, 5-6, 9-10.

Burrows, R., 1972, 'Early stages of virus infection: studies in uiuo and in vitro. In Twenty-second Symposium of the Society for General Microbiology, H. Smith and J. H. Pearce, Eds, Cambridge University Press, Cambridge, pp. 303-332.

BVI & MERIAL, 2014, Aftovax inactivated vaccine against Foot-and-mouth disease in ruminants, <http://www.bvi.co.bw/products/aftovax.html>, accessed July, 2014.

Callahan, J.D., Brown, F., Osorio, F.A., Sur, J.H., Kramer, E., Long, G.W., Lubroth, J. & Ellis, S.J., 2002, 'Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of Foot-and-mouth disease virus', *J Am Vet Med Assoc* 220, 1636–1642.

Cameron, A.R. & Baldock, F.C., 1998a, 'A new probability formula for surveys to substantiate freedom from disease. FreeCalc, *Prev Vet Med* 34(1), 1-17.

Cameron, A.R. & Baldock, F.C., 1998b, 'Two-stage sampling in surveys to substantiate freedom from disease ', *Prev Vet Med* 34(1), 19-30.

Cannon, R.M. & Roe, R.T., 1982, *Livestock Disease Survey: A Field Manual for*

Veterinarians, Canberra Australian Bureau of Animal Health, pp 30.

Carrillo, C., Tulman, E.R., Delhon, G.L.Z., Carreno, A. & Vagnozzi, A., 2005, 'Comparative genomics of Foot-and-mouth disease virus', *J Virol* 79, 6487-6504.

Clavijo, A., Wright, P. & Kitching, P., 2004, 'Developments in diagnostic techniques for differentiating infection from vaccination in Foot-and-mouth disease', *Vet J* 167, 9–22.

Condy, J. B., Hedger, R.S., Hamblin, C., Barnett, I.T.R., 1985, 'The duration of the Foot-and-mouth disease carrier state in African buffalo (i) In the individual animal and (ii) In a free-living herd', *Comp Immunol Microbiol Infect Dis* 8, 259–265.

Condy, J.B. Hedger, R.S., Hamblin, C. & Barnett, I.T., 1985, 'The duration of the foot-and-mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd', *Comp. Immunol. Microbiol. Infect. Dis* 8, 259–265.

Council Directive 2003/85/EC., 2003, 'Community measures for the control of Foot-and-mouth disease repealing Directive 85/511/EEC and Decisions 89/531/EEC and 96/665/EEC and amending Directive 92/46/EEC', *Official J Eur Union* 46.

Dawe, P.S., Flanagan, F.O., Madekurozwa, R.L., Sorenson, K.J., Anderson, E.C., Foggin, C.M., Ferris, N.P. & Knowles, N. J., 1994, 'Natural transmission of Foot-and-mouth disease from African buffalo (*Syncerus caffer*) to cattle in a wildlife area of Zimbabwe', *Vet Rec* 134, 230–232.

De la Rua, R., Watkins, G.H. & Watson P.J., 2001, 'Idiopathic mouth ulcers in sheep (letter) *Veterinary Record* 149, pp. 30–31

Dekker, A., Nielen, M., Molendijk, M. & Kroonenberg, F., 1996, 'Foot-and-mouth disease airborne transmission prediction model: data and model considerations', Report of the Session of the Research group of the European Commission for the Control of Foot-and-mouth disease, Kibbutz Ma'ale Hachamisha, Israel, 2–6 September, FAO, Rome, 176–182.

Di Nardo, A., Knowles, N.J. & Paton, D.J., 2011, 'Combining livestock trade patterns with phylogenetics to help understand the spread of foot and mouth disease in sub-Saharan Africa, the Middle East and Southeast Asia ', *Rev sci. tec. Int. off. Epiz* 30, 63–85.

Dimitriadis, I.A. & Delimpaltas, P., 1992, 'Dimitriadis, P. Delimpaltas Epizootiology of Foot- and-mouth disease during a quarter century (1962-1988) in Greece Berliner und Münchener tierärztliche Wochenschrift 105 , pp. 90–95.

Directorates of Veterinary Services 2005, Annual report 2005, Ministry of Agriculture and Forestry, Windhoek, Namibia.

Domanski, R., Fitko, R. & 1959, 'Disturbances of the pituitary and other hormonal glands in cows after Foot-and-mouth disease', *Proceedings of the 16th International Veterinary Congress*, Madrid, 421.

Domingo, E., Mateu, M.G Martínez, M.A., Dopazo, J Moya, A & Sobrino, F., 1990, Genetic variability and antigenic diversity of Foot-and-mouth disease virus," in *Applied Virology Research*, E. Kurstak, R. G. Marusk, S. A. Murphy, and M. H. V. Van Regenmortel, Eds., vol. 2, pp. 233–266.

Donaldson, A., 2001, 'Relative resistance of pigs to infection by natural aerosols', *Vet. Rec*, May 12, 3, 600–602.

Donaldson, A.I. & Sellers, R.F., 2000, Foot-and-mouth disease, in W. B. Martin & I. D. Aitken (eds.), *Diseases of sheep*, Blackwell Science, Oxford, United Kingdom, pp. 254-258.

Donaldson, A.I., 1987 'Foot-and-mouth disease: The principal features', *Irish Veterinary Journal* 41, 325-327.

Edwards, J.T., 1934, 'Further experiments with hedgehogs', Foot-and-mouth disease research committee, UK, Unpublished papers, 214B & 223B.

Eskildsen, M.K., 1969, 'Experimental pulmonary infection of cattle with Foot-and-mouth disease virus', *Nord. Med. Vet* 21, 86–91.

Ferris, N.P. & Dawson, M., 1988, 'Routine application of ELISA assay in comparison with CFT for the diagnosis of FMD and swine vesicular disease', *Veterinary Microbiology* 16 (3), 201-209.

Fogedby, E., 1963, 'Review of epizootiology and control of Foot-and-mouth disease in Europe, 1937–1961. Eur. Comm. Control of FMD, FAO, Rome.

Foreman, S., Le Gall, F., Belton, D., Evans, B., François, J.L., Murray, G. & Sheesley, D., 2009, 'Moving towards the global control of Foot-and-mouth disease: An opportunity for donors', *Rev. sci. tech. Off. int. Epiz* 28(3), 883-96.

Fracastorius, H., 1546, *De alijs differentijs contagionis. De Sympathia et Antipathia Rerum Liber Unus. De Contagione et Contagiosis Morbis et Curatione (libri iii)*, pp. 36–38

Gailunas, P. & Cottral, G.E., 1966, 'Presence and persistence of Foot-and-mouth disease virus in bovine skin', *J. Bacteriol* 91, 2333-2338.

Gall, F.L. & Leboucq, N., 2004, 'The role of animal disease control in poverty reduction, food safety, market access and food security in Africa, OIE.

Garland, A. J. M., 1974, 'The inhibitory activity of secretions in cattle against Foot and-mouth disease virus'. Ph.D. Thesis, London School of Hygiene and Tropical Medicine, University of London. Henderson, W. M. (1952).

Geering, W.A. & Lubroth, J., 2002, Preparation of Foot-and-mouth disease contingency plans. Chapter 6, Food and Agriculture Organization, Rome, ISBN: 9789251048672, pp. 39-57.

Geering, W. A., 1967, 'Foot- and- mouth disease in sheep', *Aust. Vet. J* 43, 485-489.

Gibbs, Y.M., 1931, 'Susceptibility of different species of animals to Foot-and-mouth

disease', ii. Hedgehogs, in 4th progress report of Foot-and-mouth disease Research Committee.

Golding, S.M., Hedge, R.S., Talbot, P. & Watson, J., 1976, 'Radial Immunodiffusions and Serum Neutralisation Techniques for the Assay of Antibodies to Swine Vesicular Disease', *Res. Vet. Sci* 20, 142–147.

Graves, J.H., 1971, 'Contact transmission of Foot-and-mouth disease from infected to susceptible cattle', *J. Infect. Dis* 123, 386–391.

Grubman, M.J. & Baxt, B., 2004, 'Foot-and-mouth disease', *Clinical Microbiology Reviews* 17(2), 465 - 493.

Hamblin, C., Barnett, I.T.R. & Hedger, R.S., 1986, 'A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against Foot-and-mouth disease virus: Development and method of ELISA', *J. Immunol. Methods* 93, 115-121.

Hesterberg, U., 2007, 'A Serological Prevalence study of important infectious diseases of cattle in rural areas of Kwazulu Natal', Thesis, Dept. of Production Animal Studies, University of Pretoria, South Africa.

Hunter, P., 1998, 'Vaccination as a means of control of Foot-and-mouth disease in sub-Saharan Africa', *Vac Hygiene London* 71, 15–34.

Hyslop, N.S.G., 1965, 'Air-borne infection with the virus of Foot-and-mouth disease', *J. Comp. Path* 75, 119–126.

International Fund for Agricultural Development., 1997, 'Northern regions Livestock development Project: Reformulation report', July, IFAD, Rome

Ivanov, Y.A. & Tekerlekov, P., 1989, 'Typing Aphthovirus by the micro-complement fixation test', *Veterinarya Sbrika* 87, 18–21.

Johnson, R.F., 1998, *Handy guide to determining the age of cattle by the teeth*, The Stockman's Handbook by Ensminger, 2nd edn, pp. 539.

Karesh, W.B., 2012, Wildlife and Foot- and- Mouth Disease A look from the Wild Side. FAO/OIE Global Conference on Foot-and-Mouth Disease Control, Bangkok, Thailand. Retrieved from [http://0-www.oie.int.innopac.up.ac.za/eng/A_FMD2012/FAO OIE Global Conference 2012 in Thailand](http://0-www.oie.int.innopac.up.ac.za/eng/A_FMD2012/FAO_OIE_Global_Conference_2012_in_Thailand).

King, A.M.Q., Brown, F., Christian, P., Hovi, T., Hyypia, T., Knowles, N.J.S., Lemon, M., Minor, P.D., Palmenberg, A.C., Skern, T. & Stanway, G., 2000, 'Picornaviridae', *In* Van Regenmortel, M.H.V., Fauquet, C. M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S. M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R. & Wickner, R.B (eds.), *Virus taxonomy: classification and nomenclature of viruses, Seventh report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, Calif, pp. 657-673.

Kitching, R.P., 2002, 'Clinical variation in Foot-and-mouth disease in cattle', *Rev Science Tech* 21(3), 499-504.

Kitching, R.P., 2005, Global Epidemiology and Prospects for Control of Foot-and mouth-disease. Compans RW, Cooper MD, Honjo TJ, Melchers F, Olsnes S, Vogt PK (Eds). Springer, Germany.

Kitching, R.P., Rendle, R. & Ferris, N.P., 1988, 'Rapid correlation between field isolates and vaccine strains of Foot-and-mouth disease virus', *Vaccine* 6(5), 403–408.

Knowles, N.J., Samuel, A.R., Davies, P.R. Kitching, R.P. & Donaldson A.I., 2001, 'Outbreak of foot-and-mouth disease virus serotype O in the UK caused by a pandemic strain', *Vet. Rec* 148, 258–259.

Korn, G., 1957, 'Experimentelle Untersuchungen zum Virusnachweis im Inkubationsstadium der Maul-und Klauenseuche und ihrer Pathogenese', *Arch. Exp. Vet. Med* 11, 637–649.

Kruger, A.S, 1997, Comprehensive assessment of environmental problems and opportunities for USAID intervention in Namibia, United States Agency for international development.

Leeuw, P.W., Van Bekkum, J.G. & Tiessink, J.W.A., 1978, 'Excretion of Foot-and-mouth disease virus in oesophageal-pharyngeal fluid and milk of cattle after intranasal infection', *J. Hyg* 81, 415–425.

Loeffler, F. & Frosch, P., 1897, 'Summarischer Bericht über die Ergebnisse der Untersuchungen zur Erforschung der Maul- und Klauenseuche', *Zentbl. Bakteriol. Parasitenkd Abt I* 22, 257-259.

Löffler, F. & Frosch, P., 1898, *Zentbl. Bakt. Abt. I Org.* 28, pp. 371. Lubroth, J., Yedloutschnig, R.J., 1987, Foot-and-mouth disease studies in the llama (Lama Glama). Mexican-US Commission FMD, Culle Hegal 713, Colonia Polanco, 11560, Mexico. *Proceedings of the United States Animal Health Association* 91.

Lubroth, J., 2002, 'Foot and mouth disease: a review for the practitioner', *Vet Clin North Am Food Anim Pract* 18 (3), 475-99.

Lunt, R.A., Linchongsubongkoch, W. & Gleeson, L.J., 1994, 'A modified liquid phase (LP) blocking ELISA used to assess type O Foot-and-mouth disease virus antigenic variation in Thailand', *Veterinary Microbiology* 42(1), 79–90.

Malan, J. S., 1995, *Peoples of Namibia*, Rhino Publishers, Indiana University.

Manocchio, M., 1974, 'Selective necrosis of the Islets of Langerhans in a cow with experimental Foot-and-mouth disease', *Veterinaria* 50, 1/3, 182.

Martin, S.W., Meck, A.H. & Willeberg, P., 1987, *Veterinary Epidemiology: Principles and Methods*, Iowa State University Press, Ames, Iowa.

McVicar, J.W. & Sutmoller, P., 1976, 'Growth of Foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation', *J. Hyg.* 76, 467–481.

McVicar, J.W., Graves, J.H. & Sutmoller, P., 1971, 'Growth of Foot-and-mouth disease virus in the bovine pharynx', *United States Animal Health Association Proceedings* 74,

230-234.

Moniwa, M., Clavijo, A., Li, M., Colignon, B. & Kitching, R.P., 2007, 'Performance of a Foot-and-mouth disease virus reverse transcription-polymerase reaction with amplification controls between three real-time instruments', *J Vet Diagn Invest* 19, 9–20.

Moonen, P., Boonstra, J., van der Honing, R.H., Leendertse, C.B., Jacobs, L. & Dekker, A., 2003, 'Validation of a LightCycler-based reverse transcription polymerase chain reaction for the detection of Foot-and-mouth disease virus', *J Virol Methods* 113, 35–41.

Namibia Tourism 2012, *Kavango People, Culture, Language and Region*, viewed 07 March 2013, from www.namibiatourism.org/people/kavango.

National Planning Commission., 2001, *Namibia 2001 Population and Housing Census*, Statistics Info Systems Centre, Government office park, Government of the Republic of Namibia, viewed 20 October 2008, from www.npc.gov.na/census.

National Planning Commission., 2002, 'Namibia Vision 2030', Government office park, Government of the Republic of Namibia.

National Planning Commission., 2013, 'Namibia 2013 Millennium Development Goals', Government office park, Government of the Republic of Namibia.

OIE 2012, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Chapter 2.1.5. Foot and mouth disease, viewed 17 July 2013, from http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2008/pdf/2.01.05_FMD.pdf.

OIE, 2006, Foot-and-mouth disease. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, ch 2.1.1, OIE, Paris, France.

OIE, 2006, Foot-and-mouth disease. In *Manual of Diagnostic Tests and Vaccines for*

Terrestrial Animals, ch 2.1.1, OIE, Paris, France.

OIE, 2009, Foot-and-Mouth Disease: Etiology Epidemiology Diagnosis Prevention and Control References 1–5. Retrieved from http://0-www.oie.int.innopac.up.ac.za/fileadmin/Home/eng/Animal_Health_in_the_World/docs/pdf/FOOT_AND_MOUTH_DISEASE_FINAL.pdf

OIE, 2014a, Criteria for the inclusion of diseases, infections and infestations in the OIE list, viewed 03 July 2014, from <http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2014>.

OIE, 2014b, *Terrestrial Animal Health Code, chapter 4.3*, viewed February 2015, from <http://www.oie.int/international-standard-setting/terrestrial-code/access-online>.

OIE, 2014c, *Terrestrial Animal Health Code, chapter 8.7*, viewed July 2014, from <http://www.oie.int/international-standard-setting/terrestrial-code/access-online>.

OIE., 2009, Foot-and-mouth disease. In *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, ch 2.1.5, OIE, Paris, France.

Paskin, R.D., 1990, 'A review of Agriculture in Kaokoland with special reference to animal husbandry and veterinary extension, Directorates of Veterinary Services, Government office park ,Windhoek.

Paton, D.J., de Clercq, K. & Greiner, M., 2006, 'Application of non-structural protein antibody tests in substantiating freedom from Foot-and-mouth disease virus infection after emergency vaccination of cattle', *Vaccine* 24:6503–6512.

Pinto, A.A., 2004, 'Foot-and-Mouth Disease in Tropical Wildlife Ann', *NY Acad. Sci* 1026, 65–72.

Potel, K., 1958, 'Neue Ergebnisse auf dem Gebiet der experimentellen Pathologie de Maul-und Klauenseuche', *Mh. Vet. Med* 13,401.

Radostitis, O.M, Blood, D.C. & Gay, C.C., 1994, *A text book of the diseases of cattle*,

sheep, pigs, goats and horses, ELBS, 8th edn, pp 968.

Rasmussen, T.B., Uttenthal, A., de Stricker, K., Bela'k, S. & Storgaard, T., 2003, 'Development of a novel quantitative real-time RT-PCR assay for the simultaneous detection of all serotypes of Foot-and-mouth disease virus', *Arch Virol* 148, 2005–2021.

Reid, S.M., Ferris, N.P., Hutchings, G.H., Zhang, Z., Belsham, G.J. & Alexandersen, S., 2002, 'Detection of all seven serotypes of Foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay', *J Virol Methods* 105, 67–80.

Rémond, M., Kaiser, C. & Lebreton, F., 2002, 'Diagnosis and screening of Foot-and-mouth disease', *Comparative Immunology, Microbiology and Infectious Diseases* 25 (5-6), 309–320.

Roberts, J.S. *Veterinary Obstetrics and Genital Diseases*. 2nd edition. India: CBS Publisher and Distributors, 197; 108-112.

Robiolo, B., 2006, 'Analysis of immune response to FMDV structural and non-structural proteins in cattle in Argentina by the combined use of liquid phase and 3 ABC-ELISA tests', *Vaccine* 24, 997-1008

Röhrer, H. & Olechnowitz, A.F., 1980, In *Maul-und Klauenseuche*, Gustav Fisher Verlag, Jena, DDR, 247–267.

Rossi, M.S., Sadir, A.M., Schudel, A.A. & Palma, E.L., 1988, 'Detection of Foot-and-mouth-disease virus with DNA probes in bovine esophageal-pharyngeal fluids', *Arch Virol* 99, 67–74.

Rweyemamu, M., Roeder, P., Mackay, D., Sumption, K., Brownlie, J., Leforban, Y., Valarcher, J.F, Knowles, N.J., Saraiva, V., 2008, 'Epidemiological patterns of Foot-and-mouth disease worldwide Transbound', *Emerg. Dis* 55, 57–72

Rweyemamu, M.M., Booth, J.C., Head, M. & Pay, J.W.F., 1978, 'Microneutralisation

test for serological typing and subtyping of FMD virus strains', *Journal of Hygiene* 81(1), 107-123.

Salman, M.D. & Meyer, M.E., 1984, 'Epidemiology of bovine brucellosis in the Mexican valley, Mexico: Literatures review of disease-associated factors', *American Journal of Veterinary Research* 45, 1557-1560.

Salt, J.S., 1993, 'The carrier state in Foot and mouth disease: an immunological review', *Br. Vet. J.* 149, 207–223.

Samuel, A.R. & Knowles, N.J., 2001, 'Foot-and-mouth-disease type O viruses exhibit genetically and geographically distinct evolutionary lineages (topotypes)', *J. Gen. Virol* 82, 609-621.

Sangare, O., Bastos, A.D.S., Marquardt, O., Venter, E. H., Vosloo, W. & Thomson, G. R. 2003. Molecular epidemiology of serotype O Foot-and-mouth disease virus with emphasis on West and South Africa.

Sangare, O., Bastos, A.D.S., Marquardt, O., Venter, E.H., Vosloo, W. & Thomson, G.R., 2001, 'Molecular epidemiology of serotype O Foot-and-mouth disease virus with emphasis on West and South Africa', *Virus* 22 (3), 345-351.

Schneider, H.P., 1994, *Animal Health and Veterinary Medicines in Namibia*, Publication by Agrivet International, Windhoek, Namibia

Schweiger, B., Zadow, I., Heckler, R., Timm, H. & Pauli, G., 2000, 'Application Scientifique et Technique Office International des Épizooties', 23, 1003-1009.

Scott, F.W., Cottral, G.E. & Gailiunas, P., 1965, 'Presence of Foot-and-mouth disease virus in the pituitary and central nervous system of experimentally infected cattle', *Proceedings of the 69th Annual Mtg US Livestock Sanitary Assoc*, pp. 87.

Scudamore, J.M. & Harris, D.M., 2002, 'Control of Foot-and-mouth disease: lessons from the experience of the outbreak in Great Britain in 2001', *Rev Sci Tech Off Int Epiz*

21, 699–710.

Seibold, H.R., 1963, 'A revised concept of the lingual lesions in cattle with Foot-and-mouth disease', *Am. J. Vet. Res* 24, 1123–1130.

Seifert, S.H., 1996, *Tropical Animal Health*, 2nd edn., Dordrecht: Kluwer Academic Publishers, pp. 358.

Sellers, R.F. & Forman, A.J., 1973, 'The Hampshire epidemic of Foot-and-mouth disease 1967', *Journal of Hygiene (London)* 71, 15–34.

Sellers, R.F. & Parker, J., 1969, 'Air-borne excretion of Foot-and-mouth disease virus in monolayer cultures of calf thyroid cells', *Nature(Lond.)* 210, 1079–1080.

Sellers, R.F., Herniman, K.A.J. & Mann, J.A., 1971, 'Transfer of Foot-and-mouth disease virus in the nose of man from infected to non-infected animals', *Vet. Rec*, 16 October, 447–449.

Sergeant, E.S.G., 2014, *Epitools epidemiological calculators*. Aus Vet Animal Health Services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease, viewed 12 March 2007, from <http://epitools.ausvet.com.au>,.

Shimshony, A., 1988 Foot- and-mouth disease in the mountain gazelle in Israel Rev. sci. tech. Int. off. Epiz., 7, 917–923

Skinner, H.H. & Knight, E.H., 1964, 'Environmental factors influencing the response of guinea pigs to modified strains of Foot-and-mouth disease virus', *Bull. Off. Int. Epiz* 61, 11–12, 1523–1543.

Sorensen, K.J., De Stricker, K. & Dyrting, K.C., 2005, 'Differentiation of Foot-and-mouth disease virus infected animals from vaccinated animals using a blocking ELISA based on baculovirus expressed FMDV 3ABC antigen and a 3ABC monoclonal antibody', *Arch Virol* 150, 805–814.

Sørensen, K.J., Hansen, C.M., Madsen, E.S. & Madsen, K.G., 1998, 'Blocking ELISAs

using the FMDV nonstructural proteins 3D, 3AB, and 3ABC produced in the baculovirus expression system', *Vet. Q* 20, 17-20.

Sorensen, K.J., Madsen, K.G., Madsen, E.S., Salt, J.S., Nqindi, J. & Mackay, D.K.J., 1998, 'Differentiation of infection from vaccination in Foot and mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus', *Arch Virol* 143, 1461-1476.

Straver, P.J., Bool, P.H., Claessens, A.M. & van Bekkum, J.G., 1970, 'Some properties of carrier strains of Foot-and-mouth disease virus', *Arch Gesamte Virusforsch* 29, 113–126.

Sutmoller, P., Barteling, S.S., Olascoaga, R.C. & Sumption, K.J., 2003, 'Control and eradication of Foot-and-mouth disease', *Virus Res* 91(1)

Sutmoller, P. & McVicar, J.W., 1981, 'Pathogenesis of Foot-and-mouth disease: The lung as an additional and simulated natural Foot-and-mouth disease infection in cattle', *J. Comp. Path* 91, 599–609.

Sutmoller, P., McVicar, J.W. & Cottral, G.E., 1968, 'The epizootiological importance of Foot-and-mouth disease carriers: Experimentally produced Foot-and-mouth disease carriers in susceptible and immune cattle', *Arch. Ges. Virusforsch* 23, 227–235.

Sutmoller, P. & Cottral, G.E., 1967, 'Improved techniques for the detection of Foot-and-mouth disease virus in carrier cattle', *Arch. Ges. Virusforsch* 21(2), 170–177.

Teklehiorghis, T., R. J. M., Moormann, K., Weerdmeester .& Dekker, A., 2014, 'Foot-and-mouth Disease Transmission in Africa': Implications for Control, a Review. *Transboundary and Emerging Diseases*, doi: 10.1111/tbed.12248 .

The Times Comprehensive Atlas of the World, 2014, 10th edn. Harper Collins

Thiermann, A., 2005, Foot- and- mouth-disease. *In: Terrestrial Animal Health Code*, 14th edn., Office International des Epizooties, OIE Standards Commission Paris,

France, pp. 111–122.

Thomson, G. R., 1996, 'The role of carrier animals in the transmission of Foot-and-mouth-disease', Comprehensive Reports on Technical Items Presented to the International Committee or to Regional Commissions, 87–103.

Thomson, G., 2008, 'A short overview of regional positions on Foot-and-mouth disease control in southern Africa, Transboundary animal disease and market access: future options for the beef industry in southern Africa', Working Paper 2, Brighton: Institute of Development Studies.

Thomson, G.R. & Bastos, A.D.S., 2004, *Foot-and-mouth- disease*, Coetzee J.A.W., Tustin R.C., In: *Infectious Diseases of Livestock in Southern Africa*, 2nd edn., pp. 1324–1365, Oxford, UK: Oxford University Press.

Thomson, G.R. 1996. 'The role of carriers animals in the transmission of Foot-and-mouth disease', Off. Int. Epiz, 64th General Session, Paris, 20-24 May, pp. 87-103.

Thomson, G.R., 1996, The role of carrier animals in the transmission of foot and mouth disease OIE Comprehensive Reports on Technical Items Presented to the International Committee or to Regional Commissions, 87–103.

Thomson, G.R., 1995. 'Overview of FMD in Southern Africa', *Revue Scientifique et Technique*. Off. Int. Epiz. 14, 503–520.

Thomson, G.R., 1994, Foot-and-mouth disease. In: Coetzer, J.A.W., Thomson, G.R. & Tustin, R.C, (Edn.), *Infectious Diseases of Livestock with Special Reference to Southern Africa*, Oxford University Press, Cape Town, London, New York, pp. 825–852.

Thornton, P.K., Kruska, R.L., Henninger, N., Kristjanson, P.M., Reid, R.S., Atieno, F., Odero, A. & Ndengwa, T., 2002, *Mapping Poverty and Livestock*, International Livestock Research Institute, Nairobi.

Thrusfield, M., 2005, *Veterinary Epidemiology*, 3rd edn, Blackwell Science, Oxford, UK.

Valdazo-González, B., Knowles, N.J., Hammond, J.D. & King, P., 2012, 'Genome sequences of SAT 2 foot-and-mouth disease viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip)', *J. Virol* 86, 8901–8902.

Van Rooyen, B. & Gartside, A., 1999, 'Findings of a survey about animal husbandry and management practices in 5 riverine villages', Livestock report, Kavango Region, Namibia.

Vangrysperre, W. & De Clercq, K., 1996, 'Rapid and sensitive polymerase chain reaction based detection and typing of Foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses', *Arch Virol* 141, 331-344.

Vigne, P., 2005, 'Reconsidering Policies to encourage formal Livestock Marketing in the Northern Communal Areas: Policy Analysis & Recommendations', NASSP Report No. 007/2005, Ministry of Agriculture, Water and Rural Development, Windhoek, Namibia.

Vosloo, W., Bastos, A.D.S., Sahle, M., Sangare, O. & Dwarka, R., 2010, Virus Topotypes and the Role of Wildlife in Foot and Mouth Disease in Africa, Chapter 10, International Union for Conservation of Nature.

Vosloo, W., Thomson, G.R., 2004, *Natural Habitats in which Foot-and-mouth disease viruses are maintained*, E. Domingo, F. Sobrino (Eds.), Foot-and-mouth disease: Current Perspectives, Horizon Bioscience, Great Britain, pp. 383–410

Vosloo, W., Bastos, A.D., Kirkbride, E., Esterhuysen, J.J., Janse van Rensburg, D., Bengis, R.G., Keet, D.F. & Thomson, G.R., 1996, 'Persistent infection of African buffalo (*Syncerus caffer*) with SAT-type Foot-and-mouth disease viruses: rate of

fixation of mutations, antigenic change and interspecies transmission', *J Gen Virol* 77, 1457–1467.

Vosloo, W., Knowles, N.J. & Thomson, G.R., 1992, *Genetic relationships between South African SAT-2 isolates of Foot-and-mouth virus*, *Epidemiol. Infect.* 109, pp. 547–558.

Watson, P., The differential diagnosis of FMD in sheep in the UK in 2001

Woodbury, E.L., 1995, 'A review of the possible mechanisms for the persistence of Foot-and-mouth disease virus ', *Epidemiol Infect* 114(1), 1-13.

Yang, P.C., Chu, R.M., Chung, W.B. & Sung, H.T., 1999, 'Epidemiological characteristics and financial costs of the 1997 Foot-and-mouth disease epidemic in Taiwan', *Vet Rec* 145, 731–734.

Appendices

Appendix A

DETECTION OF NSP ANTIBODIES TO FMD VIRUS

The FMD Ceditest® NSP ELISA has been developed and described by (Sorensen *et al* 1998). Based on these reagents Cedi Diagnostics B.V has made a ready-for-use ELISA test kit. All reagents need was equilibrated to room temperature before use. All glassware and tips used were also cleaned and sterilised. Fresh tips for each sample or reagent transfer were used. Prior to the test, individual micro plates were marked and numbered to show proposed plate layout.

Prepare day 1 working solutions:

Dilution buffer

Dilute concentrated dilution buffer supplied 1:1 (v/v) in deionized water.

Additive

Reconstitute the lyophilized additive with the demineralised water supplied. Volume as stated on the vial. Gently agitate the vial to dissolve any remaining material. Allow the solution to stand at least 15 minutes at room temperature before use. Unused prepared additive should be stored between -5 and -30 °C.

ELISA buffer

Add reconstituted additive to dilution buffer to a final concentration of 10% (v/v). Prepare 24 ml per test plate. Store unused ELISA buffer at +2-8°C.

Test Procedure

Day 1

Plates were removed from the bags and dispense 80µl of prepared ELISA buffer to all wells.

Dispense 20µl of negative control to well A1 and B1 or to the designated well in duplicate. Dispense 20µl of weak positive control to wells C1 and D1 or to the designated well in duplicate. Dispense 20µl of positive control to well E1 and F1 or to the designated well in duplicate. Dispense 20µl of test serum samples to the designated well in duplicate. Plates were sealed using the supplied plate sealer. The plates were then shake gently and incubate at +20-25 °C at room temperature for 16-18 hours.

Day 2

Prepare day 2 working solutions:

Washing buffer

Dilute the concentrated washing fluid 1/200 in deionised water (see label on vial). The kit contains enough concentrated washing fluid to prepare 12 Litres.

Conjugate

The working dilution of the conjugate was freshly prepared following the instruction on the label of the vial in ELISA buffer.

Empty the well contents into the sink using an abrupt downward hand motion. Wash plates with washing buffer or PBS for three times manually or using plate washer. Slap the inverted plate onto lint free absorbent toweling to remove residual contents.

Dispense 100µl of prepared conjugate to all wells of the plate, seal or cover the plate and incubate at +20-25°C for an hour.

Plates were washed as above:

Dispense 100µl chromogen/substrate solution (provided ready for use) in numerical order to all wells of the plate. Incubate for about 20 minutes at +20-25°C.

During the chromogen incubation time, switch on the plate reader and check the appropriate filter (450 nm) is present.

Dispense 100µl of stop solution (provided ready for use) to all wells in the same order as the chromogen/substrate solution was added. Tap the side of the plates to ensure even mixing.

Measure the optical density (OD) of the wells at 450 nm preferable within 15 minutes after color development has been stopped. Blank the spectrophotometer against air then initiate the reading sequence and read all test plates.

Calculation and interpretation of results.

Calculate the mean OD₄₅₀ value of wells A1 and B1 (negative control wells) (= OD max.)

Calculate Percentage Inhibition (PI) value of test samples and controls manually or automatically by the software installed in the computer which connected to the plate reader using the following formula:

$$PI = 100 - \left(\frac{OD_{450} \text{ of test or control sample}}{OD_{450} \text{ max.}} \right) * 100$$

Print out OD₄₅₀ values and percentage inhibition (PI) values (if applicable) of each of all plates. Label printouts appropriately to show plate plan.

Accept the assay if OD₄₅₀ or PI values of controls meet the following criteria

- a. The mean OD₄₅₀ of wells A1 and B1 (negative control, OD₄₅₀ max.) must be > 1.000
- b. The mean PI value of the weak positive control must be > 50%

c. The mean PI value of the positive control must be $>70\%$

Calculate the mean PI of each sample and interpretation of test results as following

PI value less than 50% Negative (No antibodies to FMDV 3ABC present)

PI value greater than or equal to 50% Positive (Antibody to FMDV 3ABC present)

If the OD of a test sample is higher than the OD_{450} max, the PI of this sample can be interpreted as 0%.

It was assumed that the Sensitivity of the test was 97.2% and the Specificity of 98.1% Paton *et al.* (2006).

Appendix B

Viral Neutralisation Test

Method

VNT was performed according to OIE's Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2007) at BVI. It was assumed that the test has a specificity of 95% and a sensitivity of 97%. A reactors was considered negative if the titer was less than 0.9 (Titer cut off <0.9) and positive if high than 0.9.

1. Prepare the number of microtitre plates required for Reference Serum Controls (RS), Virus Controls (VC) and Test Plates (TP). On duplicate RS plates label sections A-H 1-4, 5-8 and 9-12. On duplicate VC plates, draw a vertical line after column nine. On one or both, make additional marks to delineate wells A 10-12 (test cell medium only), B 10-12 (diluent only) and C 10-12. (cell controls). Label these plates appropriately (Neutralisation Plate Layout Controls). Choose the most appropriate TP format, and label the remaining plates accordingly (Neutralisation Plate Layout Test Sera)

2. Cover plates with loose-fitting lids to keep them sterile.

3. Add some diluents to a sterile reservoir and keep loosely covered when not required.
4. Add 50 μ L of diluent to all wells on the RS plates except where using a weak serum, when row H should be omitted. Add 50 μ l to all wells in columns 1-9 on the VC plates. Dispense 150 μ l into the diluents controls (wells B, 10-12) and 100 μ l into the cell controls.
5. Inactivated reference sera should be diluted so that the end-point in neutralisation assays occurs approximately halfway up the microtitre plate when tested with 100 Tissue Culture Infective Doses (TCID₅₀). Store this pre-dilution at -20°C between tests.
6. Thaw reference sera immediately before use and invert several times to mix thoroughly before dispensing. Add 50 μ l to every well in row H of the RS plates, and additionally to row G if using a weak serum. Make serial twofold dilutions by mixing, and transferring 50 μ l from row H to G and so on until row A, when 50 μ l must be discarded (dilute from row G if using a weak serum).
7. Cover the plates until the next step.
8. Add diluents to the TPs according to the format required:

EXAMPLE:

- a) Test large numbers of sera in blocks of six wells at single serum dilution, 16/plate. Add 50 μ l of diluent to the first vertical pair of wells (toxicity controls), and 25 μ l to the third pair. Manageable numbers of sera may be tested against additional serum dilutions simply by replicating the second and third vertical pairs in the most economical arrangement. When using one serum dilution, add 50 μ l of 1/4 test serum to the first and second (1/8 final dilution) vertical pairs and 25 μ l to the third (1/16 final). If also testing against other serum dilutions, repeat the additions to the second and third pairs.
Or
- b) When testing vaccinates or convalescents, etc., use a 6/plate format. For each serum add 50 μ l of diluent to rows B-H in duplicate, and 50 μ l of 1/4 sera likewise to A and B, then dilute from row B and discard after row H. Alternatively, using a 12/plate format,

only dispense 50 µl of diluents into rows B-D and F-H. Add 1/4 test sera in duplicate to rows A and B, dilute as far as row D, and discard. Similarly, add 1/4 sera to rows E and F, dilute as far as row H, and discard.

9. Cover all plates until required.

Reference virus isolates

1. If the last known log₁₀ titre of the reference virus/es, is 10^{-4.8} ('4.8'), equivalent to 1 TCID₅₀ per unit volume, then subtracting 2.0 gives the dilution 10^{-2.8} ('2.8') containing approximately 100 TCID₅₀. Together with a twofold (log₁₀ 0.3) or fourfold (log₁₀ 0.6) step either side, this gives the three virus Challenge Dilutions (CDs) which must all be reacted with the RS.

Unknown sera are assayed against one or more of these CDs.

2. Always start antigen dilution by adding 0.5 ml working stock to 4.5 ml diluent to make a 10⁻¹ step ('1'). To ascertain further dilutions, use the Logarithm to Arithmetic conversion chart and dispense the correct volumes of diluent into bottles. For example, if the three CDs selected are '2.5', '2.8' and '3.1', make a log₁₀ 1.5 (2.5-1.0) or 1/32 step by adding 0.5 ml of the 10⁻¹ dilution to 15.5 ml of diluent to make the 2.5 dilution. The second and third CDs are simply made by making two further twofold steps. After the third CD, make six fourfold steps to dilute the virus past its 50% end-point.

3. Add 50 µl of the highest virus dilution to every well in column 9 of both VC plates. Similarly, add 50 µl of the next highest step to every well in column 8, and so on until the third CD, which should be dispensed into column 3 of the VC plates and section A-H 9-12 of both RS plates, then to any test serum dilutions if appropriate (but not to toxicity controls). Similarly, add the second or central CD to column 2 of the VC plates and section A-H 5-8 of both RS plates, as well as to the test sera if necessary. Finish by adding the first CD to column 1 of the VC plates, section A-H 1-4 of both RS plates and to the test sera, if applicable.

5. Put the plates into piles of up to four, loosely cover them and incubate at 37°C for approximately 60 minutes.

6. Prepare an appropriate cell culture suspension of the required concentration which should be sufficient to achieve 100% confluency after 24 hours. (It is important that the cells are agitated frequently). A suspension of 10% cells/ml is usually appropriate for IBRS-2 cells but each laboratory should determine its own optimal dilution of indicator cells.
7. Add 150 μ l of test cell medium to wells a 10-12 on one VC plate. Dispense 50 μ l of cell suspension to the cell controls (C 10-12 on this VC plate), then to every well of the virus and reference sera titrations and of the TPs
8. Re-stack and loosely cover the plates and return them to the incubator for about 10 minutes.
9. Remove and tightly cover the plates with thin plastic backing tabs, and return them to the incubator.
10. Plates should be incubated at 37°C for three days and inspected daily for evidence of Cytopathic Effect in Cell Culture (CPE). Microscope readings may be feasible after 48 hours; the plates may be finally fixed and stained routinely on the third day. Fixation is effected with 10% formalin-saline for 30 minutes. For staining, the plates are immersed in 0.05% methylene blue in 10% formalin for 30 minutes. An alternative fixative/stain solution is a 0.4% (w/v) naphthalene blue black solution in 8% (w/v) citric acid in saline. The plates are rinsed finally in tap water.

Preparation

Diluent

Eagle's Minimum Essential Medium (Eagle's MEM)

This can be formulated according to standard tissue culture protocols or, alternatively, supplied complete as Minimum Essential Medium Eagle (HEPES Modification), from Sigma Ltd (500 ml).

Disinfectant solution

0.4% FAM in tap water made up daily, or 0.2% citric acid in tap water.

Preparation of test samples

Sera may arrive as whole bloods in vacutainers. To separate the serum, either leave overnight at +4°C and tip off into bottles or centrifuge the tubes in a bench centrifuge at approximately 600g (1-3000 rpm) for 5-10 minutes. In the latter case, pour off the serum directly into a bottle or use a pipette. Always ensure contamination from less tightly packed loose red blood cells is kept to a minimum. Label each serum.

For testing, sera (and reference control sera) are diluted 1:4 in diluent and heat inactivated at 56°C for 30-45 minutes. Samples are held at 4°C until required.

If sera are to be kept for some time before testing they should be stored undiluted at -20°C.

Cell culture

Appropriate cell cultures, grown as monolayers in tubes or flasks, should be prepared as required in a dedicated tissue culture facility. Description of tissue culture methodology and procedures is beyond the scope of this module. Any suitably sensitive cell line may be used. At the WRL for FMD IBRS-2 cells are used as a routine although BHK cells are an alternative. It may be necessary to passage the virus several times on the selected cell line to achieve a suitable level of adaptation.

Chemicals

FAM disinfectant (Evans Vanodine International). Citric acid (standard grade, any make).

Antibiotics

Eagle's Minimum Essential Medium (Eagle's MEM): This can be formulated according to standard tissue culture protocols or, alternatively, supplied complete as Minimum Essential Medium Eagle (HEPES Modification), from Sigma Ltd (500 ml).

Biological Reagents

Standard reference viruses: Stocks of standardised reference virus strains are grown in tissue culture and stored at -20°C after addition of an equal volume of glycerol. The

stocks are repeatedly titrated to obtain a running mean titer which is constantly updated each time an assay, including virus titration, is run.

Standard reference sera: Stocks of standardised bovine convalescent sera for each reference virus are maintained. The running mean titers of the reference serum are derived by repeated titration and are updated as described above.