SERO-PREVALENCE AND RISK FACTORS OF FOOT AND MOUTH DISEASE IN GOATS IN NGAMILAND AND NORTH EAST DISTRICTS OF BOTSWANA

BY

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DECLARATION

The contents of the dissertation are the author's own works. The dissertation has not previously been submitted for the award of degree to any University.

Gabasenye Juliah Raletobana

Date

DEDICATION

This work is dedicated to my lovely daughter, husband and mother. I love you all.

ABSTRACT

Foot and mouth disease (FMD) is an economically and highly contagious viral disease that affects cloven-hoofed domestic and wild animals. Although adult animals generally recover, the morbidity rate is high in naïve populations, and significant pain occurs in other species.

A cross sectional study was conducted in the North East and Ngamiland districts of Botswana between September and November 2012, to determine the sero-prevalence and identify potential risk factors associated with FMD in goats. Results of the study showed that out of the 640 goats sampled, 238 animals tested positive for FMD virus antibodies in the two study areas representing an overall sero-prevalence of 37.2% (95%, CI =33.43-40.94). In Ngamiland district the sero-prevalence was 25.2% (95%, CI= 20.74-29.67), while in the North East district, the sero-prevalence was 53.09% (95%, CI=47.17-59.01). There was a significant difference in sero-prevalence between the two study areas ($p<0.01$). Variations were also observed in the sero-prevalence of the disease among the villages. Antibodies to all the three Southern African Territories (SAT) serotypes were detected in the goats in the study areas.

Variables that were found to be important predictors of goats being sero-positive to FMD were age and district (location). It was found that goats from Ngamiland district were 0.298 (95%, CI=0.213-0.416) times less likely to be sero-positive for FMDV antibodies than those from the North East district ($p<0.01$). Adult goats were found to be 1.327 times more likely to be sero-positive than weaner goats ($p = 0.006$) while kids were found to be 4.744 (95%, CI=2.194-10.257) times more likely to be sero-positive than weaners ($p < 0.01$).

These results show that FMD is prevalent in goats in Botswana and that goats may play an important role in the epidemiology of the disease. It is therefore, recommended that this animal species should also be included in the routine vaccination programmes against FMD.

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LIST OF ABBREVIATIONS

BVI	Botswana Vaccine Institute
CFT	Complement Fixation Test
ELISA	Enzyme-Linked immunosorbent assay
FAO	Food Agriculture Organisation
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
GDP	Gross Domestic Product
IgG	Immunoglobin G
Kb	Kilobyte
Km	Kilometers
LPBE	Liquid Phase Blocking ELISA
NSP	Non-Structural Proteins
OD	Optical density
ODp	Optical density percentage
OIE	World Organisation for Animal Health
OPD	*O*-Phenylenediamine dihydrochloride
OVI	Onderstepoort Veterinary Institute
PBS	Phosphate buffered saline

PBST	Phosphate buffered saline tween
PI	Percentage Inhibition
SAT	South African Territories
UK	United Kingdom
VNT	Virus Neutralization Test
WRL	World Reference Laboratory

CHAPTER 1.0

1.0 INTRODUCTION

Foot and Mouth Disease (FMD) is a highly infectious viral disease of cattle, pigs, sheep, goats, buffalo *(Syncerus caffer)* and artiodactyls wildlife (Grubman and Baxt, 2004). The disease is caused by the FMD virus of which there are seven serotypes namely, O, A, C, South African Territories (SAT) 1, 2, and 3, and Asia 1. The disease is a major global animal health problem and the most contagious trans-boundary animal disease (Thompson, 1994: Murphy *et al*., 1999; Radostits *et al.,* 2000). Although seldom lethal in adult animals, it causes serious production losses and is a major constraint to international trade in livestock and livestock products. Severe mortality may occur in young stock, particularly lambs and piglets (FAO, 2002).

In Botswana, African buffaloes (*syncerus caffer*) occur in large numbers along the Okavango delta, the Chobe and Nata river basins and these areas constitute the main FMD high-risk zones in the country (Baipoledi *et al.,* 2004; Hyera *et al.,* 2006). Clinical FMD has not been observed in buffaloes resident in these areas but all SAT serotypes of FMD virus have been isolated from clinically healthy buffaloes (Hedger *et al.,* 1969; Mapitse, 1998). Consequently, cloven-hoofed livestock, particularly cattle being raised in the vicinity of these areas/zones, are at constant risk of becoming infected with SAT serotype of FMD virus (Baipoledi *et al.,* 2004; Hyera *et al.,* 2006).

Small ruminants can play an important role in the spread of FMD virus (FMDV) but it is not clear whether the virus can be maintained in these species for long periods in the absence of infection in cattle (OIE, 2008). Natural infection and carrier status has been reported in both sheep and goats (McVicar & Sutmoller 1968, Hedjazi *et al.,* 1972; Ganter *et al*., 2001). In general sheep and goats develop persistent infection less frequently, and for shorter periods than cattle, and the carrier state

last for one to five months only (Burrows, 1968). In sheep the carrier state may last for up twelve months (McVicar and Sutmoller 1968). Persistent outbreaks of FMD have occurred in some parts of Botswana over the years (Baipoledi *et al*., 2004). Goats were never implicated in these outbreaks except for the last outbreak which occurred in 2011 in the North East region of the country in Matsiloje village, where goats tested sero-positive to both liquid phase blocking Enzyme-Linked immunosorbent assay (ELISA) and Non-Structural Protein ELISA (NSP-ELISA) (Anon, 2011). In these outbreaks, a combination of stamping out and vaccination of cattle against the disease was done. Other species affected in this outbreak included cattle and sheep. However, despite having confirmed the disease in goats in this outbreak, the actual role that goats play in the epidemiology of FMD in Botswana is still unclear. In addition, the factors that may lead to goats being infected or to remain carriers of the disease remain unknown. This study therefore aimed at determining the sero-prevalence and risk factors associated with goats being sero-positive to FMD in Ngamiland and North East districts of Botswana.

1.1 General Objectives of the study

To determine the sero-prevalence and risk factors of FMD in goats in Ngamiland and North East Districts of Botswana.

1.2 Specific Objectives of the study

- To determine the sero-prevalence of FMD in goats in the Ngamiland and North East Districts of Botswana.
- To determine the risk factors associated with FMD infection in goats.

CHAPTER 2.0

2.0 LITERATURE REVIEW

2.1 Livestock production system in Botswana

Botswana occupies about 572 000 km^2 of raised plateau at a mean elevation of 1 000 m above sea level. About 450 000 km^2 of this area is rangeland, supporting a cattle population of around 3 million and a sheep and goat population of approximately 2.6 million. (http://www.ilri.org/InfoServ/). Mean annual rainfall varies from 700 mm in the North-East, dropping to 400 mm in the East and 200 mm in the South-West. Rain falls in the summer months from October to April and fluctuates widely between and within seasons. The beef cattle industry is based on the use of the natural pasture produced by this environment (http://www.ilri.org/InfoServ/).

Ruminant livestock production systems are dominated by cattle and small-stock (goats and sheep). The sector is divided into traditional, mixed small holder and commercial producers (FAO, 2006). Traditional systems are dominated by the cattle-post system, where a farmer, or group of farmers water livestock at a central watering point (most often a well, or a borehole), and the livestock wander freely over the grazing land around the watering point. There are some areas where transhumance is still practised, mainly in the eastern hardveld. People there usually practice crop and livestock production. Arable land is fenced, but livestock are permitted onto the fields once the crops have been harvested. Many members of the family move from the villages to their lands

during the ploughing, planting and harvesting period, but go back home during the fallow season when livestock are herded by herders who remain in the grazing areas (http://www.ilri.org/InfoServ/).

Mixed small-holder systems are an integral part of the traditional livestock sector. People who live in areas where surface water is available, either on a year-round, or a seasonal basis, have some livestock and some small fields. This is most noticeable in the North-west, the North and the Eastern parts of the country where there are seasonal and perennial rivers. Much of the land is either freehold or reserve land in areas where there is perennial water (http://www.ilri.org/InfoServ/). Livestock are normally grazed in the dry areas away from the rivers, but watered either from wells or in dry, sandy river beds. Crop production is normally from small plots on river banks which are thorn fenced, and watered from wells.

Commercial production systems are practised mainly on leasehold and freehold land by people who are relatively wealthy and have access to finance and lucrative markets. Within the commercial sector, productions systems include intensive livestock production systems, such as feedlots. Stall-feeding, tethering and other systems are rarely used, except in one or two instances where dairy cows are kept in extremely dry conditions, and are fed with green silage and imported grains and fodder.

Exports of animal products from Botswana, mainly in the form of fresh meat, account for 17 percent of the gross domestic product (GDP) and almost one half of the value of exports (Mapitse, 2008). Before the recent expansion in the mining industry, the livestock sector was even more important to the economy. The success which has been achieved in beef marketing is due largely to a history of effective disease control, particularly against foot and mouth disease, and the absence

of Rinderpest, Contagious Bovine Pleuropneumonia, tsetse fly transmitted trypanosomiasis, and tick-borne disease such as East Coast fever (Theileriosis) (http://www.ilri.org).

2.2 Wildlife production systems in Botswana

Botswana's wildlife production systems comprise national parks and Game reserves. The Okavango delta is one of the most popular tourist attraction places in Botswana. On the surrounding mainland and among the islands of the Delta, the predators live among their prey. Lions, elephants, hyenas, wild dogs, buffaloes, hippos and crocodiles share the space with many antelope species and other small wild animals (Anon, 2011). Maun is the gateway to the Okavango Delta and the Moremi Game Reserve. For that reason, most visitors to Botswana pass through Maun at one point or another. Situated on the Chobe River, Kasane is the main gateway to the world-renowned Chobe National Park. No boundary fences separate the park from the villages. Elephants and hippos frequently wander through the town's streets; hence this makes the area at risk of diseases like FMD because of wildlife-livestock interaction. Moreover the buffaloes which are known to be the maintenance host for FMDV (Hedger *et al.*, 1972) are found in the Okavango delta, the Chobe and Nata basin. Cattle raised in the vicinity of these areas are at constant risk of becoming infected with FMDV (Hyera *et al*., 2006). However the veterinary administration in Botswana has introduced a fencing policy for all livestock ranches in the country to avoid the contact of livestock with wildlife, especially the African buffalo (Baipoledi *et al*.**,** 2004).

2.3 Aetiology of FMD

The FMDV is a member of a family *Picornaviridae*, genus *Aphthovirus (*Andrews *et al.,* 1978; James *et al*., 2012). It has a single-stranded, positive-sense RNA genome of approximately 8.4 Kb. There are 7 immunologically distinct serotypes of FMD with a large number of variants spread

over several regions in the world (Alexandersen *et al.*, 2003; Grubman and Baxt, 2004). These are O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1 (OIE, 2007). Of these seven FMDV serotypes, O is the most prevalent worldwide (Reid *et al*., 2001).

All the serotypes produce a disease that is clinically indistinguishable but immunologically distinct. There is no cross-immunity among serotypes (FAO, 2002). They can be differentiated by various serological tests, including the virus neutralization test (VNT), the complement fixation test (CFT) and ELISA (FAO, 2002). Within each serotype there is a spectrum of antigenic variation with strains of close or distant relationship to each other. Antigenic variation tends to be greatest within type A. Analysis of strains of FMDV by antigenic and genetic profiles is important in epidemiological studies and for the selection of the most appropriate vaccine strains for a region where vaccination is practised (FAO, 2002).

2.4 Worldwide distribution of FMD

Foot and Mouth Disease is endemic in the Middle East, Iran, the Southern countries of the former Soviet Union, India and South East Asia and Africa (Aiello, 1995). Table 1 below shows worldwide distribution of foot and mouth disease serotypes.

Table 1; worldwide distribution of foot and mouth Disease serotypes

Serotype	Representative country (ies)	Referencece
SAT 1	South Africa, Southern Zimbabwe, Mozambique	Vosloo *et al.*, 1995
	Botswana ,Namibia, Zambia, Western Zimbabwe	Bastos *et al.*, 2001
	Zambia, Malawi, Northern Zimbabwe	
SAT 2	SouthAfrica, Mozambique, Southern Zimabwe	Bastos *et al.*, 2003b
	Namibia, Botswana, Northen and Western Zimbabwe	Vosloo *et al.*, 1995
	Botswana, Zambia Zimbabwe	
SAT 3	South Africa, Southern Zimbabwe	Vosloo *et al.*, 1995
	Namibia,Botswana, Western Zimbabwe	Bastos *et al.*, 2003a
	Malawi and Northen Zimbabwe	
	Zambia	
O	Brazil, Angola, Tanzania, Uganda	Sahle, 2003
	Iran	Sangare *et al*, 2001
	Philippines	
	South Africa	
A	Mauritania, Mali, Côte d'Ivoire, Ghana, Niger, Nigeria, Cameroon, Chad, Senegal, Gambia, Sudan	Knowles and Samuel, 2003
	Angola, Algeria, Morocco, Libya, Tunisia, Malawi	Knowles and Samuel 2003
	Ethiopia	Knowles *et al.* 1998
	Uganda, Kenya, Ethiopia, Sudan, Eritrea	
C	Kenya	Reid *et al.* 2001
	Ethiopia, Kenya	Knowles and Samuel 2003
	Angola	
C	Angola	Knowles and Samuel, 2003
	Kenya	FAO, 2007
	Ethiopia	
Asia 1	Afghanistan, Bahrain, Iran, Pakistan, Turkey	OIE, 2012

The Pacific nations and the Caribbean are free from the disease (FAO, 2002). In the Sub-Saharan Africa, Madagasca, Mauritius and Seychelles are free from FMD, with a recognized status of FMD freedom without vaccination (Vosloo *et al*., 2002).

Six serotypes, namely O, A, C and SAT 1, SAT 2 and SAT 3 are endemic in most sub-Saharan African countries with marked difference in distribution (Kitching, 1998., Vosloo *et al*., 2002). Serotypes SAT 1, SAT 2, A and O are the most frequently occurring, while serotype C rarely occurs (Rweyemamu *et al.,* 2000; Vosloo *et al.,* 2002). In some parts of Africa, virus persistence in wild African buffalo (*Syncerus caffer*) makes eradication unfeasible. Several studies in Southern Africa have shown that the African buffalo is capable of maintaining silent infection of serotypes SAT 1, SAT 2, SAT 3 and one buffalo can become infected with all three of the SAT serotypes of FMD virus and this poses a threat of infection to other susceptible cloven-hoofed animals (Bengis, *et al*., 1986; Hargreaves *et al.,* 2004, Vosloo, 2002). Most living populations of African buffalo in Southern Africa have high infection rates with SAT serotypes of FMDV (Esterhuyse *et al*., 1995). In the Kruger National Park in South Africa, rates of persistent infection of buffalo are estimated to be as high as 60% (Hedger *et al.,* 1972; Hedger *et al*., 1976). These animals are usually persistently infected in the oro-pharnx, sometimes with multiple serotypes and often for long periods (Condy *et al.,* 1985).

In Botswana clinical FMD has not been observed in African buffaloes and all SAT serotypes of the virus have been isolated from clinically healthy animals of this species (Hedger *et al*., 1969; Mapitse, 1998). Consequently, cloven-hoofed livestock particularly cattle being raised in the vicinity of the areas where buffaloes reside are at constant risk of becoming infected with SAT type of FMD virus (Baipoledi *et al.,* 2004; Hyera *et al*., 2006). Sporadic outbreaks of FMD have occurred in disease-free countries, with the exception of New Zealand, Greenland, Iceland and the

smaller islands of Oceania. The last United States outbreak occurred in 1929 (OIE 2007). The map below distribution of FMD sero-types between 1990 and 2002.

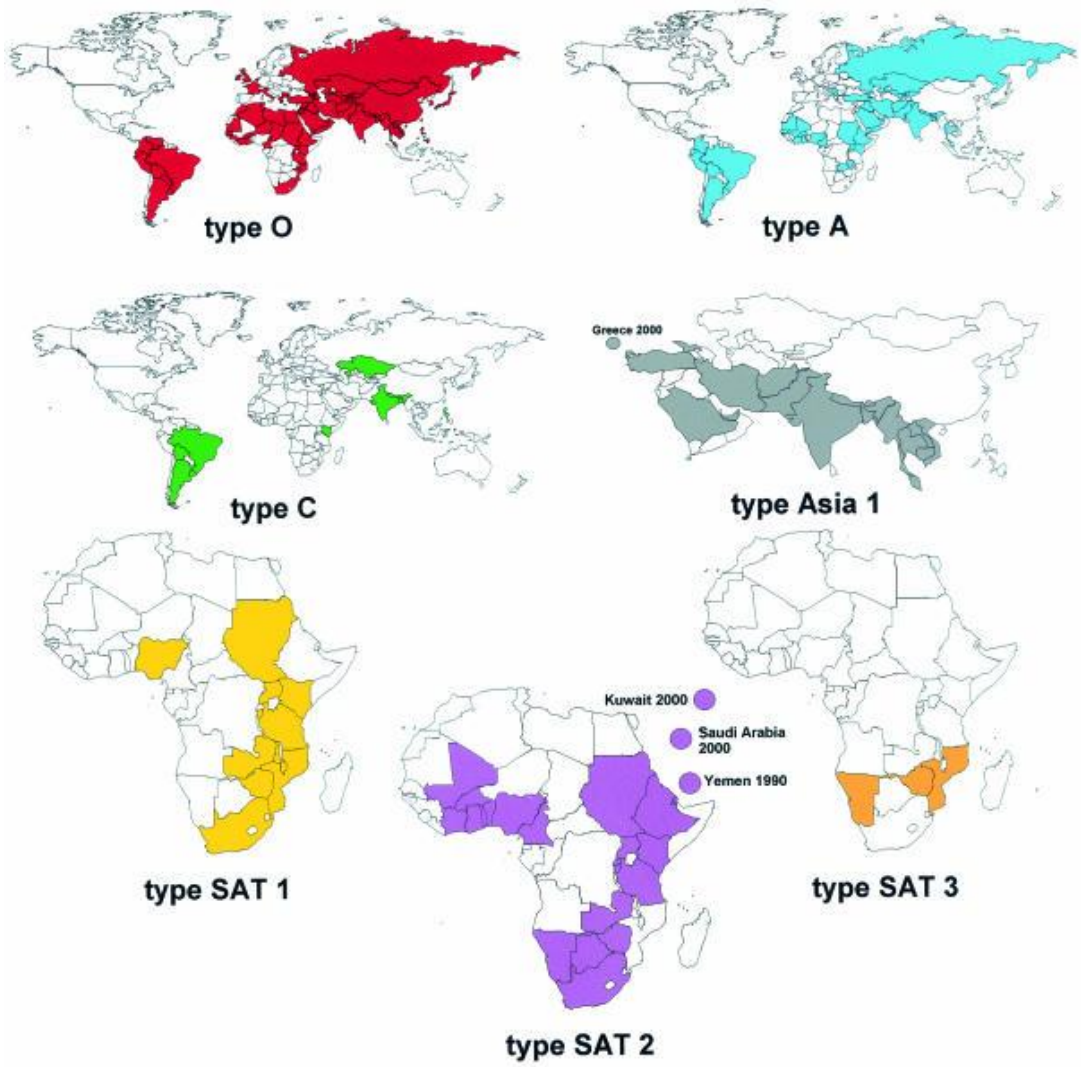


Figure 1; Distribution of FMD sero-types between 1990 and 2002

Sourced from; http://www.ncbi.nlm.nih.gov/core/lw/2.0/html/tileshop_pmc_inline.

2.5 History of FMD in Botswana

Foot and mouth disease in Botswana was first officially confirmed in 1933 by Walker (1934). He reported that during the rainy season of 1932-1933, the rainfall was exceptionally low which led to severe drought experienced for many months. Wildlife, especially wildbests, were forced to seek water from cattle watering points; it was therefore assumed that cattle picked the disease from these animals since some wildebeests showed the same lesions (Walker, 1934). Serotype O was isolated in this outbreak. According to Baipoledi *et al.,* (2004), this was the first and last recorded outbreak of a serotype O FMDV in both cattle and wildlife in Botswana.

During the period 1948-1970, eight outbreaks of FMD occurred in various parts of the country caused by either SAT 1 or SAT 3 serotypes (Baipoledi *et al*, 2004). These outbreaks were associated with cattle having been in contact with buffaloes (Falconer, 1972). The first outbreak of FMD caused by a combination of SAT 1 and SAT 2 serotypes in Botswana was recorded in 1977 (Annon, 1977). In the subsequent two years, two other outbreaks of the disease occurred and these outbreaks were also caused by the same combination of SAT serotypes of FMDV (Annon, 1978; 1979). Contrasting with these, the 1980 FMD outbreak was caused by SAT 2 alone, this was apparently the first outbreak of FMD in cattle in this country to be associated sorely with SAT 2 type of FMDV (Annon , 1980).

After a year, the disease caused by SAT 2 resurfaced in Chobe district and North East district, in 2005 and 2006, respectively. The latter being an FMD free without vaccination and export zone. The disease reoccurred in 2007 and 2008 in zone 1 which is an FMD free without vaccination.

In 2011, the disease resurfaced in Central and North East districts, which were FMD free zones in the North eastern part of the country. The virus was identified as serotype SAT 2 and topo-type 1,

which was the first report of this topo-type in the country. The virus was closely related to an FMD outbreak of 2010 in Mozambique (Annon, 2012).

Outbreaks of FMD in small stock have always been relatively rare compared to those in cattle (Hyera, 2006). When FMD occurred in small stock, it was mainly attributable to SAT 1 and was very mild (Hyera, 2006). In the recent outbreaks of 2011 in zone 6 and 2, although sheep and goats did not show any clinical signs, some animals tested positive to NSP antibodies against FMD virus in non-vaccinated population in crushes that had clinical cases in cattle (Annon, 2011).

2.6 Epidemiology

The epidemiology of FMD is influenced by a cycle in which wildlife plays a role in maintaining and spreading the disease to other susceptible domestic animals and wild ungulates and a cycle that is maintained within domestic animals that is independent of wildlife (Thomson *et al.,* 2003). Outbreaks of FMD in cattle caused by SAT serotypes are usually associated with wild buffalo known to be the reservoir host (Thomson *et al.,* 2003). According to Condy and Hedger, (1969), FMDV infections may be maintained in cattle subclinically by strengthening of the virus through serial passage in the same species and depending on the density of naïve cattle, epidemics may occur.

Potential risk factors which have been found to be associated with FMD include, farming system, age category of animals, breed type, sex and seasonal influence (Sarker *et al*., 2011). Other risk factors pointed out by Intha, (2009) include management of the farm, feed source of the animal, trade of animal and husbandry practices. The semi-intensive farm systems or smallholder livestock in developing countries are prone to FMD. The reasons might stem from either the increased contact between animals infected and animals susceptible to the infection or

from higher virus survival in the more humid microclimate around water sources (Geering and Lubroth, 2002; Williams, 2003). Furthermore, raising goats with cattle may increase the risk of FMD infection because goats are highly susceptible to the FMDV and spread the virus via aerosol (Kitching and Hughes, 2002). This may be the mode of virus transmission to other animals that are raised together in the same area. Once an animal is infected, the virus can be disseminated into the environment including field pastures, water resources and soil. Sharing of pasture and water source is common in most African countries especially Botswana where farmers (small holder) feed their animals by letting the animals roam freely in communal pastures. This promotes the spread and infection of FMD. Moreover, FMDV infection in cattle is mainly transmitted via infected animal and susceptible animals in the same area by aerosol, because cattle are sensitive to respiratory infections (Kitching, 2005).

Purchasing cattle from animal markets is believed to be a risk factor of outbreaks of FMD into a herd. Bronsvoort *et al* (2004) reported that cattle raised in a herd that brought in new cattle from other places were more likely to have the disease when compared to the cattle in a herd that did not bring in new cattle. Recently, the danger of spread of FMD by animal movement was clearly illustrated by a shipment of sheep from the UK that disseminated the virus to other animals in a rest-station in western France (Sutmoller *et al.,* 2003). In addition, the chances of an animal getting infected increases with the age of the animal (Gelaye et al., 2009, Sarker *et al*., 2011). This had been attributed to younger animals being herded in homestead while adults are out most of the time, constantly being re-exposed to infection (Mackay *et al*., 1998).

The use of molecular epidemiology to elucidate the source of the FMDV has become common in recent years (Knowles and Samuel, 2003). Molecular epidemiology using phylogenetics relates FMDV isolates from outbreaks or persistently infected animals with viruses available at

the viral gene bank at Onderstepoort Veterinary Institute (OVI) in Pretoria, South Africa, and World Reference Laboratory (WRL) for FMD at Pirbright in the United Kingdom. However, this does not reflect the true epidemiology of the disease because it does not account for the underlying factors. Bronsvoort *et al.*, (2004) suggests structured sampling of endemically infected populations to understand complex epidemiological situations with multiple serotypes of FMDV and various degrees of diversity within serotypes circulating in a region or herd.

2.6.1 Susceptible species

Domestic livestock species susceptible to FMD include cattle, water buffaloes, pigs, sheep, goats and deers (FAO, 2002). The disease is generally most severe in cattle and pigs. Camelidae (camels, llamas and vicuñas) have a low susceptibility. Although rare, FMD in elephants, hedgehogs and some rodents has been documented (FAO, 2000). African buffaloes (*Syncerus caffer*) commonly become infected with FMD virus of the SAT serotypes. Human infections have been reported but are extremely rare and mild. However, people may harbour the virus in their respiratory tract for more than 24 hours without ever developing clinical disease (FAO, 2002).

2.6.2 FMD in animal products

Although the FMD virus is inactivated in the meat carcasses that undergo the normal post-slaughter acidification processes, it can retain infectivity for very long periods in frozen or chilled lymph nodes, bone marrow and residual blood clots, and for shorter periods in offals (FAO, 2000). Other products in which the virus can retain infectivity for long periods include uncooked salted and cured meats, green-salted hides, unpasteurized milk and some other dairy products (FAO, 2002).

2.6.3 The role of domestic animals in the Epidemiology of FMD

The role of domestic animals in the maintenance and spread of FMD in sub-Saharan Africa has not been studied in detail. However, it is accepted that domestic animals play a significant role in the epidemiology of FMD in East and West Africa due to uncontrolled domestic animal movement within and between countries, inadequate vaccination coverage to prevent disease transmission, and the fact that cattle, sheep, and goats can become FMD carriers (Vosloo *et al*., 2002). In Zimbabwe (Southern Africa) for example, FMD spread seemed to have been perpetuated by domestic animal populations since the initial possible spread from buffalo in September 2001 (Vosloo *et al*., 2002).

Small ruminants can also play an important role in the epidemiology of FMD (Ganter *et al.*, 2001). In adult sheep and goats, FMD is frequently mild or inapparent and the cardinal signs mimic other diseases which makes a clinical diagnosis difficult. However high mortality can result in young animals (Kitching and Hughes, 2002). Their ability to become carriers represents a reservoir for further infection and spread of disease, and so trade of live sheep and goats present a major risk of entry of FMD to disease free countries (Barnett and Cox, 1999). In Turkey, 18.5% of the total FMD cases reported in 1996 were associated with small ruminants (Taylor *et al.*, 1996), and in Greece, during the 1996 FMD epidemic, 5,000 sheep and goats were destroyed (Kitching, 2002). In the 2001 epidemic in Great Britain, the first species infected on the affected farms was almost always sheep (53%) or cattle (45%) rather than pigs (Ferguson *et al*., 2001). In an epizootiological study of FMD, in Sudan conducted by Habiela *et al.*, (2010), liquid phase blocking ELISA revealed that antibodies to four serotypes (O, A SAT 1 and SAT 2), were present in goats and sheep. In Botswana the role that small ruminants play in FMD transmission is not known although previous studies have reported evidence of exposure to the virus in goats (Hyera *et al*., 2006).

2.6.4 FMDV maternal antibodies in various livestock

In the investigation carried out by Prasanna et al., (2002), FMDV quadrivalent double emulsion (Montanide ISA 206) vaccines were tested in sheep. The oil adjuvant elicited a better immune response at any time than did aluminum hydroxide gel vaccine, and the response developed quicker. The animals maintained their neutralizing antibody titers at >3 $\log_{10}$ for the duration of the trial (90 days).

The half-life of Maternal Derived Antibodies (MDA) was shown to be approximately 22 days, suggesting that under field conditions significant MDA titres are likely to persist for 4-5 months. A trial carried out in Brazil in which the primary course of two inoculations, 4 weeks apart, was initiated when the calves were 5-6 months of age, resulted in the reduction of FMD in the calf population from 11% to 0.9% over a 12-month period (Nicholls *et al,* 1984). In piglets however, maternal antibodies were found to persist for at least 3 weeks (Francis and Black, 1984).

2.7 Transmission

Transmission of FMD is between infected and susceptible animals (Merck & Co, 2005). FMDV can be found in all secretions and excretions from acutely infected animals, including expired air, saliva, milk, urine, faeces and semen. Pigs, in particular, produce large quantities of aerosolized virus (OIE, 2007). Animals can shed FMDV for up to four days before the onset of clinical signs (OIE, 2007). The virus can also be transmitted on fomites including vehicles, as well as mechanically by animals and other living vectors. Airborne transmission can occur under favourable climatic conditions. FMDV is thought to have been transmitted via aerosols from Brittany to Jersey (approximately 30 miles or 48 km) and for approximately 70 miles (113 km) from Jersey to the Isle of Wight (Bartley *et al.,* 2002).

Infected herds which practice transhumance or are nomadic can spread the infection to other herds long before diagnosis of the disease is established (Ganter *et al*., 2001). Shipping and trade with live sheep and goats is much more common than in other FMD susceptible species (Ganter *et al*., 2001). Ability to become carriers for a period of time represents a reservoir for further infection and spread of the disease, and so trade of live sheep and goats presents a major risk of entry of FMD to disease-free countries (Barnett *et al*,. 1999). Lack of registration of all sheep and goat herds (especially of small hobby herds) and lack of individual identifications signs (ear tags) may result in difficulties in controlling the disease (Ganter *et al*., 2001).

Another important factor in the transmission of FMD virus is its relative stability under the right environmental conditions (Cottral, 1969). Relative humidity levels above 55%, cool temperatures and approximately neutral or slightly alkaline conditions favour prolonged survival of infective aerosols and fomites (Donaldson, 1986).

In cattle, the incubation period varies from two to 14 days, depending on the dose of the virus and route of infection. In pigs, the incubation period is usually two days or more, but can be as short as 18-24 hours (OIE, 2007). The incubation period in sheep is usually 3 to 8 days. Incubation periods as short as 24 hours and as long as 12 days have been reported in this species after experimental infection (OIE, 2007).

2.8 Clinical signs

Clinical signs of FMD are more severe in cattle and intensively reared pigs than in sheep and goats, resulting in the disease being frequently ignored or misdiagnosed in small ruminants (Aiello, 1995). Although the disease is frequently mild or unapparent in adult cattle, sheep and goats, FMD can cause high mortality in young animals (Kitching *et al*., 2002). In the mouth, vesicles are

particularly prominent on the tongue, dental pad and gums. In severe cases, most of the mucosa of the dorsal surface of the tongue may slough. The painful stomatitis associated with unruptured and freshly ruptured vesicles causes excess salivation, lip smacking and cessation of eating. There is rapid loss of body condition (FAO, 2000). According to Radostits *et al*., (2000), vesicles are also formed around the coronary band and skin of interdigital spaces. Fluids from ruptured vesicles spread to areas of abraded skin, for example that of mammary glands. Vesicles often rupture rapidly, becoming erosions. Pain and discomfort from the lesions leads to a variety of symptoms including depression, anorexia, excessive salivation, lameness and reluctance to move or rise. Lesions on the coronary band may cause growth arrest lines on the hoof (OIE, 2000).

2.9 Diagnosis

Diagnosis is initially based on clinical signs (OIE, 2011). Susceptible animals exhibiting excess salivation, lameness and other suggestive clinical signs should be examined carefully for vesicular lesions. If these are found, FMD should strongly be suspected and appropriate action taken immediately to secure a definitive diagnosis and prevent any further spread of the disease while this is being done (FAO, 2008). A presumptive clinical diagnosis associated with laboratory tests such as serology, virus isolation, and antigen detection are the basis for the diagnosis at herd level. Serological tests i.e., virus neutralization and liquid phase blocking ELISA (LPBE) are not time-consuming, but they are indirect tests that do not always allow for differentiation between infected and vaccinated cattle due to the use of poor vaccine or previous infection in endemic areas. Serological techniques are not the first choice to detect an acute infection (Sutmoller *et al*., 2003; OIE, 2008). A definitive diagnosis is based on detection of virus in fluids or epithelium from vesicular lesions or oesophageal-pharyngeal fluid collected with a probang (Rémond *et al.,* 2002;

OIE, 2008). Virus isolation is the most reliable diagnostic method, but it is labour-intensive, time-consuming, and requires properly equipped facilities. Sandwich ELISA is a much faster approach to detect viral antigens, but it has low sensitivity, so its primary indication is to confirm and type the FMDV after isolation in cell culture (Rémond *et al.*, 2002). Therefore, there has been a lot of effort in developing faster diagnostic methods for FMD based on amplification of specific sequences of the viral genome by reverse transcription polymerase chain reaction (RT-PCR) (Vangrysperre *et al*., 1996 Reid *et al*., 1999), which can be applied to different kinds of biological samples such as fluids and tissues. This approach allows identification of infected animals even before development of clinical signs or positive virus isolation as well as identification of positive cattle at the end of the course of infection when virus isolation may be negative (Callahan *et al*., 2002; King *et al.*, 2006). In addition, in a study in which results were compared between reference laboratories, RT-PCR results were more consistent among laboratories as compared to virus isolation and ELISA results, which varied from low to high sensitivity when aliquots of the same samples were processed by 5 different laboratories (Ferris *et al.*, 2006). Reverse transcription polymerase chain reaction is also useful for typing FMDV isolates (Mohapatra *et al*., 2006). However, conventional RT-PCR does not have optimal results in terms of specificity and sensitivity (Alexandersen *et al*., 2003).

FMD serology is particularly important because it can detect antibodies in a range of livestock infected as well as in animals with mild infection, where collection of oral lesions is not feasible and has been recommended as a standard test (Brocchi *et al*., 2006). Similarly, the demonstration of specific antibodies to structural proteins in non-vaccinated animals is indicative of prior infection with FMD. This is particularly useful in mild cases or where epithelial tissues cannot be collected (OIE, 2009). Tests for antibodies to some non-structural proteins (NSP) of FMD are

useful in providing evidence of previous or current viral replication in the host, irrespective of vaccination status. The NSP unlike the structural proteins are highly conserved and are not serotype specific. Since the FMD inactivated vaccine is partially purified virus antigen (free of NSP), antibody response to NSP in cattle serum is indicative of an infection status rather than response to vaccination. As such the prevalence of FMD can be detected serologically by measuring the antibody level to 3-ABC non-structural protein (Diego *et al*., 1997).

NSP ELISA has been used to detect antibodies to the polyproteins 3ABC of FMDV as an indicator of past or present infection with any of the SAT serotypes of the virus existing in Botswana, whether or not the animal has been vaccinated (Mackay *et al*, 2001). The problem is that vaccines used are not purified and the repeated vaccinations that exist may lead to NSP being produced and thus providing challenges in the interpretation of NSP test results. According to Paton *et al.*, (2006), acute infection cannot be detected on serology until antibodies develop necessitating the use of paired serology to identify rising titres of antibody. The LPBE that detects antibodies to viral structural proteins is used at the Reference Laboratories e.g. BVI and OVI. Botswana National Veterinary Laboratory has also been using the test. LPBE is also being used at the Botswana National Veterinary Laboratory. LPBE is serotype specific, highly sensitive, detects antibodies elicited by both vaccination and infection and is a prescribed test for trade because it is appropriate for confirming previous or on-going infection in non- vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field if purified vaccines have been used (Hamblin *et al*., 1986; Bronsvoort et al., 2008). The LPBE has a sensitivity of 100% and specificity of 95% (Hamblin *et al*., 1986).

FMD is difficult to diagnose in small ruminants as infected animals do not always show typical clinical signs or as the cardinal signs mimic other diseases (Ganter *et al*., 2001). The difficulty in

making a clinical diagnosis should encourage the development of more rapid screening tests to assist in future control programmes (Patil *et al*., 2002).

2.10 Prevention and Control

The most important resource in the prevention of FMD is the informed animal owner or manager. Livestock owners at all levels of production, and traders should be familiarized with the basic features of FMD, including the recognition of the essential signs of the disease, how and where to seek help if they suspect the disease (FAO, 2002).

Many countries free of FMD have a policy of slaughter of all affected and in-contact susceptible animals and strict restrictions on movement of animals and vehicles around infected premises. After slaughter, the carcasses are either burned or buried on or close to the premises and the buildings are thoroughly washed and disinfected with mild acid or alkali and fumigated (Aiello, 1995).

In endemic countries, vaccination is the best control strategy that may be applied with quarantine. Vaccines must be formulated taking into account the virus type and subtypes prevalent in the area. Vaccination programmes must cover not less than 80% of the susceptible population, preferably 100% of cattle so as to maintain a reliable hard immunity status (OIE, 2000).

Research and epidemiological studies continue to be necessary in order to both prevent the entry of the virus and to assist in control should the disease reoccur (Bannert *et al*., 1999). Vaccines with oil adjuvant were found to elicit a better immune response at any time than did aluminium hydroxide gel vaccine, and the response developed quicker (Patil *et al*., 2002). The animals maintained their neutralizing antibody titers at >3 log (10) for the duration of the trial (90 days). Sheep have been found to be late responders to serotypes A, C, and Asia-1; a clear

upward shift in titer was observed at 60 days post vaccination. However, development of the immune response to serotype O in sheep has been found to be superior to that in cattle and goats (Patil *et al*., 2002).

In Botswana, to reduce the incidence rate of FMD, all cattle in high risk or endemic areas are routinely immunised, at least twice in a year using a trivalent vaccine prepared from SAT virus strains (Baipoledi *et al.*, 2004). For effective FMD prevention, the country has successfully implemented the concept of zoning or regionalisation with disease control fences as efficient barriers between high-risk zones and disease-free zones (Mapitse, 2008).

Vaccination is practised in the FMD-high risk area while in the FMD-free area vaccination is not practiced, as defined in the OIE Terrestrial Animal Health Code (Anon, 2001). The zones are separated by disease control fences which are maintained by government. There are strategically-placed livestock quarantines also manned by government officials, and these are used to quarantine FMD-vaccinated cattle before slaughter and also non-vaccinated cattle before movement to the vaccinated zone (Mapitse, 2008).

An "*Animal and Animal Products Movement Protocol"* has been designed to guide extension officers and the general public on movement of susceptible animals within the country and between disease control zones. The protocol is an instrument that aids in the implementation of the Diseases of Animals Act and the Diseases of Stock Regulations (Mapitse, 2008).

CHAPTER 3.0

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted from the Ngamiland and North East districts of Botswana. (Figure 2). These districts were selected because they are the FMD high risk zones with Ngamiland being the most important since this area has wildlife-livestock interaction.

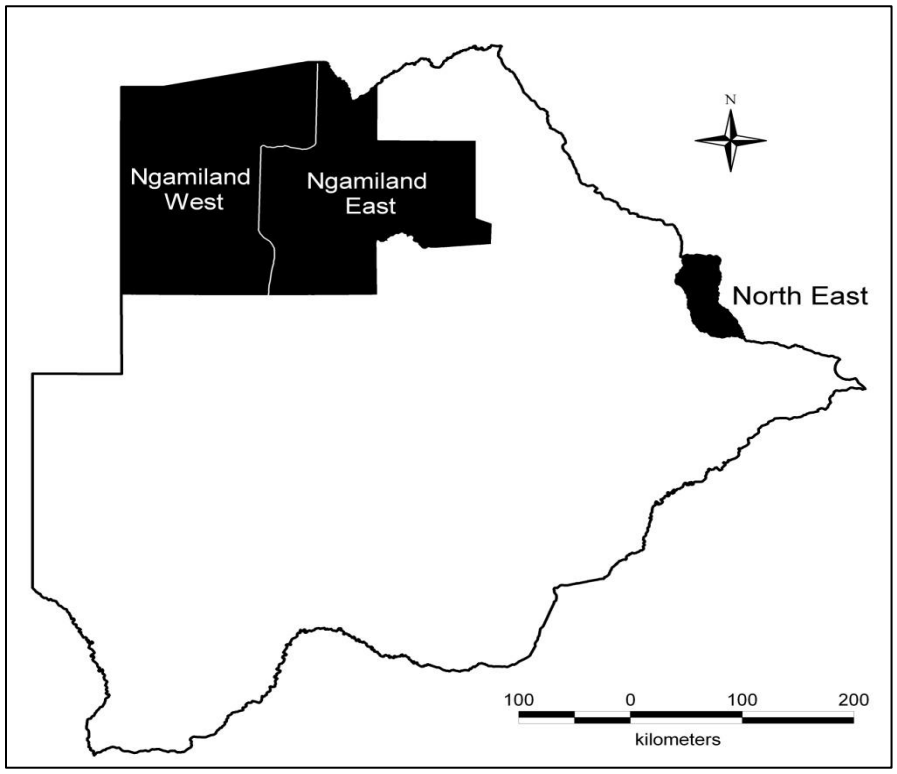


Figure 2; Ngamiland and North East districts of Botswana (Study areas)

***Sampling was done from both Ngamiland west and Ngamiland East**

Ngamiland district lies in the Northwest part of Botswana between 19 degrees 30’ South, and 23 degrees 30’ East. It covers a total area of 129 930 square kilometres. It shares borders with Namibia on the Northern and Western sides. Domestically, it borders the Central, Ghanzi and Chobe Districts on the East and Eastern sides

(eashttp://en.wikipedia.org/wiki/North-West_District Botswana).

North-East district lies 21 degrees South 5’ South and 27 degrees 30 ‘ East of Botswana. It covers a total area of 2,500 square kilometres. It borders the Matabeleland South Province of Zimbabwe in the East along the Ramokgwebana River. In the South and West, North-East district borders Central district of Botswana along the Shashe River. The Nata River flows through the North-East District and is a significant gathering place for wildlife including birds. The Nata River continues to flow to the Makgadikgadi Pans, where it discharges (http://en.wikipedia.org/wiki/North-East_District_Botswana). This region (North East district) is a containment region.

3.2 Study Design and Sampling

A cross sectional study was undertaken from September to November 2012. The sampling unit was the flock. There were twenty-eight crush pens in total from 6 villages in Ngamiland district. From the 6 villages 5 were randomly selected and fourteen crushes were selected randomly from these 5 villages. From the North East district all the crush pens were sampled (6) from 6 randomly selected villages. A total of 36 and 23 farms were selected from Ngamiland and North East district respectively. From Ngamiland district a total of 365 goats were sampled where as in the North East district 275 goats were sampled (table 2).

Table 2; Sampling strategy

District	Village		Crush		Farms		Number of goats sampled
	Total	Sampled	Total	Sampled	Total	Sampled	
Ngamiland	6	5	28	11	NK	40	365
North East	12	6	6	6	NK	19	275

NK = Not known

The total number of goats to be sampled was determined using the formula described by Dohoo *et al*, (2003). To maximise the number of goats to be sampled, the sample size was estimated by assuming a prevalence of 50%. The precision was decided to be 5% and the confidence level at 95%. The minimum sample size after adjusting for the loss (by a factor of 1.1) and stratification by a factor of 0.5 was 424. However a total of 640 goats from Ngamiland and North East districts were sampled in the study.

Blood samples were collected from goats with no history of FMD vaccination. From Ngamiland district samples were collected from the following 5 villages; Gumare (69 goats), Kareng (31 goats), Komana (195 goats), Toteng (23 goats), Nokaneng (47 goats). In the North East district, samples were collected from 6 villages; Matsiloje (12 goats), Mhatane (79 goats), Matopi (46 goats), Patayamatebele (89 goats) and Tatisiding (18 goats). A total of 11 villages were sampled (Table 3).

Table 3; Number of goats tested per village

District	Name of Village	Number of animals sampled
Ngamiland	Gumare	69
	Kareng	31
	Komana	195
	Toteng	23
	Nokaneng	47
	Sub-total	**365**
North East	Matsiloje	12
	Mhatane	79
	Matopi	46
	Patayamatebele	89
	Tati-Siding	18
	Lephane	31
	Sub-total	**275**
	Total number of animals Sampled	**640**

Blood samples were collected from individual goats aseptically from the jugular vein into 10 ml sterile plain vacutainer tubes. Consent was always sought from the owner before sampling and samples were only collected from those where permission was granted. The owner(s) and the animal health assistant handled the animals during sampling. Each tube used was identified clearly. After collecting the blood samples were allowed to clot by placing them over night at room temperature. Serum was harvested from the clotted blood into 5 mls storage tubes. The tubes were properly labelled and delivered to the Botswana National Veterinary Laboratory on ice in cooler boxes. At the laboratory, it was stored at -20 degrees Celsius until analyses.

At the same time as blood sampling, a questionnaire was administered to individual farmers to help in the identification of possible risk factors. Variables under consideration included, the breed, location of the animals, system of rearing, husbandry practices, proximity to national parks and types of animals kept with goats, roaming wild life, watering points and mixing with other animals.

3.3. Laboratory testing

LPBE (Prionics, Switzerland) was used in the laboratory analysis of the serum samples. It is a serotype specific serological test which aims at detecting the specific antibodies to structural proteins elicited by infection with FMDV (OIE, 2008). It detects antibodies against all SAT serotypes of FMD virus in serum samples collected from cloven-hoofed animals (Bovine, ovine, caprine and antelopes) (OIE, 2008).

The procedure was carried out according to the manufacturer's instructions (Prionics, Switzerland). Flat-bottomed microplates were used (Nunc immunoplate Maxisorp) for ELISA. Briefly, one plate was used for each particular FMD serotype (SAT 1, SAT 2 and SAT 3). About 50 µl of Rabbit sera against SAT 1 was diluted (pre-determined) in coating buffer (carbonate bicarbonate pH 9.6+/-0.05) to all wells. The plates were incubated for 1 hour at 37 degrees Celcius with shaking. Sera samples were diluted with 215 µl of Phosphate Buffer Saline Tween (PBST) in U – bottom plate (sample dilution plate). About 10 microlitres of the diluted sera samples were added to all the wells except the controls. Another U–bottom plate (control dilution plate) was prepared and was treated like the first plate and 10 µl of the negative and positive controls was added appropriately where it had been indicated with different colours for different SAT types. Another set of U – Bottom plates were prepared, clearly labelled. Control wells were also marked according to the plate layout, these were carrier plates. About 25 µl of diluted samples was

transferred from sample dilution plate to the 3 labelled carrier plates corresponding to the original dilution plate in accordance with the plate layout. Thereafter 25 µl of the PBST was removed in positive and negative control wells. 25µl of diluted control sera was transferred from control dilution plate to the carrier plates corresponding to the original dilution plate in accordance with the plate layout (see appendix 2), and then 25µl of PBST was added to the same plates in all the wells to make 50µl in total. This made a dilution of 1/45. Antigen of the corresponding SAT types was prepared by diluting according to the predetermined working dilution in PBST and to the carrier plates. About 50µl of the diluted antigen was added to make a total of 100µl of diluted samples and controls. This made a dilution of 1/90. Plates were incubated overnight at +37 ^{o}C.

The coated ELISA plates were washed three times manually i.e. plates were flooded with washing buffer and then the contents were discharged after filling. The washing was repeated two more times. The plates were inverted and slapped onto a lint-free absorbent surface to remove residual contents. Using a multichannel pipette, 50µl of goat serum sample was transferred from the carrier plates to the corresponding wells of the coated ELISA plates. Plates were stacked up and the top plate was covered with a lid. Plates were incubated at 37°C for 1 hour on an orbital shaker.

After incubation, plates were washed with Phosphate Buffer Saline (PBS) (pH 7.2 - 7.6) (3 cycles as above). Guinea pig SAT 1 antisera was diluted initially to 1/100 in ordinary buffer and stored. To make a working solution, it was then diluted further to 1:200 in pre-blocking buffer and incubated for 30 minutes at 37±2^{o}C and 50µl of the diluted guinea pig antiserum was added to all wells of all the plates. The plates were stacked up and the top plate covered with a lid and incubated at 37°C for 1 hour on an orbital shaker.

After incubation, the plates were washed with PBS (pH 7.2 – 7.6) as previously described. Conjugate was diluted initially to 1/100 in ordinary buffer and stored at -50 ^{0}C. For working purposes, it was then diluted further to 1/200 by pre-blocking with a buffer and incubated for 30 minutes at 37±2^{0}C and finally diluted to a predetermined working dilution with Phosphate Buffer Saline Tween–M (PBST-M). About 50 µl of diluted conjugate was added to all wells of all plates. Plates were stacked up and the top plate was covered with a lid. Plates were incubated at 35^{o}C °C for 1 hour on an orbital shaker.

The *O*-Phenylenediamine Dihydrochloride (OPD) solution (5ml per plate) was prepared. Then plates were washed with PBS (4 cycles with a soak period of at least 60 seconds after the 3rd wash). Appropriate amount of 30-33% hydrogen peroxide was added to the prepared chromogen solution. About 50µl of substrate/chromogen solution was added to all wells of all plates including blank plates/wells. Plates were incubated at room temperature for 15 +/-5 minutes, and an opitical density (OD) of 1.0 or above were obtained in the antigen control well. During the colour development, ELISA plate reader was turned on to allow warming up. It was also checked if the appropriate interference filter (490nm) was in place. To stop further colour development, 50µl of acid stopper solution (1.25M sulphuric acid) was added to all wells of all plates in the same order as the addition of substrate/chromogen.

Optical densities of samples were read using microplate reader at wavelength 492 nm. The range of OD for antigen control was from 1.0 – 1.5. The optical density percentage (ODp) of each individual sample was calculated using the following formula:

ODp = Sample OD/ Mean OD Antigen control X 100%

From each plate, the mean OD value of the antigen controls (wells F5, F6, G5 and G6) was calculated and used according to the above formula to establish individual sample ODp value.

The range OD for antigen control was >1.0. Positive control should give ODp values >50% of Antigen control average and the negative control should give ODp values <50%. The positive control should be accepted if the mean ODP values fall within the set range derived from verification of each batch of antisera (Doc: BNVL/MS.WI 0645 SERO). If the positive controls fall outside the set range, a non-conformance should be raised and investigated.

Samples with ODp values higher than or equal to 50% were considered negative, while those with ODp values lower than 50% were considered positive. They were titrated and NSP test done if the Titration is positive.

*Same procedure was followed for SAT 2 and SAT 3.

3.4 Data analysis

Data collected was entered into Microsoft Excel and coded for analysis. Data was analysed using Stata SE 12 *(*Prionics®-Check PrioSTRIP™*)*. Descriptive statistics were generated for each of the variables under investigation. Categorical variables were compared using fisher's exact test. Continuous variables were compared using the t-test. The step-wise binary logistic regression model was used to determine predictors (risk factor) of being serologically positive for FMD. The Logit link function reported the coefficient, p value, odds ratio (OR) and 95% lower and upper confidence interval values for the OR. Criteria used in determining whether each of the constructed models adequately fitted the data were, a non-significant Hosmer and Lemeshow Test ($p > 0.05$)

and a significant Omnibus Test of Model Coefficients ($p < 0.05$). All statistical tests were considered significant at $p<0.05$.

CHAPTER 4.0

4.0 RESULTS

4.1 FMD sero-prevalence at individual animal level

A total of 640 goat serum samples from 59 flocks in the two study areas (Ngamiland and North East district) were subjected to the FMD LPBE. The overall prevalence of FMDV antibodies in the two study areas was 37.2% (95%, CI =33.43-40.94). In Ngamiland district a total of 92 samples were positive for FMDV representing a sero-prevalence of 25.2% (95%, CI= 20.74-29.67), while in the North East district, 146 animals tested positive representing a sero-prevalence of 53.09% (95%, CI=47.17-59.01) (Table 4). There was a significant difference in sero-prevalence between the two study areas ($p < 0.01$).

From the two districts, eleven villages were sampled, six from the North East and five from Ngamiland district. In Ngamiland district the highest sero-prevalence was recorded in Nokaneng village 38.3 % (95%, CI = 27.32 – 63.00), while no goats were found positive for FMDV Abs in Toteng village. In North East District, the highest sero-prevalence of 69.6% (95%, CI=59- 40 - 79.85) was recorded in Mhatane, while no goats tested positive for FMDV antibodies in and Tati-Siding village. There were significant differences in sero-prevalence between the villages in both districts ($p<0.01$ in Ngamiland and $p=0.002$ in North East) (Table 5) .

All SAT serotypes (SAT 1, 2 and 3) were detected in both districts. In Ngamiland 13.70% goats tested positive for serotype SAT 1, 25.75% tested positive for SAT 2 and 25.50% tested positive

for serotype SAT 3. In the North East district 38.1%, 24.40% and 35.64% goats tested positive for SAT 1, 2 and 3 respectively (See table 6).

Table 4; Sero-prevalence of FMDV Abs at individual goat level in Ngamiland and North East Districts.

n=Sample size

District	n	Prevalence	95% CI for prevalence	p-Value
Ngamiland	365	25.20%	20.74-29.67	<0.01
North East	275	53.09%	47.17-59.01	
Total	640	37.2%	33.43-40.94	

Table 5; The sero-prevalence of FMDV in different villages

District	Village	N	Prevalence	95%CI:	P-value
Ngamiland	Gumare	69	23.2	20.43-32.90	0.002
	Kareng	31	35.5	11.17- 49.70	
	Komana	195	24.1	11.92 -31.56	
	Nokaneng	47	38.3	27.32-63.00	
	Toteng	23	0	-	
North East	Matsiloje	12	66.7	38.80-94.58	<0.01
	Mhatane	79	69.6	59.40 -79.85	
	Lephane	31	45.2	33.13-94.27	
	Patayamatebele	89	65.2	55.20-75.14	
	Tati-Siding	18	0	_	
	Matopi	46	23.9	10.12-41.49	

n = sample size

Table 6; Distribution of the SAT serotypes of FMD antibodies in Ngamiland and North East districts

	serotypes detected		
District	SAT 1 (%)	SAT 2 (%)	SAT 3 (%)
Ngamiland	13.70	25.75	25.50
North East	38.1	24.40	35.64

The sero-prevalence of FMDV Abs in adults, weaners and kids was 33.1% (95%, CI=29.09-37.72), 18.64% (95%, CI=86.02-28.67%), 60.83 %(95%, CI=52.05-69.62) respectively (Table 7). There was significant difference in sero-prevalence among the three age groups ($p<0.01$). The Tswana, boar and mixed breeds goats had sero-prevalence 37.47% (95%, CI =33.47-41.49), 38.89 % (95%, CI=15.67-62.11) and 33.90 % (95%, CI=21.69-46.10), respectively to FMDV Abs. (Table 8). There was no significant difference in sero-prevalence between the three breeds of goats ($p = 0.876$). The sero-prevalence of FMD was significantly higher in males 44.50% (95%, CI = 37.42-51.58) than in female goats 34.08%, (95%, CI = 29.68-38.47%). There was a significant difference between the sexes ($p=0.016$) (Table 7).

Table 7; Sero-prevalence of FMDV in different age groups of goats

Age	N	Prevalence %	95% CI	P-value
Adult	461	33.1	(29.09-37.72)	<0.01
Weaner	59	18.64	(8.60-28.67)	
Kid	120	60.83	(52.05-69.62)	

n = sample size

Table 8; Sero-prevalence of FMDV in different breeds of goats

Breed	N	Prevalence %	95% CI	P-value
Tswana	563	37.47	(33.47-41.49)	0.876
Mixed	18	38.89	(15.67-62.11)	
Boar	59	33.90	(21.69-46.10)	

n = sample size

Table 9; Sero-prevalence of FMDV in male and female goats

Sex	N	Prevalence	95% CI	P-value
Male	191	44.50	37.42-51.58	0.016
Female	449	34.08	29.68-38.47	

n = sample size

4.2 Overall sero-prevalence at flock level

A total of 59 flocks were tested, 40 from Ngamiland and 19 from North East districts. A flock was deemed sero-positive if atleast one goat in it tested positive for FMD. Out of the 40 flocks tested from Ngamiland, 23 were positive for FMDV Abs giving a flock level sero-prevalence of 57.7% (95%, CI=41.6-73.3), while in North East, 14 tested sero-positive, giving a flock sero-prevalence of 73.7% (95%CI=52.7-94.5) (Table 10). There was no significant difference in flock sero-prevalence between the two districts (p=0.26).

Table 10; Foot and mouth disease Virus sero-prevalence at flock level in both districts

Variable	Level	No. Tested	Prevalence (%)	95%CI:	P-value
District	Ngamiland	40	57.7	(41.6-73.3)	0. 26
	North East	19	73.7	(52.7-94.5)	

Fifty eight, out of the 59 farmers reported that they got authority from the veterinary department before selling their animals to areas far or across borders. Out of these farmers 36 62.07% (95%, CI= 49.20-74.93) flocks serologically positive for FMDV Abs.

A total of 35 flocks used borehole as their watering point, while the other 24 sourced their drinking water from the river. Of those who sourced their drinking water from the boreholes, 57.41% (95%, CI=40.15-74.13) flocks tested sero-positive to FMD, while among those who sourced their water from elsewhere, point 70.83% (95%, CI=51.86-89.80) flocks tested sero-positive. There was no association between the source of drinking water and being sero-positive to FMD, (p=0.41) (Table 11).

Table 11; Foot and Mouth disease sero-prevalence at flock level in flocks which drink from boreholes.

Variable	Level	No. tested	Prevalence (%)	95%CI:	P-value
Availability of borehole	Yes	35	57.41	(40.15-74.13)	0.41
	No	24	70.83	(51.86-89.80)	

From both districts, 44 farmers confirmed that they had seen wildlife roaming around their farms. Out of these, FMDV Abs 28 contained at least one sero-positive goat in their flocks 63.64% (95%,

CI=48.95-78.32) while for those farms that did not indicate seeing wildlife roaming within their vicinity, 60% (95%, CI=33.79-86.21) had at least one goat testing positive for FMDV Abs. There was no significant difference in sero-prevalence between flocks where roaming wildlife had been seen within the vicinity of the farms and those that had not seen roaming wildlife (p=1.000).

Table 12; Foot and mouth disease prevalence at flock level in farms which reported roaming wildlife and those that did not.

Variable	Level	No. Tested	Proportion (%)	95%CI:	P-value
Any roaming wildlife	Yes	44	63.64	(48.95-78.32)	1.000
	No	15	60.00	(33.79-86.21)	

Of the 21 flocks that reported having had experienced an outbreak of FMD in their premises previously, the sero-prevalence of FMDV Abs was 71.42% (95%, CI=51.21-91.65), while in those that did not report having had an outbreak of FMD previously, the sero-prevalence was 57.89% (95%, CI=41.65-74.14). There was no significant difference in sero-prevalence between the two groups (p=0.402). (Table 13).

Table 13; Foot and mouth disease prevalence in flocks with a previous experience of an outbreak and those without the disease.

Variable	Level	No. Tested	Proportion (%)	95%CI:	P-value
FMD experience	Yes	21	71.42	(51.21-91.65)	0.402
	No	38	57.89	(41.65-74.14)	

About 33 farmers reported that they vaccinated their cattle against FMD while the rest did not. Of those who reported vaccinating their cattle against FMD, 54.55% (95%, CI=36.93-72.17) of flocks were positive for FMDV Abs, while for the 26 farms that reported not vaccinating their cattle against FMD, the sero-prevalence was 73.08% (95%, CI=55.32-90.83). There was no statistical significant difference between their sero-prevalence (p=0.181). (Table 14).

Table 14; Foot and mouth disease prevalence at flock level in vaccinated farms.

Variable	Level	No. Tested	Proportion (%)	95%CI:	P-value
FMD Vaccination	Yes	33	54.55	(36.93-72.17)	0.181
	No	26	73.08	(55.32-90.83)	

Out of the 33 farmers who vaccinated their cattle against FMD, eight reported vaccinating every month, ten every two months and fifteen every three months. Sero-prevalence of FMDV Abs in

goats was 50% (95%, CI=12.17-87.83) was recorded among those who vaccinated their cattle monthly and every two months, while a sero-prevalence of 60% (95%, CI=33.79-86.21) was recorded among those who vaccinated every three months. Despite those farms that regularly vaccinated their cattle monthly and every two months recording a lower sero-prevalence than those that vaccinated every three months, this difference was not statistically significant (p=0,474) (table 15).

Table 15; Foot and mouth disease prevalence at flock level and frequency of vaccination

Variable	Level	No. Tested	Proportion (%)	95%CI:	P-value
Frequency	N/A	26	73.08	(55.32-90.83)	0.474
	Every month	8	50.00	(12.17-87.83)	
	Every 2 months	10	50.00	(16.64-83.36)	
	Every 3 months	15	60.00	(33.79-86.21)	

Farmers kept many other domestic animals apart from goats. To determine whether there was an association between the species of livestock kept by the farmer and the prevalence of FMD in goats, farms were grouped according to the species that were kept on the farm. From the 59 farmers interviewed in this study, it was found that 14 kept Cattle, goats, sheep and donkey, 5 kept cattle and goats, 16 kepts Cattle, goats and donkey, 15 kept cattle, goats and sheep, four kept goats only, one kept goats and donkey, 3 kept goats and sheep and 1 kept goats and lastly one kept sheep and donkey. Sero-prevalences were recorded as 64.28% (95%, CI=37.68-90.89), 60.0% (95%,

CI=10.97-109.03), 56.25% (95%, CI=30.61-81.89), 80 % (95%, CI=58.60-101.40), 50% (95%, CI=-7.78-107.78), 66.67% (95%, CI=-33.33-100.06) and 100% respectively. There was no association between the species of livestock kept on the farm and the sero-prevalence of FMDV Abs in goats (p=0.547).

4.2 Risk factors associated with being seropositive to foot and mouth disease

A step-wise binary logistic regression model was used to determine and quantify the risk factors associated with goats testing positive for FMDV antibodies. Hosmer and lemeshow test was insignificant (p = 0.696) and omnibus test for model coefficients was significant (p<0.01) indicating that the model fitted the data.

From the results, it was found that goats from Ngamiland district were 0.354 (95% CI=0.213-0.416) times less likely to be sero-positive for FMDV Abs than those from the North East district. Age was also found to be significant risk factors for being positive for FMD. Adult goat were 2.669 times more likely to be sero-positive for FMDV Abs than a weaner goats (p = 0.006) while kids were found to be 4.744 (95% CI=2.194-10.257) times more likely to be sero-positive than a weaners (p<0.01). Other variables under investigation were not significant predictors of being sero positive for FMDV Abs. At flock level, none of the variables was found to be a significant predictor of goats being positive to FMDV Abs (Table 16).

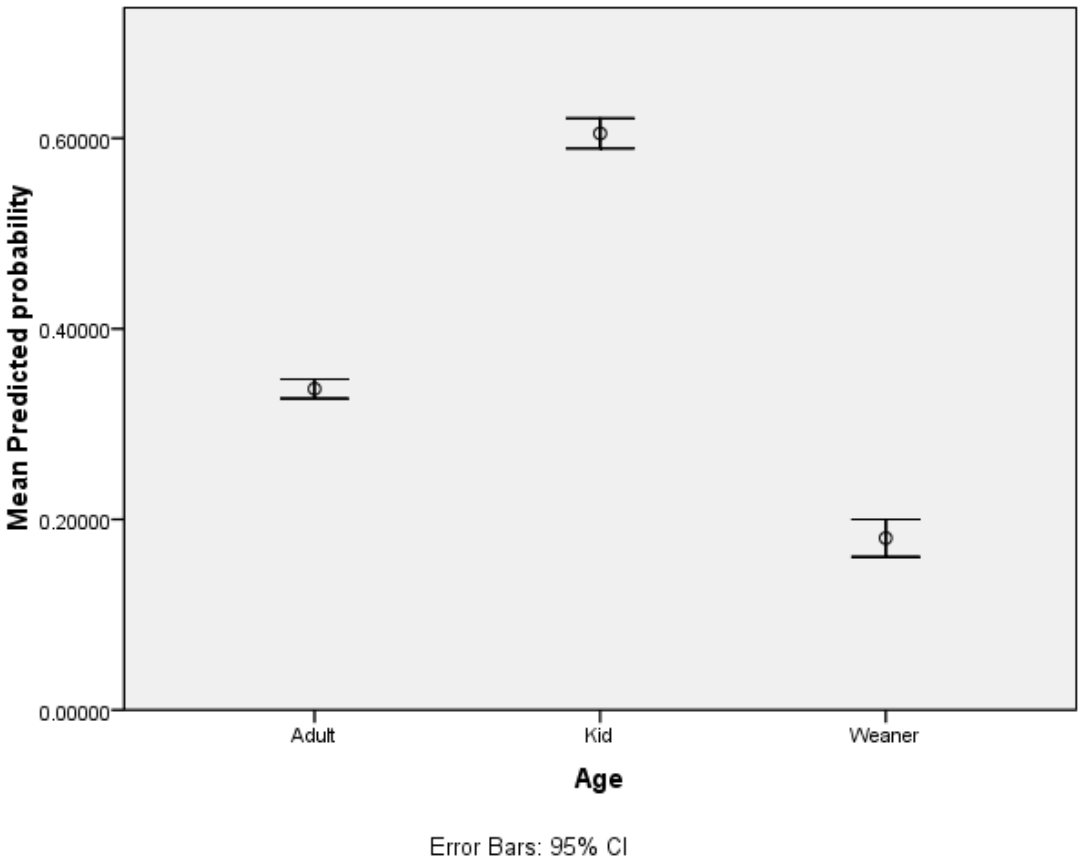
From the step-wise binary logistic model, the mean predicted probability of a goat being sero-positive for FMDV Abs for each variable under study was calculated. It was found out that kid goats had the highest mean predicted probability of being sero-positive for FMDV Abs 0.61, (95%, CI = 0.59 – 0.62) followed by adults at 0.34 (95%, CI= 0.32 – 0.35) and the weaners at 0.18 (95%, CI = 0.16 – 0.20). (Figure 3). Goats in North East district had a higher mean predicted probability

of being sero-positive for FMDV Abs 0.53 (95%, CI = 0.52 – 0.54) than those from Ngamiland district 0.25 (95%, CI = 0.24 – 0.26) (Figure 4)

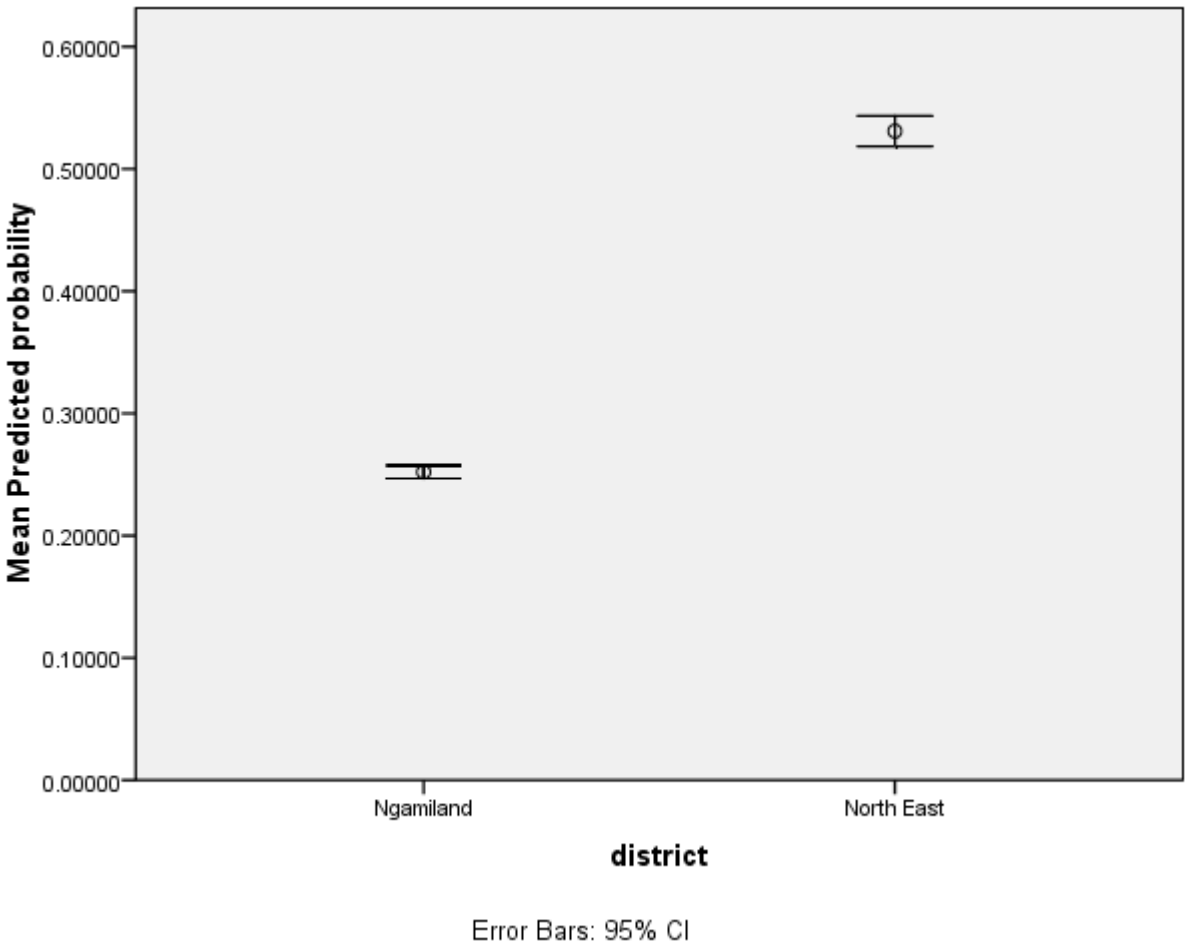
Table 16; Maximum likelihood estimates of the binary logistic regression model of factors for the prediction of goats being sero-positive to FMD

Variable	category	Odds Ration	p-value	95.0% C.I for Odds Ratio	
				Lower	Upper
District	North East*		<0.01		
	Ngamiland	0.35	<0.01	0.24	0.51
Age	Weaner*		<0.01		
	Adult	2.67	0.006	1.33	5.37
	Kid	4.74	<0.01	2.19	10.26
	Constant	0.37	0.05		

* = Reference category



Figure; 3 Mean predicted probability of kid, weaner and adult goats being positive to FMD antibodies



Figure; 4 Mean predicted probability of a goat from Ngamiland and North East districts being positive for FMD antibodies

CHAPTER 5.0

5.0 DISCUSSION

FMD is an important disease of livestock in Botswana with significant impact on trade in livestock. The majority of the outbreaks of the disease that have been reported in the country have occurred in cattle (Baipoledi *et al*., 2004). However, the role that small ruminants (goats and sheep) play in the epidemiology of the disease in the country is still not well understood even thought previous serological studies (Hyera *et al*., 2006) reported evidence of goats having been exposed to the FMD virus. In this study, a sero-prevalence survey was conducted in known FMD endemic areas of Botswana (Ngamiland and North East districts) (Anon, 2011). The results obtained indicate that goats from both districts have been exposed to the FMD virus. However, the prevalence of FMD was found to be higher in the North East district (53.09%) than in Ngamiland district (25.20%). This difference in sero-prevalence between the two districts could have been because sampling in the North East district was done when the disease was active unlike in Ngamiland where testing was done after vaccination of cattle had been carried out. Vaccination is known to reduce the transmission of FMD virus from infected animals to other susceptible animals (Orsel, 2005). In a study in calves in Netherlands (Orsel, 2005), it was observed that the odds for the non vaccinated group of calves to be infected by the FMD virus were 2.52 times whereas in the vaccinated groups, the odds of being infected by the FMD virus were 0.18 times. This is an indication that vaccination may successfully be applied as an intervention tool to reduce virus transmission in a future epidemic of FMD. Results of this study also showed that goats in the two study areas were exposed to all the three SAT serotypes. A previous serological survey of FMD in goats in Botswana (Hyera *et al*., 2006) reported exposure of goats to only SAT 1 and SAT 3. All

the three SAT serotypes have been isolated from clinically health buffaloes (Hedger *et al*., 1969) and they all have been previously associated with epidemics of the disease in livestock, mostly in cattle (baipoledi *et al*., 2004) in this country. However, only SAT 2 was responsible for the outbreak of FMD in cattle at the time of this study (Anon, 2012).

Variation in prevalence among the villages sampled in the two districts was observed, with the highest prevalence being recorded in Mhatane village from the North East district and no goats tested positive in Toteng and Tatising. The highest prevalence recorded in Mhatane village was due to the fact that a lot of kids more than adults and weaners were sampled from this village. In addition and as mentioned above sampling from this village was done when the disease was active in cattle. It is therefore possible that there was spill over of the infections from cattle to goats, espcially naive kids, as vaccination had not been carried out at the time of sampling. One of the villages (Tati-Siding) with no sero-positive animals was also from the North East district, however the active disease was confined mostly to North eastern part of the district while this village was from the Southern part of the district.

No significant difference in prevalence between the three breeds of goats was observed in this study. These findings are in disagreement with findings from Samuel and Knowles (2002) in Ethiopia and Sarker *et al*., (2011) in Rajshahi where it was reported that breed was associated with FMD outbreaks, with indigenous breeds being the most affected. They attributed this variation in sero-prevalence according to breed to poor management practices given to the indigenous as compared to the exotic species. In our study, the lack of a significance difference between breed of the goats and FMD prevalence could have been because all the goats were under a similar type of management (traditional system).

There was no association between sero-prevalence of FMD in goats and the source of water for goat drinking despite most of the farmers reporting that there was mixing of domestic animals in all the districts. Additionally, in Ngamiland, it was reported that domestic animals shared the same watering points (rivers) with wildlife. Some of the wild animals that were mentioned to share watering points with domestic animals included kudu, impala and antelope. These animals are known to play a major role in the transmission of FMD, with impala described as being most sensitive to the virus (Bengis and Thomson, unpublished data). Intha (2009) in Vientiane, capital of the Lao People's Democratic Republic, reported that FMD outbreaks were common where cattle were comingled with goats and where animals could move to areas where they could mingle with wildlife. Therefore, further studies with much larger sample size, are needed to investigate whether an association exists between wildlife-livestock interaction in Botswana.

The prevalence of FMD was significantly higher in male than in female goats. This difference in prevalence could be explained by behaviour of male goats that are constantly wondering from one flock to other seeking mating partners. It is widely accepted that contact is one of the common means in which FMD is spread between susceptible and infected animals. Mokopasetso (2005), in Botswana found that contact transmission is the most likely way of introduction and spreading FMD infections among livestock herds in Botswana. The findings are also in constant agreement with reports of Remond *et al.,* (2002), where the association between FMD and sex in cattle was observed, with highest prevalence in male cattle.

The variables that were found to be significant predictors of a goat being positive for FMD were age and district. Both kids and adult goats were found to have a higher mean predicted probability and more at risk of being sero-positive to FMD than weaners. Kids are naïve (Sarker *et al*, 2011) to FMD infections, therefore more susceptible. For adult goats, the high predicted probability of

being sero-positive to FMD is likely to be due to exposure to virus for long periods. These findings are partly different from those reported in cattle by Gelaye *et al* (2009) and Sarker *et al* (2011) who reported an even increasing prevalence as age increased. It has been reported that maternal antibodies persist in various livestock eg in lambs they can persist for 6 weeks (Prasanna et al., 2002) and in piglets for at least 3 weeks (Francis and Black 1984). Although not mentioned in kids it is also possible that the higher sero-prevalance is due maternal antibody persistence.

FMD in humans is considered very rare (Capella, 2001).Disease in humans has been reported mainly in connection with consumption of unpasteurised milk, dairy or unprocessed meat products from infected animals or as a result of direct contact with infected animals (e.g. farmers and veterinarians) (Health Protection Agency (HPA), Foot and Mouth disease, 2012). It is one of the diseases which has impacted badly on farmers in most African countries Botswana included. It does not result in high mortality in adult animals but it has debilitating effects, including weight loss, decrease in milk production, and loss of draught power, resulting in a loss in productivity for a considerable amount of time. Mortality, however, can be high in young animals, where the virus can affect the heart (Grubman et al., 2004). Livestock production is the backbone of rural livelihoods in Botswana (Mapitse, 2008). Agriculture has been the main employer in the rural areas, and livestock being the major contributor to the agricultural GDP, the sector has remained important to the government strategy of rural development and food security in Botswana (Mapitse, 2008).

CHAPTER 6.0

6.0 CONCLUSION AND RECOMMENDATIONS

This study found that FMD was prevalent in the Ngamiland and North East districts of Botswana, with all the SAT serotypes being present. Risk factors associated with FMDV in goats (location and age) were identified. It is therefore recommended that goats are also included in the vaccination programmes against the disease to reduce transmissibility of the disease to naive animals. Further studies should be conducted to determine how long the goats in Botswana can remain carriers of the disease. Experimental studies need to be conducted to find out if goats are able to transmit FMDV to other susceptible species especially cattle. Farmers also need to be made aware of the fact that goats also can get FMD and there is a possibility that they can even spread it to other species like cattle.

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8.0 APPENDICES

Appendix 1: Questionnaire

Title; Sero-prevalence and Risk Factors Of Foot and Mouth Disease in Goats In The North -West and North East Districts Of Botswana

Self Introduction;

Student name; Gabasenye Juliah Raletobana

University; University of Zambia

Programme of study; Msc One Health Analytical Epidemiology

Purpose of Research; To determine the sero-prevalence of FMD in goats in Botswana

Determine risk factors associated with FMD infection in goats.

To outline the spatial distribution of the disease.

Confidentiality assurance; Assure the farmers that the information collected will not be disclosed to other anyone.

SECTION 1

FARMER'S DETAILS

Name of farmer…….....

Date……

Age…….

District.....

Location of Farm…….

Phone number……..

1. Number of children and dependents

2. Name of person interviewed

Relationship with owner

a. Owner

b. Family member

c. Care taker

Section 2

LIVESTOCK PRODUCTION

1.Which animals do you keep on your farm

a. Cattle

b. Goats and Sheep

c. Pigs

d. Poultry

e. Others, specify----------

2. Number of goats----------

3. Breed----------

Goat Population dynamics

Adult females---------- Adult males---------- Kids---------- Weaners ----------

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SECTION 3

GENERAL QUESTIONS

6. Do you depend on your animals for payment of their school fees?

7. How long ago did you sell animals?

8. When you sell animals across borders or across the region do you get release or authority from the Veterinary department or you just sell.

9. Apart from livestock rearing what do you do for a living?

10. What type of farming/grazing do you practice?

a. Extensive

b. Intensive

c. Semi-intensive

11. If it's extensive farming does your flock mix with cattle?

11. Do your animals mix with animals from other kraals when grazing?

12. Do you have a borehole?

a. Yes

b. No

13. If no where do your animals drink?

14. Are they any other animals drinking from the same water source?

a. Yes

b. No

15. If yes what other animals use the same water source?

16. Is there any roaming wildlife in your area?

a. Yes

b. No

17. Are they any national parks and game reserves in your area?

SECTION 3

Disease control

18. What are the common diseases of goats in the area?

19. Do you vaccinate against any of these diseases?

20. If yes, who does the vaccination?

21. What other disease methods do you employ in your flock (list diseases and possible control methods)?

22. Have you ever experienced foot and mouth disease in your flock?

23. If yes how many goats were affected?

24. What ages were affect?

25. Did you experience FMD in any other animals other than goats?

a. Yes

b. No

26. If yes, which a animal was the disease noticed first?

27. Do you vaccinate your animals for FMD?

28. How often do you vaccinate?

29. Which species do you vaccinate?

Apendix 2 Plate layout for spot test

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	**Neg .con**	**Neg** .con
D	Sample	Sample	Sample	Sample	**Pos. con**	**Pos. con**	Sample	Sample	Sample	Sample	**Neg .con**	**Neg. con**
E	Sample	Sample	Sample	Sample	**Pos. con**	**Pos. con**	Sample	Sample	Sample	Sample	Sample	Sample
F	Sample	Sample	Sample	Sample	**AC. con**	**AC. con**	Sample	Sample	Sample	Sample	Sample	Sample
G	Sample	Sample	Sample	Sample	**AC. con**	**AC. con**	Sample	Sample	Sample	Sample	Sample	Sample
H	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample