The Epidemiology of Rabies in Domestic Ruminants in Botswana

By

Benjamin Ditsele

College of Veterinary Medicine

School of Veterinary and Life Sciences

Murdoch University

Western Australia

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Declaration

I hereby declare that the work presented in this thesis has been performed by me, except where otherwise clearly stated in the text, and that it has not been previously submitted for application for a degree at any University.

Benjamin Ditsele
Abstract

Rabies is a fatal viral disease of world-wide significance affecting warm blooded species, including humans, and is of major concern in Botswana. The objectives of the research reported in this thesis were to: determine the distribution of rabies in domestic ruminants in Botswana; identify risk factors associated with the disease in cattle; and assess the knowledge, attitudes and practices (KAP) of farmers in a high-risk area.

Data on cases diagnosed by the Botswana National Veterinary Laboratory between 2000 and 2010 were analysed. An average of 35 cases per year was detected in ruminants during this study period. There was a strong positive correlation between the number of cases in ruminants and jackals ($r = 0.78$, $p < 0.005$), as well as in cattle and goats ($r = 0.96$, $p < 0.0001$).

Cases of rabies (340) in ruminants were concentrated in the northern part of Botswana (88.7% of all cases). The North East District had the highest proportion of affected ruminants (0.029%) and 77.6% of these cases were in the peri-urban villages clustered around Francistown.

Livestock from farms in peri-urban villages (OR 10.6; 95% CI 4.2, 26.9), free-roaming livestock (OR 3.1), dogs attacking livestock (OR 3.1) and the presence of herding dogs (OR 4.5) were all significantly associated with a history of rabies in ruminants in a multivariable logistic regression model.
Farmers who could name at least one clinical sign of rabies in cattle were 5.7 times (95% CI 3.1, 10.5) more likely to have reported a case of rabies than those not knowing any clinical signs; however most farmers knew the clinical signs of rabies in dogs. This highlights the need for further education on the disease, including methods to recognise and control it in the farming community, as well as in the general public.

It is concluded that management and husbandry factors, along with environmental factors associated with the presence of canids, results in the disease being a problem in Botswana. Implementation of appropriate education, along with regular vaccination programmes of all dogs, should help minimise the impact of the disease on the community in Botswana.
Acknowledgements

First and foremost I wish to thank the Government of Australia for awarding me a scholarship and an opportunity to study in my area of interest. Similarly I am grateful for the financial support and assistance accorded to me by Murdoch University and the Government of Botswana, especially the Department of Veterinary Services during my field work. I wish to thank the Director of Veterinary Services, Dr. Letlhogile Modisa, and Deputy Director, Dr. Chandapiwa Marobela-Raborokgwé, for allowing me access to the National Veterinary Diseases Database. Without such support the study would not have been possible.

My sincere gratitude to my supervisors Professor Ian D. Robertson, assistant supervisor Dr. Kobedi Segale and Dr. Bernard Chizuka Mbeha for their guidance and constructive criticisms throughout the course of this work. Special thanks also to Cecilia Opelokgale, Tony Dingalo and Dr. Samantha Kejelepula for their technical assistance on data management and mapping.

I want to thank all my friends, especially Petrus Malo Bulu, Ernest Mochankana and Karma Rinzin, for their commitment and willingness to help all the time. In the same manner I want to say thank-you to my Perth friends who made studying possible for me.

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Chapter 1: Introduction

1.1 General background of Botswana

Botswana is a land-locked country surrounded by Namibia to the west, Zambia and Angola to the north, Zimbabwe to the east and the Republic of South Africa (RSA) to the south (Figure 1.1). Although Botswana is equivalent in size to France, there are only 2.2 million inhabitants in an area of approximately 581,730 square kilometres of land located between latitudes 17°S and 27°S and longitudes 20°E and 30°E (CIA, 2014). The eastern part of the country is a flat hard veld area extending from the south to the north and contains the largest human settlements and livestock populations. In contrast, the western part of the country is mostly the sandveld area of the Kalahari Desert constituting approximately 70% of the total land mass of Botswana (Figure 1.2). The sandveld areas are where the majority of the wildlife population thrive in game reserves, national parks and in land gazetted for hunting (DWNP, 2012). In the past, human occupancy in the sandveld areas was very low, however over the years it has increased with the establishment of livestock farms, villages and new settlements.

Since independence in 1966, Botswana has consistently sustained one of the world’s highest rates of economic growth. It was classified as one of the poorest country at independence, however it is now considered to be a middle-income country with a per capita gross domestic product (GDP) of USD16,400 in 2013 (CIA, 2014). This change resulted from development of mining, tourism and agriculture. The sale of diamonds generates more than one-third of the country’s GDP. Unlike many African countries with unstable political and socio-economic structures which affect disease control
programmes (Swanepoel et al., 1993), Botswana is fortunate in having few such problems.

Figure 1.1: Map of Africa showing the position of Botswana. [http://maps-africa.blogspot.com/2012/05/map-of-africa-political-pictures.html](http://maps-africa.blogspot.com/2012/05/map-of-africa-political-pictures.html) (accessed October 28th 2014).
Botswana is divided into ten districts which are further subdivided into subdistricts (Figure 1.3). A subdistrict is composed of a group of villages which are further made up of extension areas. In every extension area communal farms are grouped according to cattle crush pens (where cattle in a common grazing area are collected by different farmers for dipping, vaccination and inspection) and commercial farms. These crush pens allow congregation of cattle, sheep and goats from different cattle posts and other communally grazed areas. Cattle posts are the most remote undeveloped areas in Botswana; however they have good vegetation for livestock grazing.
The Department of Veterinary Services (DVS) is responsible for the control of all livestock diseases in the country and consequently has well-established extension area networks throughout the country. The extension areas are headed by trained animal health officers, whose roles include: vaccination of livestock and pets against important diseases, such as rabies in dogs and cats; attending to clinical cases of disease; and advising farmers on veterinary issues. The subdistrict heads and supervising extension officers are all veterinarians under the direct supervision of a district level senior veterinarian who reports to the Director of DVS (Fermet-Quinet et al., 2010).
1.2 Brief overview of agriculture in Botswana

Agriculture, through the exportation of cattle and beef, was the chief foreign currency earner for Botswana in the 1960’s and 1970’s, contributing approximately 40% of the nation’s GDP. This contribution subsequently diminished to 1.8% following the discovery and exportation of minerals and the growing tourism industry (CSO, 2009). However, agriculture remains the main source of employment in the country, particularly with the low unskilled groups. Agriculture in Botswana, especially the livestock sector, highlights and fosters the social and traditional values and practices of Batswana (indigenous people from Botswana) producing organically and naturally grazed beef that is highly desirable in Europe. While cattle play an economic role for the country, goats and sheep play a significant role at the household level, providing meat and milk for home consumption and income from their sale. Agriculture has considerable potential in terms of provision of food, employment creation, investment opportunities and diversification needs for the country (DCP, 2010). In some areas livestock are also an important source of draught power and transportation (DCP, 2010). The location of the majority of the country’s population in the eastern part of Botswana has occurred as a result of the better soils in this region and the availability of surface water. It has been the accessibility to surface water in the form of rivers, dams, lakes and springs that has influenced the location and establishment of human settlements and farms. However, the same water catchment areas also attract wildlife causing an interesting interface between humans, domestic animals and wildlife with the potential for conflicts to arise and the occurrence of inter-species disease transmission (Bengis et al., 2002). In some situations, as in the case of the Limpopo and Chobe Rivers, the waters traverse multiple countries attracting a range of animal species with different diseases and disease frequencies. In these areas the water and
vegetation are for the general public use and as a result fencing of such areas by private individuals or entities would seriously disadvantage the majority of the community. Because of the scarcity of surface water in Botswana, there have been deliberate efforts by the government to provide communities and individuals with assistance in sourcing reliable water supplies. The exploitation of ground water sources by drilling boreholes has allowed considerable expansion of the national livestock activities into the western Kalahari sandveld areas. Most commercial farms are now located in these regions because of the presence of grass and bush savannah vegetation suitable for livestock grazing (APRU, 1980). The climatic conditions of Botswana are semi-arid with annual rainfall varying from 600mm in the north to 250mm in the Kalahari desert and summer (October to March) temperatures exceeding 40°C (APRU, 1980). However, recent extended dry spells have resulted in non-irrigated arable farming being considered a risky business in Botswana. Droughts, along with occasional floods and losses associated with pests and diseases, have hindered the development of the agricultural sector in Botswana (CSO, 2011). Even with such challenges, farmers in Botswana still focus on the rearing of livestock.

1.3 Livestock production systems in Botswana

In Botswana there are two main systems for beef production: commercially raised animals on ranches; and animals raised under a communal production system. The proportions of production in each system are outlined in Table 1.1. Beef production is highly dependent upon communally raised cattle which represent 96% of the national beef cattle population (CSO, 2009).
In a commercial production system the domestic ruminants are fenced, restricting their areas of grazing. Farmers adopting this system are usually literate with adequate resources to care for their animals on commercial ranches. Management includes supplementary feeding and watering of animals at specific watering points. However, it is not uncommon for some ranches to have porous boundary fences. The services of private veterinarians are often used, along with vaccinations which are paid for. However routine vaccination and other livestock activities are also performed by the DVS. The DVS is the responsible authority for animal health and provides annual vaccinations against anthrax, brucellosis, clostridial diseases and foot and mouth disease (FMD) (in specific areas) to livestock and against rabies to dogs and cats. The DVS also inspects livestock prior to issuing permits, undertakes livestock identification and trace-back procedures and attends clinically sick animals on request.

The communal production system is a traditional way of raising cattle, sheep and goats in Botswana. Domestic ruminants are allowed to roam grazing areas in search of palatable grass, forbs and water. Traditionally these ruminants would be raised in cattle posts where they are grazed freely with herders during the day but restricted through kraaling in the evenings. This practice helps minimise interactions with wild carnivores, as well as avoiding unsafe grazing areas. In addition, farmers often keep a dog for safety and to guard their livestock. However, over time the traditional communal production system has changed. Farmers started competing with wild carnivores for meat by hunting animals such as springboks (*Antidorcas marsupialis*) and greater kudus (*Tragelaphus strepsiceros*). The increase use of dogs for hunting and the fact that more farmers and herders owned guns resulted in a significant reduction
in the number of wild herbivores around the cattle posts. At the same time the cattle population steadily increased in these areas due to reduced competition for vegetation from wild herbivores. In contrast to the wild herbivores, the wild carnivores, which were spared during hunting, started to opportunistically prey upon the domesticated ruminants. As the farmers and herders became accustomed to hunting and developed a preference for bush meat, they were required to travel further for hunting and needed more dogs for protection during these travels and for tracking animals. This resulted in the domestic ruminants being more independent in sourcing pasture and watering sources.

Concurrently, the discovery of minerals and the establishment of mining towns and their associated schools attracted a lot of farmers and herders and their families, resulting in domestic livestock being unattended during the week and only being seen on the weekends. Because of this movement between towns, villages and cattle posts, which often involved dogs, rabies was introduced into new areas, potentially resulting in infection of wild animals. The working farmers, appreciating the distances between their homes and the cattle posts and the growing frequency of livestock predation by wild carnivores, moved their animals closer to their more densely populated villages. However the predatory relationship between the domestic ruminants and the jackals was already established and jackals are now almost inseparable from livestock areas in Botswana, where they sometimes kill young animals or scavenge on dead animals (Kaunda, 2001). Today most farmers, especially those rearing livestock close to villages and cropping fields, only herd their animals during the ploughing season (November-March) and after harvest the animals are allowed to move about freely. However some
communal farmers use water and licks to attract ruminants back to their base kraals to avoid their animals spending nights in the grazing areas.

Table 1.1: Number of domestic ruminants in communal farms versus commercial farms in Botswana (CSO, 2011).

<table>
<thead>
<tr>
<th></th>
<th>Communal</th>
<th>Commercial</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>2,260,262</td>
<td>294,102</td>
<td>2,554,364</td>
</tr>
<tr>
<td>Goats</td>
<td>1,736,011</td>
<td>33,800</td>
<td>1,769,811</td>
</tr>
<tr>
<td>Sheep</td>
<td>283,075</td>
<td>12,819</td>
<td>295,894</td>
</tr>
</tbody>
</table>

1.4 The disease situation in livestock in Botswana

In the 2010 livestock census in Botswana conducted by the Division of Planning and Statistics, Ministry of Finance and Development, the population of cattle, goats and sheep were 2,554,364; 1,769,811; and 295,894, respectively (CSO, 2011). In contrast the DVS census in 2010 was vastly different for small stock but comparable for cattle (Tables 1.1 and 1.2). Cattle, sheep and goats are the most common animals raised in Botswana generating the highest earnings for agricultural commodities (Jacques, 1995). However, drought and disease are the biggest challenges for livestock, with cattle succumbing more to drought than small stock (Jacques, 1995). Foot and mouth disease (FMD) is well known by farmers because of its characteristic clinical signs, its adverse impact on trade resulting in severe financial losses, and the movement restrictions and stringent measures taken against farmers who do not comply with the necessary prevention and control procedures. Other diseases, such as heartwater,
pasteurellosis, botulism and other tick borne diseases, have received priority since the 1970’s from both farmers and veterinary officials because of their ability to kill large numbers of stock (DAHP, 1973).

Table 1.2: Livestock census in Botswana in 2010 (DVS, 2010).

<table>
<thead>
<tr>
<th>District</th>
<th>Cattle</th>
<th>Goats</th>
<th>Sheep</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>North East</td>
<td>255,800</td>
<td>92,800</td>
<td>15,573</td>
<td>12,974</td>
<td>2,259</td>
</tr>
<tr>
<td>Central</td>
<td>807,831</td>
<td>253,007</td>
<td>48,368</td>
<td>36,328</td>
<td>7,759</td>
</tr>
<tr>
<td>Chobe</td>
<td>14,390</td>
<td>2,379</td>
<td>792</td>
<td>1,701</td>
<td>190</td>
</tr>
<tr>
<td>Ngamiland</td>
<td>342,465</td>
<td>79,316</td>
<td>14,090</td>
<td>6,639</td>
<td>305</td>
</tr>
<tr>
<td>Ghantsi</td>
<td>248,548</td>
<td>27,393</td>
<td>8,339</td>
<td>1,723</td>
<td>138</td>
</tr>
<tr>
<td>Kgalagadi</td>
<td>172,067</td>
<td>196,007</td>
<td>55,442</td>
<td>5,505</td>
<td>204</td>
</tr>
<tr>
<td>Kweneng</td>
<td>242,125</td>
<td>82,681</td>
<td>17,165</td>
<td>11,937</td>
<td>822</td>
</tr>
<tr>
<td>Southern</td>
<td>289,192</td>
<td>95,029</td>
<td>38,805</td>
<td>21,425</td>
<td>5,225</td>
</tr>
<tr>
<td>South East</td>
<td>19,112</td>
<td>16,216</td>
<td>2,909</td>
<td>13,787</td>
<td>1035</td>
</tr>
<tr>
<td>Kgatleng</td>
<td>75,727</td>
<td>22,590</td>
<td>4,199</td>
<td>2,450</td>
<td>1102</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2,467,257</strong></td>
<td><strong>867,418</strong></td>
<td><strong>205,682</strong></td>
<td><strong>114,469</strong></td>
<td><strong>19,039</strong></td>
</tr>
</tbody>
</table>

Rabies, which is likely to kill individual animals on different farms at varying frequencies, is not likely to be considered a serious disease of ruminants by farmers. Furthermore, because of the inability of domestic ruminants to inflict bite wounds to humans and other animals, little research has been undertaken on rabies in domesticated ruminants. However, in Botswana the number of domestic ruminants
succumbing to rabies is significant (Masupu, 1992) and these animals are likely indicators of the importance of the disease in the country. In Botswana rabies is endemic and it is likely that black backed jackals (*Canis mesomelas*) are a major contributor to the dispersal of rabies, although in most cases dogs play a vital role in the transmission of the disease to humans (Maganu and Staugard, 1985) and possibly ruminants as well. However there is a deficiency of epidemiological information on the disease in ruminants in Botswana and this lack of knowledge formed the central reason for undertaking the research reported in this thesis.

1.5 Rabies management in Botswana

In Botswana the management of rabies in animals, as with other veterinary diseases, is through the Ministry of Agriculture by the DVS. Rabies surveillance in Botswana is mostly passive as the DVS response is only based on reporting by the farmers or dog owners. The same is true on wildlife rabies surveillance. Although rabies in humans is managed by the Ministry of Health (MoH), testing of human brain specimens for rabies is conducted by the DVS. Wild animals are managed by the Department of Wildlife and National Parks (DWNP) through game reserves and national parks as well as in areas outside protected zones where specific hunting licenses are given to the public. However, reactive disease investigation in wildlife is carried out by the DVS, especially when livestock are threatened. Human and wildlife rabies surveillance in Botswana are not built into the national disease surveillance system and therefore are not effectively implemented with little to no coordination between the veterinary, human health and wildlife departments (WHO, 2013a).
Rabies is maintained in two epidemiological cycles: an urban and a sylvatic cycle. The urban rabies cycle has been reported to predominate in African countries (Moagabo et al., 2009), however in Botswana, where the government has intensively and effectively campaigned against dog rabies, it is likely that the sylvatic rabies predominates (Moagabo et al., 2009). The sylvatic rabies cycle can be relatively stable occurring as a slow moving epidemic with often one and occasionally up to three wildlife species being responsible for perpetuating a particular strain of rabies (CFSPH, 2009).

Rabies is a notifiable disease in Botswana and all dog and cat owners are obligated by law to immunise their animals annually against the disease. Those who do not comply stand to face severe penalties which may include imprisonment, significant fines or having their animal(s) destroyed without compensation. To facilitate the control of rabies, the Government of Botswana provides free rabies immunisations to dogs and cats through campaigns implemented by the DVS every winter in urban areas, villages, farms and cattle posts. However, lack of a national identification system for dogs and cats makes it hard to monitor vaccination status of dogs and cats. Immunisation of dogs and cats at the cattle posts is usually undertaken simultaneously with cattle vaccinations against anthrax, black quarter and brucellosis. Stray dogs are usually not a problem at cattle posts, compared to in towns and villages. The vaccine used is Rabisin® produced by Pfizer which is designed to induce antibodies lasting for three years, however in Botswana its annual usage is designed to counteract the less than optimum coverage attained during vaccination campaigns and to induce high levels of protective titres (Tremlett, 1993).
Some pet owners, however, prefer to visit a private veterinarian for the vaccination of their pet(s) against rabies. The owners of all immunised dogs and cats receive a vaccination certificate which is necessary for international, national and zonal movements of their pets. Depending on the situation of rabies in an area, the local DVS office may destroy unvaccinated and stray dogs and cats immediately following a vaccination campaign. In addition the population control of dogs and cats in cities, towns and major villages is the responsibility of the local municipality who have specific by-laws authorizing these activities. However, enforcement of these laws has been challenging. Despite these efforts to curb rabies in dogs, it is a continuing battle to control the disease due to the roles played by either stray dogs or the sylvatic rabies cycle (Tremlett, 1993). Furthermore, there are some challenges encountered including vaccine efficacy (old vaccines), rural residents who own packs of dogs for hunting purposes who may refuse to vaccinate them because of a belief that the rabies vaccine renders their dogs powerless and a lack of road-access to rural areas which negatively impacts upon the vaccination coverage (Tremlett, 1993).

In the event a dog or cat bites a human or is reported to have signs indicative of rabies, a record of its vaccination history must be produced by the owner and the animal must be restrained and monitored for 10 days. If signs of rabies are observed or the animal dies, the brain is removed and specimens sent to the laboratory and the medical doctors treating the bitten human informed. If no signs of rabies are observed in the animal then it is released back to its owner (Tremlett, 1993). Stray animals or wild animals suspected of rabies are destroyed immediately and appropriate samples taken and sent to the laboratory for confirmatory diagnosis (Tremlett, 1993).
The clinical signs of rabies in dogs and cats frequently observed in Botswana are aggressiveness and salivation, whereas in small stock aggression, salivation and bleating are common (Moagabo et al., 2009). Bellowing, aggression and salivation are common findings in cattle, while wild animals may become less wary of people or display aggression toward people and other animals (Moagabo et al., 2009). Persistent bellowing in cattle or excessive vocalization in sheep or goats or excessive salivation are not pathognomonic signs of rabies, and farmers can be easily led to think of diseases other than rabies. Furthermore farmers may decide to salvage the meat from their sick animals or from freshly deceased animals, especially given that animal health officials burn the carcasses of animals suspected to have rabies. There are documented procedures guiding the extension officers of the DVS on the collection of samples for rabies testing, along with the packaging, transportation and submission of samples to the Botswana National Veterinary Laboratory (BNVL) in Gaborone (BNVL, 1990).

Investigation of rabies in southern Africa initially focussed on the disease in dogs, however subsequently the importance of other host species in the distribution of the disease was recognised (Swanepoel et al., 1993). Southern Africa, including Botswana, presents a special mix of both urban and sylvatic forms of rabies. There are no specific rabies surveillance and monitoring programmes for wild animals in Botswana (WHO, 2013a) and reports of wild animals with clinical signs suspicious for rabies relies heavily upon chance encounters by the general public, farmers, wildlife workers and even tourists. However some people who may observe unusual clinical signs in wildlife may be involved in illegal hunting activities and consequently are unlikely to report cases
suspicious of rabies. These features are likely to lead to underreporting of the disease in wildlife.

All specimens from suspected cases of rabies are tested and confirmed at the BNVL. Specimens of the brain are examined first for the presence of viral antigens by the direct fluorescent antibody test (FAT) (Masupu, 1992). Only the FAT negative samples are then subjected to a mouse inoculation test (MIT) but positive samples are sometimes subjected to MIT only for validation purposes. The inoculated mice are observed daily for up to 30 days for evidence of clinical signs of disease (Mushi and Diteko, 1992). Brain samples harvested from dead and/or surviving mice are also investigated for rabies viral antigen by the direct FAT (Mushi and Diteko, 1992).

1.6 Statement of the problem

Rabies is endemic throughout Botswana with the principal reservoir hosts not only being dogs, but also wildlife including jackals and other carnivores such as the genet (Genetta genetta) and yellow mongoose (Cynictis penicillata) (Moagabo et al., 2009). Furthermore, a molecular epidemiological study conducted in Botswana has confirmed the existence of two major rabies biotypes: the wildlife-associated biotype (Mongoose subtype); and the canine-associated biotype. These biotypes dominate the southern and northern parts of the country, respectively (Johnson et al., 2004). Interestingly in Botswana, cattle, sheep and goats are the most common domestic animals to contract rabies virus against a declining number of cases of canine rabies. Over 70% of all rabies cases in Botswana are in domestic ruminants, with cattle being the most commonly affected animal (Johnson et al., 2004). However no research has been undertaken in
Botswana to identify risk factors which may be associated with the high proportion of rabies in domesticated ruminants, nor has the spatial and temporal distribution of the disease in ruminants in Botswana been described. Although there have been significant efforts made to ensure pets and their owners are safe from rabies through rabies vaccination and educational programmes, campaigns targeting farmers on rabies in ruminants are seldom conducted in Botswana.

1.7 Objectives of the current research

The overall objective of the research reported in this thesis was to determine the distribution of rabies in domestic ruminants in Botswana in terms of geographical location and temporal factors (seasonal times). The research also addressed the question "why domestic ruminants in Botswana were experiencing the highest numbers of cases of rabies when compared to other species". This was determined by identifying the risk factors associated with this disease. The final part of the study was designed to assess the knowledge, attitudes and practices of the farmers in specific high risk areas and to see if there were any factors contributing to the high rate of rabies in certain groups of ruminants compared to those not affected by the disease in the same high risk area.

1.8 Rationale of the study

With increased knowledge on the factors likely to cause rabies in ruminants, and the geographical and seasonal trends of rabies in ruminants, better control programmes can be designed to reduce the disease in ruminants. This would benefit livestock farmers in Botswana, as well as those in neighbouring regions and countries. By
helping to raise awareness of rabies among farmers, this study could contribute to potentially reducing transmission of rabies to humans and thereby reducing some of the economic burden of this disease. Developing and implementing improved disease control measures and improving farmer’s awareness of the disease should result in reduced disease in both humans and other animals. A reduction in animal disease would concurrently result in extra income for individual farmers.
Chapter 2- Literature Review

2.1 Introduction to rabies

Rabies is a fatal neuropathic viral disease affecting warm blooded species, including humans. The word rabies came from either the Sanskrit word rabhas meaning “to rage” or a Latin word Rabera meaning “to rave” (Leung et al., 2007). Rabies has been recognised for over 4,000 years through an association of a “mad dog bite” leading to the death of the individuals bitten (Schnell et al., 2010). In 1802 the transmission of rabies from the saliva of a rabid dog to a healthy dog and later inter-species transmission were demonstrated by George Zinke and Pierre-Victor Galtier, respectively (Schnell et al., 2010). The most important breakthrough occurred in the 1805 when Louis Pasteur established that the rabies virus could be isolated from the brain of affected individuals and subsequently it was harvested and used to produce the first vaccine against the disease (Schnell et al., 2010). It is the presence of Classical Rabies Virus (RABV) on all continents (except Antarctica and Australia) and its zoonotic potential that make rabies a disease of global concern.

Canine rabies is the biggest source of both human and livestock infections in the developing countries of Asia, Latin America and Africa (Shwiff et al., 2013). However recent findings indicate that other species, especially wild animals, are playing a major epidemiological role in the maintenance and spread of rabies. Animals, including bats, skunks, raccoons and foxes in North and South America, and jackals, foxes and mongoose in Africa, have been identified as reservoirs of disease (Sabeta et al., 2007, Zulu et al., 2009, Van Zyl et al., 2010). More than 55,000 human deaths from rabies are
reported worldwide each year, with 99% of these caused by dogs and 95% of the dog-induced rabies deaths occurring in Asia and Africa (WHO. 2013b). However, rabies is a neglected disease of poor and vulnerable populations whose deaths are often rarely reported (Taylor, 2013). Consequently rabies is more likely to occur in remote rural communities where measures to prevent dog to human or dog to livestock transmission have not been implemented (Taylor, 2013).

2.1.1 The agent

The causative agent for rabies belongs to the genus Lyssavirus in the order Mononegavirales and family Rhabdoviridae (Schnell et al., 2010). Rabies virus has a non-segmented, bullet shaped, negative stranded RNA genome that is packed into ribonucleocapsid structures. It is composed of: an RNA dependent RNA polymerase (L), a single surface glycoprotein (G), a nucleoprotein (N), a phosphoprotein (P), and a matrix protein (M) (Rieder and Conzelmann, 2009) as displayed in Figure 2.1.

Rabies virus is easily inactivated by any fat soluble solvents such as liquid soap, ether, chloroform, and acetone and is susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 45-75% ethanol, iodine preparations, formaldehyde, low pH, ultraviolet radiation or heat for 1 hour at 50°C (CFSPH, 2009).
Rhabdoviruses infect a wide range of hosts including vertebrate, arthropods and plants, but the genus *Lyssavirus* infects mammals and possesses a different amino acid sequence to other rhabdoviruses (Rieder and Conzelmann, 2009). Lyssavirus are divided into 13 genetically different viral types. The first group is the most common rabies virus (RABV), “rabies proper” or classical rabies known as *Lyssavirus* genotype 1 and the others viral types are classified as rabies-related lyssaviruses. The rabies-related lyssaviruses include 1) Lagos bat virus (LBV), (2) Mokola virus (MOKV), (3)
Duvenhage virus (DUVV), (4) European bat lyssavirus type 1 (EBLV-1), (5) European bat lyssavirus type 2 (EBLV-2), (6) Australian bat lyssavirus (ABLV), (7) Irkut virus (IRKV), (8) Aravan virus (ARAV), (9) Khujand virus (KHUV), (10) West Caucasian Bat Virus (WCBV), (11) Shimoni bat virus (SHIV), is yet to be classified (Banyard et al., 2011). However there are two more new rabies related viruses namely Bokeloh virus which appears to belong to phylogroup I and Ikoma virus which seems to be related to West Caucasian bat virus, although a full analysis is not yet available (CFSPH, 2012). The lyssaviruses can be divided into three phylogroups (I, II and III) (Figure 2.2) (Banyard et al., 2011) and vaccines specific for Classical RABV (phylogroup I) have no cross protection to phylogroups II and III.
Mokola Virus is the only lyssavirus which has never been isolated from bats (Banyard et al., 2011). However, in spite of this association with bats, it is only in America where classical rabies virus, RABV, is maintained and transmitted by bats, with lyssaviruses in the rest of the world, including Africa, Asia, Australia and Europe, existing in the absence of bat-associated RABV (Banyard et al., 2011). Importantly the rabies-related lyssavirus have never been observed or confirmed to spill-over to cattle, sheep or goats, with spill-overs to other domestic animals being rarely reported. In these latter
instances no other species have become reservoirs for the virus, as has been reported for bats in America (Banyard et al., 2011). Consequently the research reported in this study will be restricted to RABV.

2.1.2 Transmission and pathogenesis of rabies

Both intra- and interspecies transmission of rabies occurs readily among mammals. The mode of transmission is usually through the bite from an infected animal. However, infrequently deposition of viral laden saliva into pre-existing cuts and wounds of susceptible animals or persons may result in infection. There is evidence to indicate aerosol transmission is possible, especially in caves where bats roost where high concentration of rabies viral particles can be dispersed into the air (Banyard et al., 2011).

Transmission through ingestion of tissues and fluids from an infected rabid animal or from a child or young animal suckling milk from their mother cannot be ruled out and therefore the consumption of such foods is considered risky and not recommended, even though the virus is destroyed by pasteurization and cooking (CFSPH, 2009). In Botswana humans that are believed to have been exposed to rabies through these uncertain transmissions (butchering and consumption of carcases) are given post-exposure prophylaxis (PEP) (Tremlett, 1993). Given their persistent oral injuries, kudus in Southern Africa have been known to potentially get rabies viral infection through browsing on contaminated thorny acacia trees and grooming of other kudus resulting in contact with virus laden fur (Swanepoel et al., 1993). Further possible transmission
in humans is through organ transplants, although this is a very rare form of transmission (CFSPH, 2009).

When the virus has entered a fresh wound of a susceptible species it attaches to animal tissue or local nerves through the G-protein. Even though rabies is known for its neurotropism, in some cases the virus assumes a very slow replicative process in the muscles before entering a peripheral nerve at a neuromuscular junction, whereas in others the virus quickly enters the central nervous system (CNS) without any prior local replication (Smith, 1996). Entry of the virus into a peripheral nerve is facilitated by the G-protein and interaction with various cell receptors such as nicotinic acetylcholine receptors at the neuromuscular junction, neural cell adhesion molecule S and the p75 neurotropic receptors on neural cell membranes (Leung et al., 2007).

The transport of the virus to the CNS involves both sensory and motor nerves in a retrograde fast axoplasmic flow of 12 to 100mm per day with the P-protein possibly playing a major role in this process (Leung et al., 2007). Once the virus reaches the brain stem and trigeminal ganglia, virus is amplified through transcription, replication and protein synthesis, assembly and budding and releasing of mature viral particles in order to invade the CNS (Leung et al., 2007). The virus is then spread through intense replication to the cerebellum, midbrain and forebrain. The successful invasion of the CNS is helped by the ability of the rabies virus to inhibit the immune response through phosphoprotein blocking interferon signals and thereby preventing the clearance of the virus in the CNS (Schnell et al., 2010). However, late in the disease process the massive replication can cause widespread neuronal inflammation and necrosis with
eosinophilic intracytoplasmic inclusion bodies known as Negri bodies (masses of viral RNA, ribosomes and virions) appearing in infected neurones (Leung et al., 2007). At the terminal stages of the disease, the virus may move in an anti-retrograde exoplasmic centrifugal flow in peripheral nerves at the rate of 100 to 400 mm/day to be released from axon terminals into the non-nervous tissues of the salivary glands, resulting in high viral load in the saliva (Smith, 1996). Immune response to rabies occurs very late in the course of the disease, usually only a few days before death. In one experimental study, the viral replication cycle was 12 hours, the average viral axonal speed was 1 mm/hour, absorption and penetration occurred within 2 hours, and it took 18 hours to reach the trigeminal ganglia and 72 to 92 hours for the intense replication and spread of the virus to all regions of the brain (Shankar et al., 1991).

2.1.3 Clinical manifestations and signs

A standard case definition of rabies is necessary because the clinical signs vary in different species. Any animal or person, who develops a sudden neurological syndrome dominated by a change in behaviour, be it furious or dumb form, resulting in coma or death in less than 10 days should be considered a potential case of rabies. The incubation period for rabies in humans averages between 2 to 3 months but can be as low as 5 days and rarely goes beyond 6 months (WHO, 2013b). However incubation periods of up to 10 years have been documented (Palanivel et al., 2011). This variation can be explained by differences in the number of viral particles initially deposited in the bite, the density of peripheral nerves close to the wound site and the proximity of the virus to the CNS. In humans, hydrophobia or aerophobia are the classical signs for rabies. Initially non-specific signs of headache, pruritus and discomfort may be
observed, however with time confusion, agitation and anxiety may set in. This progresses to insomnia, abnormal behaviour, hypersensitivity to light and sound, delirium, hallucinations, slight or partial paralysis, hypersalivation, difficulty swallowing, pharyngeal spasms upon exposure to liquids and convulsions (WHO, 2013b).

In dogs and cats the incubation period is 10 days to 6 months with most cases occurring before 3 months. However incubation periods ranging from 25 days to more than 5 months have been reported in vampire bat-transmitted rabies in cattle (CFSPH, 2009). A similar incubation period in cattle of 20 to 165 days has been reported in Croatia, where rabies is endemic and maintained in red foxes (Vulpes vulpes) with occasional transmission to cattle (Lojkic et al., 2013). However it is often difficult to confirm the incubation period in naturally infected cattle due to the difficulty in identifying bite marks.

Clinical signs in animals also start with nonspecific signs of apprehension, restlessness, anorexia or an increased appetite, vomiting, dilation of the pupils and hyper-reactivity to stimuli which may last 2 to 5 days. These signs are then followed by behavioural and temperamental change where animals become either aggressive or friendly representing the furious or paralytic forms of rabies, respectively. In the paralytic (“dumb”) form there is progressive paralysis affecting the throat and facial and spinal nerves. This form of rabies is frequently seen in cattle with signs such as profuse salivation, inability to swallow, inappetence and facial paralysis or dropping of the lower jaw. Animals may move slowly, display ataxia and incoordination, separate from
the herd, show signs of depression, may stop ruminating and have faecal and urinary retention (Reis et al., 2003). One study reported that a constant sign in both furious and dumb rabies in cattle was ascending paralysis seen first as an incoordination of the hind legs, resulting in stumbling and staggering and eventual recumbency (Radostits, 1964).

In one experimental study 70% of affected cattle developed the furious form of rabies with signs of excessive salivation (100%), behavioural changes (100%), muzzle tremors (80%), vocalization (bellowing 70%), aggression, hyperaesthesia and/or hyperexcitability (70%) and pharyngeal paresis/paralysis (60%) (Hudson et al., 1996). In addition rabies induced in sheep had the following signs: muzzle and/or head tremors (80%); aggression, hyperexcitability, and/or hyperaesthesia (80%); trismus (60%); salivation (60%); vocalization (60%); and recumbency (40%) (Hudson et al., 1996). The furious form in dogs is marked by restlessness, wandering, howling, barking, bellowing, excessive salivation and attacks on animals, people or inanimate objects. Animals with the furious form may also display pica with ingestion of objects such as sticks, stones, straw or faeces. The classical signs of rabies in cattle and buffalo are bellowing, anorexia, pyrexia and hypersalivation, whereas in dogs changes in behaviour, aggressiveness and anorexia are the most common clinical signs (Wahan et al., 2012). Domestic animals with multiple signs have a higher probability of testing positive to rabies than those with a single clinical sign (Wahan et al., 2012). Irrespective of the form of rabies, an affected animal invariably dies within 4 to 8 days of developing clinical signs.
Wild animals in Africa often lose their fear of humans and attack humans or domesticated animals that they would normally fear (Moagabo et al., 2009), while nocturnal animals may be seen during daylight hours. In one study of affected wild animals the odds of aggression were highest in larger terrestrial species such as grey foxes (*Urocyon cinereoargenteus*), red foxes and coyotes (*Canis latrans*) (OR - 10.4 to 27.6), whereas smaller animals such as raccoons (*Procyon lotor*), woodchucks (*Marmota monax*) and skunks (Family *Mephitidae*) had low odds of displaying these signs (OR - 2.8 to 8) compared with rabid stray cats (Wang et al., 2011). Therefore aggression is a good indicator of rabies in wild animals in Africa and across the world.

### 2.1.4 Diagnosis of rabies

Laboratory confirmation of rabies in animals generally involves the detection in the brain of viral antigen and isolation of the virus in cell cultures or in laboratory animals. In contrast, in humans the intra-vitam test is used as a confirmatory diagnosis along with other tests to detect viral-specific antibodies in the cerebrospinal fluid or serum of unvaccinated people, or detection of nucleic acids by molecular methods in samples (brain biopsy, skin and saliva) (Leung et al., 2007).

The detection of viral antigen is the most common and widely used rabies confirmatory method which employs a rapid, sensitive, specific direct fluorescent antibody technique (FAT). The technique is the gold standard for diagnosing rabies in animals and humans, although the accuracy of the test depends upon the examiner’s expertise and the quality of chemicals and the type of microscope used. Impression smears from the brain stem and cerebellum give the best results, although sometimes the hippocampus is included, even though it is not essential. The smears are stained
using an anti-rabies monoclonal antibody conjugated with fluorescein isothiocyanate and the detection of virus specific-antigen on microscopic examination at a magnification of 3400 and 31000 (Smith, 1996). Rabies antigen appears as round to oval intracytoplasmic inclusions with smooth bright margins and lightly stained central areas. Another test which is able to reproduce similar results in most cases with comparable sensitivity and specificity to the FAT is the direct rapid immunohistochemistry test with a sensitivity of approximately 94.7% (Wahan et al., 2012). Histopathological examination of sections stained with haematoxylin and eosin (H & E) for Negri bodies has a sensitivity of 50 to 80% compared with the FAT (Smith, 1996).

Specimens which test negative for viral antigen can be further subjected to viral isolation tests which could amplify any virus present, confirm the results of previous tests and if needed characterize the virus. Virus isolation is conducted in cell cultures such as neuroblastoma cells or by the intracranial inoculation into mice. Culture in neuroblast cells is just as accurate as animal models (animal inoculation) and offers the advantage in that a diagnosis can be confirmed within 1 to 2 days compared with the 10 to 21 days with animal inoculation (WHO, 2013b). With animal inoculation, injection into suckling mice less than 3 days old can give more rapid results because of their high susceptibility to the rabies virus compared with older mice (WHO, 2013b). The BNVL utilises both the FAT and MIT to confirm the rabies status of samples submitted from the field throughout Botswana (Masupu, 1992).
2.1.5 Treatment of rabies

There is no effective treatment for a suspected rabid animal. Because of the seriousness of the disease, animals which attack or bite humans should be treated as being rabid until proven otherwise. Owned dogs should be confined and monitored for 10 days while stray dogs or wild animals should be killed and tested for the presence of the virus (Smith, 1996). A similar argument could be made for domesticated ruminants which pose a danger to humans and these should be destroyed and tested for rabies, whereas those that are manageable should be confined and monitored and if they don’t die within 10 days more aggressive investigations should be pursued for other diseases affecting the nervous system.

For humans bitten by a rabid animal, PEP should begin immediately to prevent the rabies virus from reaching the CNS. The first step is cleansing of all wounds with soap and water and administration of anti-rabies immune globulin from hyper-immunized human plasma donors at a dose rate of 20 IU/kg body weight with half of the dose given intramuscularly (IM) and the other half infiltrated into the wound(s) (Smith, 1996). Smith (1996) further suggested that a purified cell culture-derived, inactivated vaccine at 1.0 ml IM given on days 0, 3, 7, 14 and 28 post-bite would elicit an excellent antibody response. However this treatment could cost up to US$2,000 per individual treated and hence is beyond the means of many individuals and countries (Smith, 1996).

High risk groups, including field veterinarians and technicians and workers in the laboratories where rabies is diagnosed, animal control facilities and veterinary clinics in
rabies endemic countries, should all receive pre-exposure immunisation. This can help minimise the need for immune serum and reduce the number of vaccine doses to two booster injections, should an individual be exposed to rabies (Leung et al., 2007).

2.2 History of rabies and trends in Botswana

Rabies in Botswana is commonly known as “Molafo”. The first case was confirmed in 1936 in a child from the North West district as a result of trans-boundary spread of the disease from Angola into Botswana, which was at the time called Bechuanaland Protectorate and was under British protection (Tremlett, 1993). Approximately 2,000 dogs in the area were immediately killed as a control measure, however subsequent surveillance detected rabies in dogs in 1937 and 1938 in the North West district (Tremlett, 1993).

In Angola the first diagnosis of rabies was in dogs in 1929, however civil unrest in that country made disease control measures impossible and probably favoured the spread of rabies within that country resulting in spill-over into other species (farm animals and wildlife) and facilitated subsequent transmission across the national borders in the 1940’s (Swanepoel et al., 1993). The rabies pandemic from Angola spread into Namibia, Zimbabwe and Botswana and rabies was recognised as a problem in all three countries in 1938. Subsequently the disease remained in these countries, reaching localised endemic levels in the 1940’s (Swanepoel et al., 1993). In RSA incursion of rabies, possibly emanating from Botswana in the early 1950’s (Swanepoel et al., 1993), supported the belief that rabies had spread to the southern parts of Botswana from the northern parts and was endemic throughout the country at that time. However,
mongoose rabies, first diagnosed in 1916 was apparently very subtle, persistent and endemic in RSA (Zyl et al., 2010). This type of rabies was recognised to be a problem in Botswana in the early 1990’s (Johnson et al., 2004) and therefore others have hypothesized that the first case of rabies in Botswana might have occurred as early as 1919 or 1922 in the southern part of Botswana (Lobatse), which shares a border with RSA (Moagabo et al., 2009). Lack of previous information on rabies may suggest that rabies in Botswana occurred much earlier than suggested. As FMD in Botswana was a disease of both high frequency and priority in terms of allocation of resources between the 1930’s and 1968, it is likely that many case of rabies during this period were not reported. Foot and mouth disease was finally controlled in 1968 (DAHP, 1972) with no more cases observed in Botswana until early 2000, allowing for reallocation of funds to other diseases, including rabies. In Botswana there are adequate data available on rabies cases after 1970, however prior to this information available is only in the form of occasional reports by the DVS. Therefore historical rabies trends are described in two phases: from 1936 to 1971; and from 1972 to 1999.

In the period from 1936 to 1971 there were confirmed reports of rabies cases in: 2 humans; approximately 300 dogs; 51 cats; and 2 sheep (Tremlett, 1993). According to Tremlett (1993), these cases were mostly in the north of Botswana. Furthermore he reported that the most common districts for rabies occurrence were Central, North East and Ngamiland. During this period an average of ten confirmed cases were observed per year, with dog rabies being the most frequently diagnosed.
In the late 1940’s rabies was not only occurring in dogs in Namibia but also in other species including cattle and jackals, however it was not until 1959 that rabies was diagnosed in domestic ruminants in Botswana when two sheep were diagnosed with the disease (Unsworth, 1960). Although there were reports of deaths of 8,308 cattle and 4,516 sheep/goats in 1958 and 9,001 cattle and 4,516 sheep/goats in 1959 from unknown causes in Botswana (Unsworth, 1960). Involvement of wildlife in rabies was mentioned in 1959 but not substantiated, however in 1970/1971 an operation to bait and reduce the population of jackals occurred, even though the number of jackals affected by the disease was not mentioned (Tremlett, 1993). In contrast clearer records of cases of rabies in jackals were available in 1972, after which cases of rabies in these animals were a constant feature each year (Tremlett, 1993). In addition Tremlett (1993) reported that more baiting activities were carried out in the late 1970’s and a large scale programme of baiting, combined with extensive dog vaccination and shooting of stray dogs, was undertaken in the 1990’s.

In the early 1950’s, with an outbreak of rabies in the North East district, vaccination of dogs with an American flury strain attenuated live rabies vaccine was instigated, however this led to rabies related deaths in dogs the following year (Tremlett, 1993). Whether this was a result of vaccine failure or a problem with the vaccination protocol/method is uncertain. In 1955 a chick embryo vaccine produced by the Research Institute, Ondestepoort (VRI) replaced the previous vaccine and a large scale vaccination programme of dogs was successfully conducted without reports of any breakdowns (Tremlett, 1993). However a lack of funds resulted in the introduction of a fee for the vaccination which resulted in subsequent low vaccination uptake and
coverage and consequently the disease spread further south. In the 1960’s funds became available and there was a bilateral vaccination programme against rabies in dogs conducted by the governments of both Botswana and Zimbabwe, resulting in the reporting of virtually no cases of rabies in domestic animals for the next four years (Tremlett, 1993).

In 1959 there was an alarming increase in the number of cases of rabies in cats which were used for protecting stored grain and other goods from rats and mice (Unsworth, 1960). This resulted in an overall high number of rabies cases in 1959 (61 cases) which included dogs, cats and sheep, however the number of cases dropped to 11 and 3 in 1962 and 1963, respectively, during years of severe drought. The same trend was observed in Zimbabwe where a large number of cases were reported in 1957 (59 cases), which reduced to only 15 in 1960 when drought was wide-spread (Rhodesia, 1961). Even though the drought may have played a role in the reduction of the number of cases, it is also likely that the bilateral vaccination programme also had a significant impact on the number. In contrast in the RSA in 1933 and 1934 heavy rains coincided with a greatly decreased incidence of rabies. This was, most likely, as a result of drowning of large numbers of meerkats (*Suricata suricatta*) in their burrows (Snyman, 1940). Therefore it is likely that heavy rains resulting in flooding seem to be playing a role in the epidemiology of rabies in southern Africa, although there is little evidence on the role of drought on the disease.

It was after the control of FMD in the 1970’s, that relatively high numbers of confirmed rabies cases were observed. The cases were very apparent in the Central, North East
and Ngamiland districts (Tremlett, 1993) with severe outbreaks encountered in the Francistown area and North East district (DAHP, 1973). Data from DVS, Botswana are suggestive that at that time rabies was claiming a significant number of human and animal lives and affecting a range of species (Figures 2.3 and 2.4). The first recorded cases of rabies in cattle, goats, jackals, donkeys and honey badgers (*Mellivora capensis*) were documented in 1972 (DAHP, 1972).

![Figure 2.3: Temporal distribution of rabies in domestic ruminants (1972-1999)](image)

Figure 2.4: Temporal distribution of rabies cases by species from 1972-1999 (Compiled from DAHP reports 1972; Tremlett, 1993; Maganu and Staugard; 1985, Mushu and Diteko, 1992; Masupu, 1992; Moagabo, 2009).

Between 1972 and 1989 an average of 74 cases were documented each year in Botswana (n = 1,326). Of these, 44.6% were in ruminants (average 33 cases per year, ranging from 8 to 84 cases per year). The average number of cases per year for dogs, jackals and humans in the same period were 28, 7 and 1.4 (n = 508, 123 and 25), respectively. During this time the trend in the number of cases in cattle and goats was similar. In contrast sheep were less frequently affected by rabies than cattle or goats (Figure 2.3).

The 1970 baiting activity appeared to have reduced the number of cases in jackals to low levels in the 1970’s, but subsequently the number increased to be comparable to that reported in dogs. Tremlett (1993) reported that there was approximately a two
year lag phase between an increase in cases in wild animals and a subsequent increase in cases in domesticated species. A second baiting activity in 1977, which coincided with the introduction of free and compulsory annual vaccination of dogs, resulted in a reduction in the number of cases in both dogs and jackals. The ability of dogs to maintain a rabies cycle in the absence of disease in jackals was highlighted by Tremlett (1993). In the 1980’s the number of cases in both dogs and jackals increased. This increase in cases of rabies in canids also coincided with an increase in the number of cases in ruminants.

In the 1990’s the number of cases in Botswana (average 194 cases per year) was far greater than that in the previous two decades with 69% of cases involving ruminants (average 134 cases per year). In contrast the average number of cases each year for dogs, jackals and humans for the same period, were only 28, 19 and 0.4, respectively. The highest number of cases in ruminants (202) was recorded in 1995, which also coincided with the highest number of cases in jackals (36).

In the early 1990’s, following an extensive programme of vaccination of dogs, public awareness campaigns, baiting to reduce the population of jackals and destroying (shooting) stray dogs, the number of cases in dogs reduced (Tremlett, 1993, Masupu, 1992). Although the baiting of jackals was effective in reducing the number of cases, the jackal population recovered quickly and represented a potential reservoir of infection for domesticated animals (Mushi and Diteko, 1992). In the period of 1979 to 1999 the average number of rabies samples submitted to the BNVL was 237 per year.
2.3 Interaction of wild carnivores with domestic ruminants

As discussed previously it was likely that the intensive hunting of wild herbivores outside the wildlife protection zones resulted in the depletion of these animals while at the same time allowing domesticated ruminants to thrive. This resulted in predators, such as jackals, turning towards livestock as a source of food and similar findings have been reported in the RSA (Brassine, 2012). A study in the RSA revealed that the density of jackals was two for every 5 km² (Brassine, 2012) and these animals were confirmed to be the only major mammalian carnivores in most parts of semi-arid Botswana (Kaunda, 2001) where abundant cattle and goats are found. Black-backed jackals (Canis mesomelas) have better hunting success as packs than as individuals, with the potential to impart serious losses to small ruminants, especially during the parturition season of winter and spring (June-October) (Kamler et al., 2012).

Another study in Botswana reported that black-backed jackals were responsible for 77% of the predator attacks at cattle posts (Gusset et al., 2009). In contrast large predators, such as lions, cheetahs, spotted hyenas and wild dogs, were responsible for only 22%. Simple improvement of husbandry practices, such as herding of livestock and kraaling of livestock at night, have been suggested as solutions that can be adopted to reduce this predation (Gusset et al., 2009). The most common diets of black-backed jackals include young wild ungulates, domestic stock and members of Insectivora, Lagomorpha and Rodentia orders (Brassine, 2012). However they have been known to scavenge on anything, especially when prey is not readily available. It is this multi-species interaction through predation that makes black-backed jackals a powerful rabies reservoir and transmitter of rabies. This animal warrants further
investigation in the epidemiology of rabies, especially in countries such as Botswana where intense vaccinations programs of domestic dogs and cats, coupled with dog control efforts seem to have been unsuccessful in controlling rabies.

Rabid wild carnivores, such as jackals, foxes and mongooses, are not only likely to attack the small and younger ruminants but also large ruminants such as cattle whose total population is more than that of sheep and goats combined. This may explain why, in Botswana, cattle are the domestic animal most frequently diagnosed with rabies. Domesticated ruminants in Botswana are particularly vulnerable to rabies because they are exposed to transmission from multiple wildlife reservoir species, as well as from domesticated dogs and cats. Cattle, being large animals which are clearly visible at a distance, are likely to attract the attention of rabid animals. Furthermore, when under attack they don’t run as much as goats and sheep and are often very inquisitive by putting their head and nose to the ground to smell affected animals, thereby predisposing their head and nose to attacks by even the smallest rabid animal, such as mongooses. Cattle which are attacked by jackals, dogs or other small carnivores are also likely to survive such an attack escaping with bite wounds (Jennens, 2002) but may later succumb to rabies, unlike small ruminants which are more likely to die as a result of the trauma inflicted during an attack and consequently the latter are less likely to be included in the official rabies statistics.

The large number of cattle in Botswana also increases the opportunity for attacks by carnivores on these animals as opposed to sheep which are less numerous. Sheep also have panoramic vision continuously watching for predators and respond in unison
thereby keeping together as a flock (Jennens, 2002). The usual responses in sheep of ceasing grazing, remaining still, quiet and alert are likely to prevent many predatory attacks. According to Jennens (2002), predators, including dogs, usually chase prey that move away from the predator or that separate from the flock or herd. Dogs attacking livestock are a common problem in many cities in the world with the majority of attacks caused by dogs living in the neighbourhood (Jennens, 2002). Stray dogs interact with both wild animals and domestic ruminants in search of food and consequently may play a significant role in the transmission of rabies (Kariuki and Ngulo, 1985).

One study in Botswana reported that jackals were more likely to move towards agricultural areas in the winter where there were opportunities to predate on dead livestock and rodents. Also at this time ruminants are not herded as crops have already been harvested and hence do not need protecting (Kaunda, 2001). Equally important in the distribution of jackals was a temporary relocation, especially during the rainy season, to new areas where there might be dead or ailing animals as a result of floods (Kaunda, 2001). Black-backed jackals in Botswana have been found to start their daily activity from 3 am with peak activity between 4 and 5 am. They usually rest from 10 am until 1 pm and then have another peak activity at around 6 pm and by 11 pm they sleep until the following cycle commences the next day (Kaunda, 2001). The growing human population in Botswana is likely to infringe on some wildlife ecosystems thus escalating the potential contact between domestic ruminants and jackals and possibly other wildlife animals.
In Namibia a rabies outbreak in dogs in 1948 was followed by an outbreak in cattle and black backed jackals south of Etosha National park in 1949 which perpetuated and led to an epidemic outbreak in kudu antelopes in 1977 which carried on until 1985 (Swanepoel et al., 1993). The widespread outbreak in kudus resulted in the death of 30,000 to 50,000 antelopes (20% of the estimated population) (Swanepoel et al., 1993). This outbreak was believed to have been started by a rabid jackal, however, because of the very high population and density of kudu, the virus was able to circulate and be transmitted among kudus through oral transmission (Swanepoel et al., 1993). Kudus browse thorny acacia trees which lead to frequent mouth injuries. Infected kudus with high concentration of the virus in the saliva contaminate the vegetation or the grooming sites of other kudu and the next kudu picks up the virus when eating or grooming the same site (Swanepoel et al., 1993). During this outbreak (1980) many carnivores in the Etosha National Park, including lions, bat eared foxes and jackals, succumbed to rabies. In 1981 the Gantsi district in Botswana, which previously had not had reports of rabies, first experienced the disease with confirmation in two jackals, followed by cattle in farming areas proximal to the Namibian Border. This was followed by an eastward spread into Botswana following the prolonged outbreak in kudus and other species in Namibia (Tremlett, 1993).

The trend established in Zimbabwe and RSA is that jackal rabies is mostly encountered on commercial farms where jackals are likely to persist in relatively high numbers in the absence of predators/competitors, whereas communal areas are usually overgrazed and deforested and in these areas dog rabies typically prevails (Swanepoel et al., 1993). The occurrence of jackal rabies seems to be almost always accompanied
by the occurrence of rabies in cattle in southern Africa. With the collapse of disease control measures as a result of civil war in Zimbabwe, dogs, jackals and cattle constituted 91.8% (7391/8047) of all cases of rabies from 1950 to 1992 and no other country has recorded as many rabid jackals and cattle (Swanepoel et al., 1993). The highest numbers of cases in jackals and cattle rabies were observed in the mid-1970s and immunization of cattle with Flury HEP (high egg passage) vaccine was attempted in 1976, however the disease in jackals and cattle remained a problem in the ranching areas of the northern Transvaal (Swanepoel et al., 1993).

The number of cases in cattle where jackal rabies occurs has been found to be three times greater than all other species combined (Swanepoel et al., 1993) and this was true in Botswana in the 1990’s when cases of rabies in ruminants and jackals reached their highest point. It was concluded in a 1977 veterinary report that where there was an outbreak of rabies in cattle, rabies in jackals was the underlying cause (Tremlett, 1993). This was solely based on the fact that ranches experiencing increased rabies incidence in cattle had effective dog management and control, however baiting efforts revealed a significant jackal population on such ranches, with similar findings being reported in RSA and Zambia (Tremlett, 1993). It was concluded that a population density of 3 to 4 jackals per km² was high enough to maintain rabies and rabies could only be controlled if there were less than one per km² (Tremlett, 1993). However the consumption of rodents and invertebrates by black-backed jackals has been seen as a benefit by some farmers in controlling crop pests (Kaunda, 2001) and therefore population control of this species may have some deleterious effects.
2.4 Principal reservoirs of rabies

2.4.1 Introduction

Domestic and wild canids, mongoose and bats are the most significant species for harbouring and transmitting rabies virus (RABV) (Banyard et al., 2011). There are theories that all rabies viruses originated from bats, however infected bats transmit the virus to other potential reservoir species only in North and South America (Banyard et al., 2011). In all other continents bats appear to be free from RABV. The rabies virus has been found to be well established and circulating among: skunks, foxes and raccoons in North America; mongooses, bat-eared foxes (Otocyon megalotis) and jackals in Africa; and foxes (Vulpes vulpes) and raccoon dogs of Eurasia (Nyctereutes procyonoides) (Banyard et al., 2011) and maintenance of RABV in these species seem to be associated with species abundance. Interestingly RABV is capable of undergoing genomic alteration when transmission to a new host species occurs and this could lead to novel rabies virus of different biotypes (Nel et al., 2003). Unfortunately, with the high rate of successful RABV spill-over between these different species, there is equally increased opportunity for infection of humans and domestic ruminants.

2.4.2 Rabies reservoirs in southern Africa

2.4.2.1 Introduction

In southern Africa two distinct RABV variants or biotypes have been established based on their affinity for specific host reservoir species, as well as differences in antigenic and genetic characteristics. These RABV variants are classified as the canid biotype which involves dogs, foxes and jackals, and the mongoose biotype which involves
members of the Herpestidae (mongooses and suricates), as well members of the Viverridae (e.g. genets and civets) (Nel et al., 2003). Mongoose rabies has been reported to contribute approximately 44% of the total confirmed rabies cases in RSA, 57% in the Caribbean Island of Grenada and only 2% in Zimbabwe (Nel et al., 2003).

2.4.2.2 Mongoose RABV biotype

Mongoose rabies has probably been in existence since at least 1885 when an association of a bite from a genet was linked with madness and death in humans bitten by these animals (Zyl et al., 2010). A recent scientific study also concluded that the mongoose variant may be approximately 135-360 years old in southern Africa (Nel et al., 2003). The increase of cases involving the mongoose and genet in 1916 led to extensive research eventually confirming the association between mongoose and rabies in humans in 1928 (Zyl et al., 2010). Following this development the yellow mongoose (Cynictis penicillata) was identified as the main reservoir and transmitter of mongoose rabies in RSA and mongoose species constitute 70% of all diagnosed cases of rabies in wildlife in RSA (Sabeta et al., 2007). In Zimbabwe the presence of mongoose RABV was confirmed in a slender mongoose (Galarella sanguinea) (Zyl et al., 2010) and in Botswana the mongoose biotype has been shown to be prevalent in the southern part of the country bordering RSA (Johnson et al., 2004).

Yellow mongooses often cohabit burrows with suricates/meerkats and ground squirrels (Xerus inaurus) (Nel et al., 2003). Mongoose RABV seems to be species specific because the rare events of viral spill-over into other canids has invariably
resulted in dead-end infections with little chance of intra-species transmission (Zyl et al., 2010). Consequently the mongoose RABV is specific to mongooses and establishment in other species has not been observed.

2.4.2.3 Canid rabies biotype

2.4.2.3.1 Introduction

At the time of the southwards incursion of rabies from Angola through Botswana into RSA, new cases of rabies were detected in other canid species including black-backed jackal (C. mesomelas) and bat-eared foxes (O. megalotis). This may have resulted from their relatively close relationship with dogs and their susceptibility to rabies (Sabeta et al., 2007).

2.4.2.3.2 Bat-eared foxes (O. megalotis)

Within a span of 10 years, the spread of rabies in O. megalotis had engulfed the western parts of RSA and Namibia, confirming the endemicity of the disease in this species (Sabeta et al., 2007). Given the stability and widespread distribution of rabies in this species it is possible that it is the principal host reservoir for rabies in this area.

In southern Africa the ancestor of all canid rabies appears to be a cosmopolitan dog virus which is endemic in the region. This was followed by virus in jackals which was confirmed in 1947 in RSA and then in bat-eared foxes in 1955. Furthermore between east and southern Africa the natural distributions of C. mesomelas do not overlap, and
this is also true for *O. megalotis*. This means that the introduction of rabies into these species was unlikely to be a result of intra-species spread from another region of Africa (Sabeta *et al.*, 2007). Some of the canid lineages adapted very well in the new species, and became very stable. They then drifted away from the main canid RABV lineage and established a new independent rabies cycle, as was demonstrated in *O. megalotis* (Sabeta *et al.*, 2007).

Because *O. megalotis* has intense socialization behaviours, oral to oral contact, sharing of dens by large groups, over-lapping of dens between groups and high population densities (1/km²) in southern Africa, there is usually a high concentration of RABV around dens which allows for significant opportunities for intra-species transmissions (Sabeta *et al.*, 2007). However transmission of rabies from *O. megalotis* to other canids is not common and only has been reported in the summer months when jackals approach closely to the dens (Sabeta *et al.*, 2007). However, other wild carnivores, such as *Felis lybica* (African wildcat), *Atilax paludinosus* (marsh mongoose) and meerkats could be involved in transmitting rabies to and from *O. megalotis* via inter-species interactions (Sabeta *et al.*, 2007).

### 2.4.2.3.3 The Black-backed Jackal (*C. mesomelas*)

The black-backed jackal is another species in southern Africa found to be harbouring and transmitting RABV. In the Limpopo province of RSA most cases of rabies have been linked with *C. mesomelas*, and continuous spill-over infections to other species, rabies remains uncontrolled, no matter how successful and effective vaccination campaigns
in domesticated dogs are (Zulu et al., 2009). Black-backed jackals reach population densities sufficient to sustain a rabies cycle on their own and consequently are a serious threat to livestock and a significant challenge for farmers (Zulu et al., 2009). A study aimed at exploring the relationship of rabies viruses from domestic dogs and C. mesomelas confirmed that more than 70% of virus isolates in this lineage originated from C. mesomelas. These findings confirmed that this viral cluster has been well established in the C. mesomelas population and the important role played by jackals in the transmission of the virus (Zulu et al., 2009). In the same study another important finding was the virus segregating into a distinct C. mesomelas rabies cluster. Similar findings were made in Zimbabwe for the side-striped jackal (Canis adustus) as well as in the black-backed jackal. Furthermore, reports in Botswana suggest that the significant decrease in the number of cases of rabies in domestic dogs appears to be compensated for by an increase in the number of cases in jackals (Tremlett, 1993) which strongly implies that there is a specific mechanistic/causal link between the two cycles. Even though there are findings supporting the argument that C. mesomelas is capable of maintaining continuous rabies infection cycles independent of domestic dogs, there is still controversy over whether jackals could reach a significant population density to maintain a rabies cycle in the absence of dogs (Sabeta et al., 2007). Black-backed jackals are not only found in the wild, but are also present on commercial and communal farms where they prey on livestock (Zulu et al., 2009). This animal has co-existed with dogs for a long time and this association has allowed for the transmission of RABV between the two species creating a potential public health threat (Zulu et al., 2009).
Population control of black-backed jackals through the use of toxic oral baiting has been attempted, but this is only a temporary measure for the control of rabies in wildlife. Jackals continue to dominate as a major source of infection for domestic dogs and livestock in southern Africa (Zulu et al., 2009). Black-backed jackals would appear to be an ideal reservoir host for rabies and a transmitter of infection to other species. It is a very territorial and aggressive predator with a high intrinsic population growth (Zulu et al., 2009). During feeding on large carcases and sharing of water sources, there is increased opportunity for contact between individual jackals and the opportunity for intra-species transmission during aggressive interactions. This is a large challenge in southern Africa that calls for a comprehensive and effective wildlife rabies control strategy, along with an extensive dog vaccination campaign with good vaccination coverage.

2.4.3 Rabies in the Americas

2.4.3.1 Introduction

In North America, rabies in domesticated animals has been controlled. However, RABV is sustained within both the terrestrial wildlife and bat populations in North and South America (Banyard et al., 2011). As a result there is a very high possibility of spill-over into humans and domestic animals. Making it even more serious for the Latin Americans is the occurrence of RABV transmitted by hematophagous bats, in particular vampire bats, as well as non-hematophagous bats. Rabies infection acquired from these bats has a significant negative impact on the beef industry, as well as human health. Importantly in South America there are no terrestrial wildlife acting as reservoirs for rabies. Therefore the transmission of RABV in the Americas (North and
South) is through RABV positive hematophagous and non-hematophagous bats, and terrestrial domestic and wildlife reservoirs. No lyssaviruses, other than RABV, have been isolated from bats in the Americas (Banyard et al., 2011).

2.4.3.2 Vampire bat-associated rabies

In central and South America three species of blood-consuming bats have been identified. These are the common vampire bat (*Desmodus rotundus*), the white-winged vampire bat (*Diaemus youngi*), and the hairy-legged vampire bat (*Diphylla ecaudata*) (Banyard et al., 2011). It is believed that population growth of bats occurred over three centuries as a result of the arrival and subsequent multiplication of cattle, goats, sheep and horses. These acted as readily-available food sources for bats close to their habitats. Cattle seem to be the prey of choice by both the common vampire bat and the hairy-legged bat, while the white-winged vampire bat favours avian species (Banyard et al., 2011). Global warming, occupation of new areas by livestock and deforestation are key elements resulting in the dispersal and increase in the vampire populations further into states of USA including Texas, Florida and Arizona (Banyard et al., 2011). The bat species are restricted in terms of distribution to specific areas in South America. Sharing of roosts in caves in Trinidad by different species of bats has been documented (Banyard et al., 2011). The hematophagous nature of these bats provides a unique mechanism of transmission of RABV. Similar to rumination in ruminants, vampire bats, during times of rest, are able to regurgitate their swallowed blood meal from their stomachs for sharing with their roosting mates. This, combined with grooming, biting, licking and inhalation of aerosolised virus, is a mechanism for
spreading the virus within a species and leads to potential viral transmission spill-over to other species (Banyard et al., 2011).

2.4.3.3 Insectivorous bat-associated rabies

Rabies virus in insectivorous and frugivorous bats was first confirmed in South America in the early 1900s and later in North America in the mid-1900s (Banyard et al., 2011). A study in Brazil revealed that the transmission of rabies in bats historically involved three bat families (Phyllostomidae, Vespertilionidae and Molossidae) involving 21 genera and 41 species (Banyard et al., 2011). Approximately 16% of wildlife rabies across USA was associated with rabies cases from these bats between 1993 and 2002 (Banyard et al., 2011). Extensive studies in the 1960’s and more recently revealed that aerosol transmission of RABV in coyotes and foxes living in caves where infected bats roost was a common phenomenon (Banyard et al., 2011).

2.4.3.4 Rabies reservoirs in terrestrial animals

The chief reservoirs and transmitters of RABV from domestic animals to humans are cats and dogs, whereas foxes, raccoons and skunks constitute the principal spill-over hosts for both humans and livestock (Banyard et al., 2011). However, the wide distribution and low density of these wildlife reservoirs means that a focused approach must be made for their management and control. Raccoons (Procyon lotor) are present across much of eastern USA; the grey fox (Urocyon cinereoargenteus) is widespread in Arizona and Texas; and both red (V. vulpes) and arctic (Alopex lagopus) foxes are present in Alaska and parts of Canada (Banyard et al., 2011). Oral vaccination
programmes have, however, successfully reduced the number of cases of rabies in foxes in some regions (Banyard et al., 2011). Terrestrial rabies associated with both raccoons and skunks, particularly the striped skunk (Mephitis mephitis) and the eastern spotted skunk (Spilogale putorius), is common in the northern central and southern central regions of the USA. In 2009, 3,930 cases, representing 64% of all cases, were reported from wild terrestrial species in the USA (Banyard et al., 2011).

2.5 Rabies control measures - vaccination and immunology

Even though recent studies suggest that transmission of rabies may not be reservoir density-dependent (Morters et al., 2013); the number of reservoir host species in a country plays a major role in the spread of the disease. Countries with more diverse reservoir host species are less likely to eliminate rabies, in contrast to those with only one host reservoir species. No matter what the situation is, the transmission of rabies can be effectively prevented through mass immunisation of both domestic and wildlife animals. Another control method, as was implemented in the 1990’s in Botswana, is increased awareness and knowledge about rabies through meetings of veterinary staff and the general public including school students. Radio broadcasts of control programmes on rabies and announcements on vaccination programmes also increased awareness by the general population and led to increased adoption of vaccinations and reporting of suspect cases (Masupu, 1992).

Vaccination campaigns of dogs and cats, restriction of animal movements, removal of stray or unwanted animals and programmes to control turnover of the dog population are effective in contributing to the control and eradication of rabies in developed
countries (Smith, 1996). Compulsory free annual vaccinations of dogs, destruction of stray dogs, restriction of animal movement and population control in jackals through baiting have been methods of choice for controlling rabies in Botswana. These efforts resulted in a total of 190,382 dogs and cats vaccinated against rabies in 1998 (Masupu, 1999, Tremlett, 1993). There are many different types of vaccines inducing between 1 and 3 years of immunity. Most vaccines are based on inactivated rabies virus to minimise the risk of animals developing rabies from the vaccine. Vaccinated dogs which are bitten by wild carnivores or other animals suspected to have rabies should be revaccinated and be kept under observation for 45 days. In contrast unvaccinated animals should either be euthanised or placed under observation for 6 months and if no clinical signs develop should then be vaccinated at least 1 month prior to release (Smith, 1996).

Population control and reduction of wildlife reservoir hosts has not resulted in the successful elimination of rabies from the sylvatic cycle. However, immunization through oral rabies vaccination (ORV) has worked in some areas of Western Europe resulting in the elimination of rabies from red foxes (Geue et al., 2008). Elimination has been successful where specific areas have been targeted and where only a single animal reservoir of low population density was involved. The great difficulty when dealing with the sylvatic cycles of rabies is that new outbreaks of disease in a different animal population may go unnoticed and become endemic (CFSPH, 2009).

The vaccines used for ORV in wildlife are manufactured from high-titre modified live virus and are used either as attenuated rabies virus or in a recombinant form (Geue et
Attenuated rabies virus used in ORV for wildlife all originate from one common ancestor, the Street Alabama Dufferin (SAD) field strain. In contrast the recombinant form, such as the vaccinia rabies glycoprotein (VRG), is a live virus (strain Copenhagen) with SAD derived glycoprotein of the Evelyn Rokitniki Abelseth (ERA) strain (Geue et al., 2008). In the early 1970’s the SAD strain was imported from the USA to Switzerland for research into making ORV. The first new strain was developed after successful cell culture in a BHK-21 cell line and the first SAD-derived ORV was named SAD Bern and initially used in foxes in 1978 (Geue et al., 2008). In the 1980’s the SAD Bern strain was distributed throughout Europe for more vaccine development and gave rise to more SAD vaccine variants subsequently used for ORV of wildlife in Europe (Geue et al., 2008).

Vaccines used initially in humans were derived from neural tissue and had serious side effects, including the potential to result in encephalitis. However, modern rabies vaccines recommended by the WHO to prevent rabies in humans are derived from human diploid cell cultures (human diploid cell vaccines - HDCV) (Brookes et al., 2005). These vaccines are much safer than the previous generation vaccines with almost 100% protection against RABV. Vaccine failure, however, can result from delayed treatment, numerous severe bites close to the head region and the infecting strain of RABV being different to that of the vaccine strain (Brookes et al., 2005). Protection against rabies through vaccination arises through production of rabies viral specific neutralising antibodies (VNA) to the rabies glycoprotein. However previous studies have shown that cross protection has occurred against phylogroup I but not group II lyssavirus genotypes by RABV based vaccines (Brookes et al., 2005). Human rabies
immunoglobulin (HRIG) is an anti-rabies antibody administered as part of PEP. This immunoglobulin has been shown to provide cross-neutralisation coverage for a wide variety of RABV isolates, EBLV-2, ABLV and DUVV, although it has reduced or no activity against EBLV-1, LBV and MOKV (Brookes et al., 2005).

A standard method to evaluate vaccine efficacy is by measuring VNA using the fluorescent antibody virus neutralisation test (FAVN). This test measures both IgM and IgG and a satisfactory threshold level of protection has been set at a serum titre of 0.5 IU/ml (Kennedy et al., 2007).

There are variations in the response to vaccination in different animals with some failing to develop immunity and others developing rare allergic reaction. The vaccine type, sampling interval, size, age and origin of animals all can affect the rate of vaccine failure (Kennedy et al., 2007). The immune response is influenced by physical and genetic factors of the vaccinated animal. Each vaccine has different failure rates due to differences in the formulation, production, concentration and integrity of the antigen content and the adjuvant used in the vaccine (Kennedy et al., 2007). Larger dogs are reported to have a higher vaccine failure rate than small dogs, possibly because of deeper subcutaneous fat at injection sites, although this needs further scientific investigation (Kennedy et al., 2007).

A reduction in immune regulation is believed to occur with age and this may explain why older dogs have a poorer response to vaccination than younger dogs (Kennedy et
The variation in antibody response following vaccination relates to the response kinetics of primary vaccination (Kennedy et al., 2007). The type of antibodies stimulated in dogs change from an IgM dominated response to an IgG dominated response with time and the measurement of antibody titre should consider this change and involve sampling at appropriate times (Kennedy et al., 2007).

Oral rabies vaccines, such as SAD B-19, have also been used in dogs since the early 1990’s, eliciting detectable antibodies in 81 to 92% of vaccinated dogs (Zhang et al., 2008). These vaccines can be used where conventional vaccines are difficult to use or too costly. However the production of such vaccines needs to be cheaper than alternatives and should stimulate a long lasting immunity.

A very low annual vaccination coverage (5 to 50%) in the canine population has been reported in some developing countries, making it practically impossible to disrupt the rabies transmission cycle (Hu et al., 2007). Furthermore the vaccines used in developing countries are often sub-standard further hindering disease control (Hu et al., 2007). Botswana has had challenges in the control of rabies in dogs and cats because of uncertainties over the population size and consequently the proportion of animals vaccinated (Figure 2.5). This could also arise from changes in the population dynamic of dogs in Botswana or problems arising during vaccination campaigns (e.g. some dogs were vaccinated twice). In the early 1990’s the dog and cat population was estimated at 350,000 (Tremlett, 1993), however in 2010 the annual census reported by the DVS was only 114,469 dogs and 19,039 cats (DVS, 2010).
Based on the reviewed national and international research, the current study was undertaken to investigate the existing situation of rabies in the Republic of Botswana. This investigation was designed to help understand the trends and associations of rabies in ruminants with that in dogs and jackals, to identify risk factors for infection, and to determine the knowledge, attitudes and practices of citizens of Botswana which may be associated with increased rabies cases in ruminants. The first objective of the research was to study the temporal and spatial distribution patterns of rabies in ruminants in Botswana and this forms the basis of the following chapter.
Chapter 3: Temporal and spatial distribution of rabies in ruminants in Botswana

3.1 Introduction

Rabies is one of the most widely distributed diseases in southern Africa with a high number of outbreaks reported (PRINT, 2008). The disease can result in the death of a significant number of dogs but can also affect a sizable number of cattle, goats, sheep, wildlife and cats. The zoonotic aspect of rabies in southern Africa is also of concern with a total of 88 human cases recorded in Angola, Lesotho, Zimbabwe, Zambia and Swaziland in 2008 (PRINT, 2008). Successful vaccination programmes require adequate planning and a clear understanding of the spatial and temporal distribution of rabies. This information can be used to guide the distribution of rabies vaccines, stockpiling of human vaccine and mobilization of scarce resources which is particularly relevant in Africa (Dzikwi and Abbas, 2012).

The distribution of rabies has previously been studied in Botswana (Tremlett, 1993, Maganu and Staugard, 1985, Mushi and Diteko, 1992, Masupu, 1992); however, these studies have focused on domestic canine rabies with minimal investigations into the correlations between cases in domestic canids, wild canids and domestic ruminants. In many other studies conducted in Africa investigations have focused on the domestic dog as it has been perceived to be the main reservoir of infection (Dzikwi and Abbas, 2012). In contrast rabies in wild animals, such as vampire bats, foxes, skunks, raccoons and coyotes, have been correlated with disease in other domestic species including
ruminants in the USA, Europe and some South American countries (Mushi and Diteko, 1992).

The aim of the study outlined in this chapter was to determine the temporal and spatial distribution of rabies in domestic ruminants in Botswana from 2000 to 2010. In addition, identification of the district with the highest number of cases of ruminant rabies was important for other aspects of the research undertaken.

3.2 Materials and Methods

The Botswana National Veterinary Laboratory (BNVL) is the only laboratory responsible for the diagnosis of rabies in the country and primarily uses the FAT for the disease’s diagnosis (Masupu, 1992). Because rabies surveillance in Botswana is passive, the capacity of the BNVL has never been thoroughly examined to confirm if data are representative of the true rabies situation. After collection brain samples are put in glass jars filled with glycerol and placed in lockable red wooden jar containers and transported to the laboratory via road and railway. The FAT is then used on the samples. This test is accurate (90 to 100% sensitive and 99.6% specific), cheap and results are obtained within two hours (OIE, 2007). Samples that are negative on the FAT are further subjected to the MIT in Botswana (Mushi and Diteko, 1992). Specimens of brain, preserved in 50% glycerol saline are sent from district veterinary or medical health offices. Impression smears of the medulla and hippocampus are dried and fixed in cold acetone at -20°C and stained with fluorescein conjugated rabies antiserum (Mushi and Diteko, 1992). FAT negative samples are further prepared and a suspension of 0.03ml is injected intracranially into anaesthetised weaned mice. The mice are then
monitored for one month and any dead mice tested for rabies by the FAT. The results are then reported to the head of the relevant veterinary district via telephone or radio and followed up with a written report. The results are then entered into a computerized disease data base.

In the current study disease data were collected from the Veterinary Epidemiology and Economic Section (VEES) of the BNVL for the period 2000 to 2010. Data included: diagnosis of rabies; district or sub-district involved; extension area of the case; the name of the crush pen of occurrence; name of the owner of the animal; the date reported; species; sex; breed; age; number at risk; number dead; number affected; notes written by the diagnosing veterinarian; the serial number of the disease report form (DRF); and the case history. There was no data on the variants (biotypes) infecting ruminants. The data derived from the data base were converted into an excel spread sheet and saved.

The data were cleaned by first evaluating the diagnostic section to ensure that the diagnosis was recorded as either positive or negative for rabies. The diagnoses were cross-checked against corresponding DRF’s and any discrepancies corrected. The discrepancies observed included no diagnoses recorded and diagnoses not corresponding with the notes written by the veterinarian. There were some rabies cases captured in the system which carried no diagnosis because no samples had been submitted to the laboratory. For example cases of dogs thought to be rabies which were sampled during the 10 day observation period in the field and which were later released to the owner having ruled out rabies as a diagnosis. Such cases were excluded from this study. However, at the end there were still some cases with no diagnosis...
recorded because the cases were negative on the FAT but contained missing information on the results of the MIT. All blank spaces for the dates of disease occurrence, districts, extensions, and crushes were completed as far as possible using the DRF reference material.

The data were then analysed using the filter function in Microsoft Excel to determine the number of cases of rabies in individual ruminant species, domestic canines and jackals during the study period. Further analyses were performed using descriptive statistical tools in Excel. For ruminants the number of cases occurring per month was also determined. A summary of the occurrence of rabies from 2000 to 2010 for the species of interest were tabulated and graphs generated. Summaries of the monthly and seasonal occurrence of ruminant rabies were also generated and charted. Seasonal climatic changes in Botswana were observed based on grouping cases for summer (November to January), autumn (February to April), winter (May to July) and spring (August to October). The distribution of rabies in ruminants was determined in different districts and extension areas and the data mapped to determine the spatial distribution of rabies in ruminants using ArcGIS version 10.2.2 (Esri). In addition the population of ruminants was determined per district from DVS, 2010 livestock census and using the number of cases occurring per district, the district with the highest disease frequency relative to the population was determined.

Pearson’s correlation coefficients and their significance were calculated for the number of cases in different species over time. An ANOVA was performed to investigate the impact of seasons on the number of cases of rabies in ruminants. These analyses were conducted in Statistix ver 10.0 (Analytical Software).
3. 3 Results

3. 3. 1 Rabies temporal distribution among different species

A total of 1277 samples were tested for rabies by the BNVL from 2000 to 2010 (average of 116 samples per year). This compared with an average of 237 per year for the period 1979 to 1999. However, for the current study period the results of 167 cases were not conclusively confirmed because there was no final definitive diagnosis for rabies and 469 cases were negative for rabies. A total of 641 samples were confirmed positive for rabies (57.7%: 95% CI 54.7, 60.6). The mean number of cases per year was 58 cases (minimum 22 and maximum 96). The distribution of cases in different host species (ruminants, dogs and jackals) over this period is summarised in Figure 3.1.

Of the 641 confirmed positives cases, 389 (60.7%: 95% CI 56.8, 64.5) were domestic ruminants (average of 35 cases in ruminants per year). Of the positive ruminant cases 225, 157 and 7 were from cattle, goats and sheep, respectively. Dogs represented 177 cases (27.6%: 95% CI 24.2, 31.2) and cats 18 cases (2.8%: 95% CI 1.7, 4.4). Twenty cases were confirmed in jackals during the study period. The remainder of cases (35) were distributed between other domestic species (donkeys and horse) and wild animals such as lions, hyenas, caracal, leopards, baboons and mongoose. Approximately one quarter of the samples (27%; 95% CI 23.6, 30.6) were confirmed on the MIT which the authorities admitted was high and that there was a need to improve the FAT. There was a strong positive correlation between the number of cases in ruminants and the number in jackals ($r = 0.78$, $p < 0.005$). In contrast there was no significant correlation
between the number of cases in ruminants and the number in dogs ($r = -0.066$, $p = 0.85$).

Figure 3.1: Comparison of the temporal distribution of the number of cases of rabies in ruminants, domestic dogs and jackals (2000-2010).

**3.3.2 Temporal variations of rabies among domestic ruminants**

In Figure 3.2 the number of cases in cattle, goats and sheep over the study period are displayed. The number of cases of cattle and goats were strongly correlated ($r = 0.96$, $p < 0.0001$) over the study period. In contrast there was no correlation between the number of cases of sheep and cattle ($r = -0.48$, $p = 0.14$) or between the number of cases in sheep and goats ($r = -0.44$, $p = 0.18$).
3.3.3 Seasonal temporal trend variations of rabies in ruminants

There were a total 107, 84, 100 and 97 rabies cases during summer, autumn, winter and spring, respectively (Figure 3.3). This equated with approximately 10, 8, 9 and 9 cases per year, respectively in these seasons. There was no significant difference between the number of cases in the different seasons. (F = 0.04, P = 0.98).
3.3.4 Spatial distribution of ruminant rabies in Botswana in the period 2000-2010

The district with the highest number of cases of rabies in ruminants over the 11 year period was the Central district (209 cases – 54.6%; 95% CI 49.5, 59.7). This was followed by the North East district (107 cases – 27%; 95% CI 23.5, 32.7). The Southern and Gantsi districts had 28 (7.3%; 95% CI 4.9, 10.4) and 15 cases (3.9%; 95% CI 2.2, 6.4), respectively, while all other districts had very low numbers of cases (< 10). The spatial distribution of the ruminant cases is displayed in Figure 3.4. Approximately 89% of the ruminant rabies cases were located in the north-eastern part of Botswana.
3.3.5 District with highest disease frequency relative to the population

The North East district had the highest level of disease (average incidence over the 11 years period) followed by the Central district (Table 3.1). Disease was least frequently detected in Chobe with no cases being reported over the 11 year study period and Kgalagadi with only 1 case in 424,000 ruminants.
Table 3.1: The number of rabies case from 2000 to 2010 in ruminants in different districts.

<table>
<thead>
<tr>
<th>District</th>
<th>Number of ruminants present in DVS 2010 census</th>
<th>Total number of cases</th>
<th>Proportion positive from 2000 to 2010 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>North East</td>
<td>364,173</td>
<td>107</td>
<td>0.029382</td>
</tr>
<tr>
<td>Central</td>
<td>1,109,206</td>
<td>209</td>
<td>0.018842</td>
</tr>
<tr>
<td>South East</td>
<td>38,237</td>
<td>5</td>
<td>0.013076</td>
</tr>
<tr>
<td>Southern</td>
<td>423,026</td>
<td>28</td>
<td>0.006619</td>
</tr>
<tr>
<td>Gantsi</td>
<td>284,280</td>
<td>15</td>
<td>0.005276</td>
</tr>
<tr>
<td>Kgatleng</td>
<td>102,516</td>
<td>5</td>
<td>0.004877</td>
</tr>
<tr>
<td>Ngamiland</td>
<td>435,871</td>
<td>9</td>
<td>0.002065</td>
</tr>
<tr>
<td>Kweneng</td>
<td>341,971</td>
<td>4</td>
<td>0.00117</td>
</tr>
<tr>
<td>Kgalagadi</td>
<td>423,516</td>
<td>1</td>
<td>0.000236</td>
</tr>
<tr>
<td>Chobe</td>
<td>17,561</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NB: These population estimates were available throughout the study period.
3.4 Discussion

The temporal distribution of rabies among different species

The average annual number of cases in ruminants in this study from 2000 to 2010 (35) was dramatically lower than that reported for the period from 1990 to 1999 (when an average of 134 ruminant cases were reported each year). This decline was most likely due to a series of continuous successful extensive dog vaccination campaigns (Tremlett, 1993, Masupu, 1992). Control measures of the dog and jackal populations through shooting and toxic baiting in the 1990’s were very restricted and only yielded temporary results. In addition, in the 1990’s educational campaigns were conducted by veterinary staff to increase the awareness of the community about the disease. The vaccination programmes were also advertised on the radio and these efforts resulted in 190,382 dogs and cats being vaccinated against rabies in 1998 (Masupu, 1999). This is probably the highest vaccination coverage achieved in Botswana since rabies control programmes were established. Stray dogs are a major problem in Botswana especially in towns and cities and rabies cases mostly account for owned dogs. Infrastructure, including access roads to rural villages, had all improved since the late 1990’s allowing greater access to remote villages and a greater vaccine coverage of domestic canids than that achieved previously (Tremlett, 1993). Consequently it is not surprising that at the beginning of the study period investigated in this analysis (2000) the numbers of cases of rabies in dogs and jackals were low, compared with those reported in the 1990’s. These low numbers corresponded with a reduction in the number of cases in ruminants to only 13 in 2004. This sharp decline in ruminant cases could also be linked with the control of FMD implemented in 2002 and 2003. Foot and mouth disease was restricted to the North East district which was also a high risk district for rabies and where 16,061 cattle were destroyed for disease control purposes for FMD (OIE, 2013).
The reduced number of cattle and the presence of surveillance teams containing veterinarians throughout the high risk districts would have ensured improved access to veterinary services which would have resulted in increased adoption of rabies vaccinations as well as restricting animal movements. Concurrently the presence of a drought in 2002/2003 resulted in a significant reduction in the number of ruminants, in particular cattle, from 3 million in 2002 to 1.8 million by the end of 2003 (CSO, 2011).

Although the number of cases of rabies in dogs was declining from 2000 to 2002, the number of cases in jackals increased over this period confirming the report of Tremlett (1993) that a decrease in cases in dogs was offset by an increase in cases in jackals. The subsequent outbreak of rabies in dogs in 2003 occurred two years after the first cases were reported in jackals, again supporting the observation by Tremlett (1993) that it takes two years for cases in jackals to spill-over to domestic species. The outbreak was then perpetuated in dogs. It has been suggested that the chief reservoirs and transmitters of RABV are cats and dogs. The subsequent reduction in the number of cases in jackals may indicate that jackals are not able to maintain the infection for a long time. It is possible that the presence of the veterinary teams controlling FMD at that time affected interaction between jackals, dogs and ruminants reducing virus spill-over and spread between the two canid species resulting in a decrease in the number of cases in ruminants.

The number of cases of rabies in ruminants increased after the increase in cases in dogs which was preceded by the increase in the number of cases in jackals. Towards the end of the study period (2006 to 2010), the numbers of rabies cases in all species were stabilizing at endemic levels similar to those of the 1970’s with few cases in
jackals, as opposed to dogs and ruminants which were averaging 25 and 30 cases per year, respectively. The increase in the number of cases in ruminants and dogs may also be a result of complacency by dog owners and veterinary staff in control efforts against rabies.

Although there is controversy surrounding the maintenance of rabies in jackals, both jackals and dogs are capable of transmitting rabies to ruminants and the close and continual interaction between the two species results in a multiplier effects on the transmission of rabies to cattle. There are four plausible mechanisms through which transmission to ruminants could occur:

1) Dogs may maintain a rabies cycle alone and allow subsequent transmission to ruminants.

2) Occasional rabies transmission from jackals to dogs results in an outbreak in dogs with subsequent transmission to ruminants.

3) Persistent and sustained infection in jackals leads to an outbreak in dogs resulting in a synergistic and multiplier effect fostering transmission patterns to ruminants by the two species.

4) Jackals may temporarily maintain rabies cycles and transmit infections directly to ruminants (Zulu et al., 2009).

More data and studies are needed to explore the mechanism of transmission between dogs and jackals, and between jackals or dogs and ruminants.

In this study period only two humans died as a result of rabies compared to 25 in the period from 1972 to 1989 and four in the period 1990 to 1999. The two human cases in the current study period were from the highest risk area for rabies in ruminants
(Francistown) during peak periods of the disease in ruminants. This raises the question “Are rabies cases in ruminants an indicator of the risk of infection in humans?” In the period of 1972 to 1999, 82.8% (n=29) of the rabies cases in humans were all from the northern districts of Botswana and a similar scenario was confirmed for the period 1978 to 1991 (Tremlett, 1993). However in the current study there was no clear correlation of increased rabies infections in humans and increased infections in ruminants, dogs or jackals, primarily due to the low number of cases in humans.

Variation in the temporal distribution of rabies between different ruminant species. Historically in Botswana more cattle have been affected with rabies than goats or sheep (Tremlett, 1993). In this study the number of cases in cattle and goats were strongly correlated ($r = 0.96$) however this was not the case for sheep and cattle or sheep and goats. This could be associated with differences in the numbers of these three species (cattle 2,634,683; goats 1,937,931; sheep 279,237 (CSO, 2011)) or due to other factors. Domesticated ruminants in Botswana are particularly vulnerable to rabies because they are exposed to transmission from multiple wildlife reservoir species, such as the black backed jackals and mongoose, as well as from domestic dogs and cats. With their large size, cattle are likely to be quickly spotted by predators and with their inquisitive behaviour and inability to run fast, the chance of being caught in attacks are higher than for small stock. However such attacks by jackals or any of these small carnivores, including dogs, are less likely to kill the cattle immediately, although the animal may die subsequently from a septic wound infection or from rabies. The large number of cattle in Botswana also increases the opportunity for attacks by carnivores in this species.
Aided by their panoramic vision and keeping a constant watch while on pastures, domesticated sheep are able to respond together, avoiding any separation and thereby evading or minimising predatory attacks (Jennens, 2002). In many instances predatory attacks on livestock are a result of locally owned or stray dogs. Stray dogs are known for their ability to scavenge and their high fecundity and consequently there are numerous interactions with both wild animals and domestic ruminants when the dogs are searching for food. This interaction would increase the risk of rabies for the ruminants (Kariuki and Ngulo, 1985).

**Seasonal effects on the number of cases of rabies in ruminants**

There was no obvious seasonal effect on the number of cases of rabies in ruminants. Slightly more cattle were affected in autumn (February to April) while fewer goats were affected during this period and more were affected in the summer (November to January). Autumn and summer are potentially important seasons for the epidemiology of rabies in ruminants because of the animals’ increased roaming and uncontrolled movement at this time when crops have already been harvested. In summer there is usually sufficient water and grazing pasture to allow livestock to graze and drink freely without the need to return to kraals. This results in increased potential for interactions with predators. In contrast in autumn water and grazing opportunities are lessened meaning that livestock must travel long distances for pasture and water and consequently animals spend significant periods away from their kraals. However drought can have a negative impact on the number of cases of rabies due to a reduced population size. Others have reported an increase in the number of rabies between
May and September, and proposed this may be due to the movement and breeding of jackals which coincides with the long distance travel of the ruminants (WHO, 2013a).

**Spatial distribution of rabies**

In this study the majority of cases in ruminants were reported in the north of Botswana, north of the Tropic of Capricorn. This supports previous studies which concluded that cases were apparent in the Central, North East and Ngamiland districts in the northern parts of Botswana, with severe outbreaks in the Francistown area or North East district (DAHP, 1973, Tremlett, 1993). The disease was also distributed on the eastern part of the nation rather than in the drier western part. This coincides with the distribution of domestic ruminants which are concentrated in the eastern region of the country (DWNP, 2012). Others have reported that the majority of cases have been located in the eastern part of the country commonly affecting domestic ruminants, dogs and jackals (WHO, 2013a). Cases of rabies in ruminants were clustered close to towns and the major villages, highlighting the likely contacts between ruminants and domestic dogs. The clustering of ruminant cases close to urbanised areas is not unexpected as in these areas the population of dogs would be expected to be higher (WHO, 2013a).

Previous studies have confirmed the existence of two major rabies biotypes: the wildlife-associated biotype (mongoose subtype); and the canine-associated biotype. These biotypes dominate the southern and northern parts of the country, respectively (Johnson *et al.*, 2004). However a 2010 dog census indicated that the dog population
was distributed in almost a 50:50 ratio between the northern and southern parts of Botswana (DVS, 2010).

**The highest risk district for ruminant rabies**

According to the analysis for the 11 year study period, the North East District had the highest risk for ruminant rabies (0.029382%) in a ruminant population of 364,173. This district has historically had a high number of rabies out-breaks in both dogs and humans (Tremlett, 1993).

One purpose of the research undertaken was to determine the risk factors which may be associated with a high risk for the disease. Understanding the reasons and risk factors for high levels of rabies are important and results of an investigation to elucidate these are reported in the following chapter.
Chapter 4: Risk factor analysis of rabies in domestic ruminants

4.1 Introduction

As demonstrated in the previous chapter, the burden of rabies in ruminants mostly occurred in the northern districts of Botswana with 340 cases reported over the 11 year period from 2000 to 2010 (88.7%; 95% CI 85.2, 91.8 of all ruminant cases). Of these cases 107 (31.5%; 95% CI 26.6, 36.7%) occurred in the North East district which represented the district with the highest risk for ruminant rabies in the country over this time period (0.029383% per 11 years). Historically from the early 1970’s rabies has constantly featured in Francistown area and the North East, Central and Ngamiland districts (Tremlett, 1993). However currently few cases are experienced in Ngamiland district, possibly because of the existing combined efforts of government and private organizations to implement intensive rabies control methods, including dog and cat population control.

The persistence of rabies in ruminants in the North East district for over 40 years (Tremlett, 1993) may be indicative of the presence of risk factors for disease and be associated with maintenance and spill-over of infection from other species. Identification of these risk factors might assist in the efficient application of control measures for rabies in ruminants and other species, and this was the basis for the study reported in this chapter.

4.2: Defining the area and the risk factors

The North East district referred to in this study refers to the veterinary disease control district before 2011 which, at that time, included Francistown as well as Nata.
Currently the North East district excludes the Nata sub-districts. The district is approximately 42,000 km², and is located in the north east of Botswana bordered to the east by Zimbabwe, to the north by Chobe district, to the west by Ngamiland district and to the south by Central district (Figure 4.1).

The district has two towns, namely Francistown in the east which is a very old mining town established in 1897, and a new mining town called Sowa in the south west which was founded in the late 1980’s. In the eastern parts of the district the Francistown area is surrounded by a series of congested clusters of sub-districts, villages and small settlements. Approximately 60 villages and settlement areas around Francistown are interspersed within an area of approximately 12,000 km² making the peri-urban areas around Francistown possibly the most expanded and congested in Botswana. Furthermore, in the same area there are approximately 100 freehold farms.

There are three major rivers with the Shashe and Tati rivers traversing through the town and with their tributaries these rivers form several lakes and dams. At the border between Botswana and Zimbabwe there is the Ramokgwebana River, which also is a water source for most of the villages along the border. These rivers are a source of water for most farms where mixed farming is practiced (crop farming and rearing of livestock). In contrast, the western part of the district is made up of the Nata area, which is approximately 30,000 km² in size. There are approximately 12 villages and 45 commercial farms in this area with little surface water present. In the Nata area farmers generally have areas of field crops that are isolated from the cattle posts. In
most cases farmers rely on underground water (boreholes) for watering their livestock. In terms of human population the eastern part of the district is heavily populated and similarly a large number of livestock are present in the already congested towns and villages in this area (Table 4.1). The numbers of dogs vaccinated against rabies in the district from 2005 to 2010 are compared in Table 4.2. The vaccination coverage in general for the North East district has been variable, with Francistown having the lowest coverage.

Figure 4.1: Map of Botswana showing the North East district (NED) veterinary boundaries.
Table 4.1: Livestock (including dogs and cats) census in the North East district (DVS, 2010)

<table>
<thead>
<tr>
<th>Region</th>
<th>Cattle</th>
<th>Goats</th>
<th>Sheep</th>
<th>Dogs</th>
<th>Cats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nata</td>
<td>57,016</td>
<td>13,184</td>
<td>1,582</td>
<td>1,219</td>
<td>140</td>
</tr>
<tr>
<td>Francistown</td>
<td>255,910</td>
<td>68,612</td>
<td>13,795</td>
<td>13,655</td>
<td>3,523</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>312,926</td>
<td>81,796</td>
<td>15,377</td>
<td>14,874</td>
<td>3,663</td>
</tr>
</tbody>
</table>

Table 4.2: Number of dogs vaccinated against rabies in the North East district (Segale, 2010)

<table>
<thead>
<tr>
<th>Area</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nata</td>
<td>2,266</td>
<td>2,004</td>
<td>1,267</td>
<td>1,018</td>
<td>3,703</td>
<td>10,474</td>
</tr>
<tr>
<td>Francistown</td>
<td>23,264</td>
<td>3,031</td>
<td>9,930</td>
<td>23,314</td>
<td>20,914</td>
<td>8,963</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25,530</td>
<td>5,035</td>
<td>11,197</td>
<td>24,332</td>
<td>24,617</td>
<td>19,437</td>
</tr>
</tbody>
</table>

NB: Census records were sometimes poorly done such that the actual vaccination turn-up was significantly higher than the numbers reported in the 2010 census in Nata.

**Waste management and disposal**

Waste management and its disposal is a challenging task for the district, as it is in other areas of Botswana and countries in Africa. This results in the attraction of animals, including dogs and wild carnivores, to such areas in the search for food (Hughes and Macdonald, 2013). Unmanaged waste encourages stray dogs and makes livestock unmanageable, especially in over-populated areas and where pastures are insufficient.
(Kariuki and Ngulo, 1985). This problem was observed in the North East district during this study (Figures 4.2 – 4.4).

Figure 4.2: Refuse dumping site for Sowa town in the North East district with evidence of cattle frequenting this place.

Figure 4.3: Unfenced settling ponds for effluent adjacent to the refuse dumping site in Sowa.
This study was designed to evaluate intrinsic and extrinsic risk factors for the transmission of rabies in ruminants. The intrinsic factors included gender and age while extrinsic factors included farm production type (commercial or communal) and location of the farm (town/village or cattle post). Equally important was determining the density of ruminant rabies in the North East district through mapping.

4.3: Methodology

A retrospective study was conducted using data of rabies cases from the North East from 2000 to 2010. The target population was all cattle, sheep and goats in the district. The study population was all cattle, sheep and goats whose samples were submitted at BNVL for rabies testing.
Data were acquired from the BNVL and handled and processed as outlined in Chapter 3. From the main data prepared in Chapter 3, data for the North East district relating to cattle, sheep and goats were extracted to form the main focus for the analyses reported in this chapter. The distribution of rabies in ruminants was determined in different extension areas and the data were mapped to determine the density of rabies cases in ruminants in the North East district.

To analyse the intrinsic risk factors the data required further preparation. Some of the age groupings in the data base were coded using the Southern African Development Community (SADC) categories which are different to OIE age categories/groupings. The SADC codes were: code 28 indicating adult animals; code 30 indicating various ages with more than one animal involved; and code 27 referring to young animals. These age codes were transformed into the more universally adopted international age coding of the OIE where, for cattle, age groups were: 0-12 months = calves; 13-24 months = heifers; 25-36 months = sub-adults; and > 36 months = adults. For small stock: 0-6 months were classified as lambs/kids; 7-12 months as before puberty; 13-18 months as sub-adults; and ≥ 18 months as adults. Where age and sex was not indicated, history notes were used to derive the age grouping and gender of the relevant animal.

Extrinsic factors, such as farm location and the type of production, were also evaluated for any association with the presence of the disease in the North East district. The crush pens where the cases originated from were matched with the crush location (cattle post, town or village). This was done in consultation with extension area officers.
and district area experts in the district. The production system was categorised as either commercial or communal.

The analyses of risk factors were conducted using standard contingency tables (e.g. 2 x 2 tables) for observational studies. Odds ratios and their 95% confidence intervals were calculated to identify potential risk factors for disease.

4.4: Results

4.4.1 Density of the disease in the North East district

The analysis revealed that the Francistown area had the majority of the rabies cases in ruminants with 83 cases (77.6% 95% CI 68.5, 85.1) compared with 24 cases (22.4% 95% CI 14.9, 31.5%) in the Nata area. The case densities increased in villages closer to Francistown city (Figure 4.5).
Figure 4.5: Rabies density map showing concentration of cases of rabies in all domestic ruminants in the North East district of Botswana.

### 4.4.2 Analysis of intrinsic factors in ruminants

Older animals were more likely to be a rabies case than young animals (all OR > 1); however these differences were not significant (all 95% CI for OR included the value 1) (Table 4.3).

Females were at a slightly greater risk of being a case than males (Table 4.4); however this difference was also not significant.
Table 4.3: Influence of age on the risk of rabies in all domestic ruminants

<table>
<thead>
<tr>
<th>Age</th>
<th>Number positive</th>
<th>Number negative</th>
<th>Odd ratios (OR)</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>66</td>
<td>39</td>
<td>1.27</td>
<td>0.49 - 3.28</td>
</tr>
<tr>
<td>Sub-adults</td>
<td>11</td>
<td>8</td>
<td>1.03</td>
<td>0.29 - 3.62</td>
</tr>
<tr>
<td>Juveniles</td>
<td>17</td>
<td>7</td>
<td>1.82</td>
<td>0.53 - 6.25</td>
</tr>
<tr>
<td>Young</td>
<td>12</td>
<td>9</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: Influence of gender on the risk of rabies in ruminants

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number positive</th>
<th>Number negative</th>
<th>Odd ratios (OR)</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>85</td>
<td>46</td>
<td>1.57</td>
<td>0.75 - 3.29</td>
</tr>
<tr>
<td>Males</td>
<td>20</td>
<td>17</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

4.4.3 Extrinsic risk factor analysis

The risk of disease in communal farms (63.7%; 95% CI 55.7, 71.2) was higher than that for commercial farms (53.8%; 95% CI 25.1, 80.8); however this difference was also not significant (OR 1.5; 95% CI 0.48, 4.69).

Ruminants from towns/villages were significantly more likely to be positive (66.9%; 95% CI 58.5, 74.6) than those from cattle posts (42.9%; 95% CI 24.5, 62.8) (OR 2.7; 95% CI 1.2 to 6.2). This equates to an attributable fraction for ruminants in towns/villages of 63%.
4.5: Discussion

In this study there was no significant impact of age on rabies, although the risk was higher in older animals. Young livestock are usually kept and protected in the kraals and never allowed to go out with their parents, and therefore their risk of exposure to rabid carnivores would be expected to be less than that for older animals. Naïve juveniles, when old enough to be released and when they are becoming acclimatized to the grazing environment, are more likely to be victims of predation or attacks than older, more experienced members of the group.

Similarly in this study gender was found not to be a risk factor for rabies to ruminants, although females were slightly more likely to be affected than males. The higher number of female cattle, goats and sheep kept by farmers and the fact that they are kept for a longer period for breeding purpose may be linked to this slight increased risk. Furthermore most males are slaughtered for specific ceremonial purposes or are sold once they reach market weight, decreasing the opportunity to be exposed to animals with rabies. A study conducted in Thailand also did not detect a statistically significant difference in the occurrence of rabies in males and females and in different age groups in cattle (Thiptara et al., 2011). Although similar to the findings of the current study Thiptara et al. (2011) reported that females and older animals were slightly more likely to be affected than males and younger animals less than one year old, respectively.

In this study there were no differences in the risk of disease in ruminants raised on commercial farms and those raised on communal farms. The commercial farms that had reported cases of rabies had damaged fences allowing comingling of ruminants
from these farms with those of local stock at cattle posts, and from local villages and towns. Some of the commercial farms practiced mixed farming, growing field crops as well as raising livestock. It has been reported that jackals have migratory preferences toward agricultural fields where they have opportunities to predate on rodents and other animals (Kaunda, 2001). Some commercial farms had a large number of staff working and residing on them and these staff owned many dogs which could not all be accounted for. The increased risk of the disease in livestock raised in peri-urban communal areas was likely to have arisen from the lower level of supervision these animals receive and the greater opportunity to be exposed to rabid animals whilst grazing. Some studies have demonstrated increased reports of rabies on commercial farms, particularly as these farms often preserve or tolerate wild carnivores and herbivores (Swanepoel et al., 1993). Swanepoel et al. (1993) also suggested that rabies originating from dogs was more likely to occur in overgrazed and deforested communal areas.

Livestock from farms which were located in the peri-urban villages and farmers with livestock in town were significantly more likely (2.7 times) to have rabies diagnosed in their cattle herd. If all cattle were removed from the urban areas and the surrounding villages 63% of the rabies in ruminants in the North East district could be eliminated (Attributable fraction = 63%).

Stray dogs are usually a problem in cities, towns and major villages where they can scavenge on waste (Hughes and Macdonald, 2013). These animals have a high level of fecundity and compete and fight with other dogs, wild animals and ruminants for food,
during which the risk of disease transmissions are high (Kariuki and Ngulo, 1985). Additionally farmers in these peri-urban villages are involved in growing agricultural field crops and rearing livestock in a very congested over-grazed environment, an area where jackals are known to move towards during their peak periods of movement (Kaunda, 2001). At these locations jackals are likely to interact with livestock or with peri-urban dogs, many of which may be unvaccinated stray dogs (Kariuki and Ngulo, 1985).

Furthermore in these peri-urban villages there is surface water from rivers, lakes and dams which is used by the general public, livestock, wildlife and dogs. These locations allow congregation of animals, especially given the free movement of ruminants, and hence there are increased opportunities for interactions between livestock, wildlife and dogs (Gusset et al., 2009). In contrast at the cattle posts there is heavy reliance on water sourced from boreholes due to the scant surface water, and there are also few dogs with stray dogs seldom posing a problem (Hughes and Macdonald, 2013). In addition, the proximity of Francistown to other villages and wilderness areas in Zimbabwe also adds some risk of intermingling of animals, especially between domestic and wild animals.

Even though in this chapter it was shown that the location of the farms is an important factor in the distribution of rabies, with those in urban or peri-urban locations having a higher risk of infection than those in sparsely populated areas, further investigations into the different management, husbandry and cultural practices is required. Furthermore it is important to understand the knowledge of and attitude towards
rabies prevention and control measures adopted by farmers within the North East district. In the following chapter a study designed to address these requirements is outlined and contributory factors to cases of rabies investigated in these communities.
Chapter 5: The knowledge, attitudes and practices of farmers in Botswana relating to rabies

5.1 Introduction

Although approximately 55,000 people die worldwide annually from rabies, it is likely that this number is higher due to underreporting, with up to 100,000 estimated in Africa and Asia alone (Davlin et al., 2014). Even though rabies is preventable, the funding priorities in poor nations often are directed towards other more important disease control programmes in humans and livestock (Davlin et al., 2014).

Botswana has rapidly developed its primary health care and modern health services are freely available to all groups of people, including the provision of a standardized post exposure prophylaxis (PEP) anti-rabies vaccine (human diploid) in all clinics (Maganu and Staugard, 1985). Even though rabies exposure of humans has been defined as a bite by a highly suspected or diagnosed rabid animal, there have been many instances of over-use of PEP in individuals who had handled or eaten meat from rabid animals (Maganu and Staugard, 1985). In 2010 approximately 14,000 doses of PEP were made available in Botswana (WHO, 2013a). Not only is Francistown a high risk area for animal rabies but humans have also been involved, as was the case in 1981 to 1982 when 15 people were exposed to rabies and 7 died as a result of not receiving PEP (Maganu and Staugard, 1985). From 1992 to 1997 there were 5,159 human exposures in Botswana (Masupu, 1999). However in 2012 during the commemoration of the World Rabies Day in Francistown, Dr Retta Ayele from the Francistown Health Management Team (FHMT) said that reports have shown that the
more populated districts, such as Francistown and Kweneng East, had 440 cases of rabies in animals in 2011 and 159 human exposures were reported (Kologwe, 2012). He further indicated that underreporting of cases of rabies hindered efforts to fight this disease (Kologwe, 2012).

The unexplained and consistently high number of cases of rabies in ruminants in Botswana (> 60% of total cases) since the 1970’s has resulted in questions being raised about the role played by jackals in the disease (Mushi and Diteko, 1992, Tremlett, 1993). However wildlife, especially wild carnivores, are perceived to be a threat/pest to livestock through predation and consequently are often killed by farmers to protect their stock (Brassine, 2012).

In Botswana, historically there have been challenges which have negatively impacted on the outcomes of vaccination programmes against rabies and these include vaccine efficacy (use of old vaccines), rural residents who owned packs of dogs for hunting purposes who refused to vaccinate them because of a belief that rabies vaccine would render their dogs powerless, and a lack of access (passable roads) to rural areas (Tremlett, 1993). As discussed in Chapter 3, complacency by dog owners and even veterinary staff in the control of rabies is possible. Understanding the knowledge and practices of farmers could help determine some of the key issues affecting the farming community, and this information could help understand factors limiting effective disease control measures.
Control measures for rabies in humans and animals are highly dependent upon understanding the signs, risks and exposure mechanisms for the disease and the currently adopted prevention and control measures (Tack et al., 2013). Botswana is a very culturally diverse country with different tribal ethnicities likely to bring about different knowledge, attitudes and practices (KAP) by the community concerning rabies. Consequently a survey among farmers was necessary to assess the KAP on rabies in the North East district. Information obtained from this survey can be used to develop educational materials and recommendations for rabies prevention relevant to the local population.

5.2 Materials and Methods

Using data that was outlined in Chapter 4 a study was developed that involved administering a questionnaire survey. As outlined in the previous chapters, the North East district had a total of 107 positive cases from 98 different farmers and these cases involved 60 cattle and 47 small stock (sheep and goats). All 98 farmers were incorporated into the plan for surveying. Using data from the VEES, 768 crush pens were identified in the North East district. These pens included both communal and commercial farmers. Livestock using a specific crush pen are considered an epidemiological unit because they graze and interact in the same area and in most cases drink from the same source(s) of water. Consequently one farmer was randomly selected from each crush pen for interview. Using Epi tools from the Ausvet website (http://epitools.ausvet.com.au/content.php?page=1Proportion, accessed 30/05/2014) the sample size to estimate a proportion of farmers with a specified precision of 95% confidence was 228, indicating that 228 farmers each representing a crush pen would
be a good representative sample for all the 768 crush pens in the entire North East district.

As 98 farmers had a history of rabies in their livestock (Chapters 3 and 4), 130 farmers who had no cases of rabies in their animals were also selected to make a total of 228. These 130 farmers were randomly selected using a multistage sampling method at the subdistrict, extension and crush levels. There are six sub-districts in the North East district and all were included in the sampling frame-work. Only 25 of the 26 extension areas were selected to achieve 95% confidence of a good representative sample frame-work. A list of extension areas were written on pieces of paper excluding those extension areas for commercial farms. Twenty five extension areas were then drawn, resulting in Sebina being excluded from the study. Five or six farmers, who had never experienced positive cases of rabies, were selected for interviewing from each extension area to select a total of 130 farmers. All crush pens for extension areas were written on individual pieces of papers and then five to six slips of paper representing the names of the crush pens were randomly selected. The same procedure was followed to choose one farmer from each crush pen. The commercial ranches in the Tati and Nata areas were each treated as one extension area and both were included in the extension areas to be sampled.

Assessment of the KAP of farmers was conducted through face to face questionnaires. The questionnaire was initially pretrialled on five farmers, although no further adjustment to the questionnaire was required. The questionnaire (Appendix A) was approved by the Human Ethics Committee of Murdoch University (2014/077). Because
of differences in literacy levels of farmers the questionnaire was translated into local language (Setswana) and administered through a face-to-face personal interview situation. Farmers were invited to participate and their consent obtained (Appendix B). If they declined to participate a replacement farmer was selected. Farmers, especially those with previously rabies tested specimens from their farms, were contacted initially via telephone by the extension officers to confirm their availability. The participants would be visited and the questionnaire administered at their farm or an alternative convenient place (Figures 5.1 to 5.5). In some cases the farmers preferred for their farm managers or animal herders to represent them on the basis that they had more knowledge on the routine activities conducted on their farm. On such farms the most senior herder in terms of length of service was selected for interviewing.

Data for the KAP analysis was collated in Microsoft Excel and checked for data entry errors. Data were summarised to assess the differences in KAPs between farmers. Descriptive statistics were used to summarise the KAP data which was reported as a percentage with 95% confidence limits calculated by the exact binomial method. Two by two contingency tables were used to determine odds ratios and their 95% confidence intervals and the chi squared test of independence or Fisher’s exact test was performed to determine statistical significance with a p < 0.05 being categorised as significant.

A multivariable logistic regression was performed to identify factors associated with a history of rabies. Model building was performed by considering variables that were statistically significant (p values < 0.20) in the initial univariable analyses, and including them in the preliminary multivariate logistic regression model. Once the preliminary
main effects model was obtained (fully saturated model) a manual backward stepwise approach was used to remove non-significant variables and only variables with p values < 0.05 were retained in the final model. Overall fit of the final logistic model was assessed with the use of the Hosmer-Lemeshow goodness-of-fit statistic. To determine possible interactions between factors, interaction factors for each variable in the final model were added one at a time to determine the impact of the interaction factor on the model. Findings were presented with odds ratio (OR), 95%CI and p-value.

Figure 5.1: Interview in session at a farmer’s house
Figure 5.2: Farmer being interviewed at her cattle post

Figure 5.3: Interview with a farmer and his children at a livestock drinking point of one of the major rivers.
Figure 5.4: Interviews at a cattle post borehole while waiting for livestock to arrive for drinking.

Figure 5.5: While the interviews were in session with one of the peri-urban farmer the dogs waited in anticipation of a meal.
5.3 Results

5.3.1: Univariable analyses

A total of 207 (of 228) farmers participated in the questionnaire (90.8%; 95% CI 86.3, 94.2). More males (153 - 73.9%) participated in the study than females. Eighty of the 98 farmers with known positive cases participated in this study (81.6%) and the remaining 127 farmers had not experienced any reported cases of rabies (based on BNVL data) in their livestock. The mean ages, educational level, number of people on the farm and the number of livestock species owned were tabulated against variables in the farmer categories (Table 5.1).

Sixty-three of the 80 farmers who had a history of rabies based on the records of the BNVL recalled cases of rabies in their livestock (79%; 95% CI 68.2, 87.1). None of the “control” farmers reported having rabies in their livestock (0%; 95% CI 0.0, 2.9).

The average age of the 207 respondents was 58 years (SD 14.2) with approximately half (51.2%) only having a primary school level of education. The mean number of people per farm was four (SD 3.5). On average 74, 29, 5 and 3 cattle, goats, sheep and dogs, respectively, were owned by the farming household. Sixteen farmers did not own any dogs or cats, conversely 189 (91.3%) owned one or more dogs (two farmers did not respond to this question). On commercial farms more cattle were owned (mean 336 cattle per farm) compared to communal farms (mean 49).
Table 5.1: Summary of respondents and mean values for age, education, number of people per farm, number of dogs and other livestock species in different farmer categories

<table>
<thead>
<tr>
<th>Type of farmer or farm</th>
<th>Total respondents</th>
<th>Mean Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m=207</td>
<td>Age n=207</td>
</tr>
<tr>
<td>Work Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full time</td>
<td>186</td>
<td>58.6</td>
</tr>
<tr>
<td>Part time</td>
<td>21</td>
<td>52.5</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>153</td>
<td>57</td>
</tr>
<tr>
<td>Female</td>
<td>54</td>
<td>58</td>
</tr>
<tr>
<td>Type of farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial</td>
<td>18</td>
<td>49.4</td>
</tr>
<tr>
<td>Communal</td>
<td>189</td>
<td>58.8</td>
</tr>
<tr>
<td>History of rabies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive cases</td>
<td>80</td>
<td>58.2</td>
</tr>
<tr>
<td>Negative cases</td>
<td>127</td>
<td>57.8</td>
</tr>
<tr>
<td>Sampling site</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Town or village</td>
<td>124</td>
<td>58.3</td>
</tr>
<tr>
<td>At Cattle post</td>
<td>83</td>
<td>57.3</td>
</tr>
<tr>
<td>Own dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No dogs owned</td>
<td>16</td>
<td>59.1</td>
</tr>
<tr>
<td>One or more dogs owned</td>
<td>189</td>
<td>58.1</td>
</tr>
</tbody>
</table>

* Not all farmers responded to all questions
Table 5.2: Categorical data, odds ratios and P values comparing farms with previous cases of ruminant rabies and those without cases.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Positive farms (n = 80) n (%)</th>
<th>Negative farms (n = 127) n (%)</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55 (36)</td>
<td>98 (64)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25 (46.3)</td>
<td>29 (53.7)</td>
<td>1.54 (0.82, 2.88)</td>
<td>0.18</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ Primary</td>
<td>45 (32.9)</td>
<td>92 (67.1)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>≥ Secondary</td>
<td>35 (50)</td>
<td>35 (50)</td>
<td>2.04 (1.13, 3.68)</td>
<td>0.02</td>
</tr>
<tr>
<td>Work Status</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-time</td>
<td>67 (36.0)</td>
<td>119 (64.0)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Part-time</td>
<td>13 (61.9)</td>
<td>8 (38.1)</td>
<td>2.89 (1.14, 7.32)</td>
<td>0.02</td>
</tr>
<tr>
<td>Farm Management</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial</td>
<td>6 (33.3)</td>
<td>12 (66.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Communal</td>
<td>74 (39.2)</td>
<td>115 (60.8)</td>
<td>1.29 (0.46, 3.58)</td>
<td>0.63</td>
</tr>
<tr>
<td>Own dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71 (37.6)</td>
<td>118 (62.4)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7 (43.8)</td>
<td>9 (56.2)</td>
<td>1.29 (0.46, 3.62)</td>
<td>0.62</td>
</tr>
<tr>
<td>Sampling site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle post</td>
<td>8 (9.6)</td>
<td>75 (90.4)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Town/village</td>
<td>72 (58.1)</td>
<td>52 (41.9)</td>
<td>12.98 (5.77, 29.23)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stray dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>22 (20.8)</td>
<td>84 (79.2)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (55.7)</td>
<td>43 (44.3)</td>
<td>4.79 (2.59, 8.89)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Herding dogs used</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>49 (32.7)</td>
<td>101 (67.3)</td>
<td>1.0</td>
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</tr>
<tr>
<td>Yes</td>
<td>25 (54.4)</td>
<td>21 (45.6)</td>
<td>2.45 (1.25, 4.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>Wild predators present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>51 (32.9)</td>
<td>104 (67.1)</td>
<td>1.0</td>
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</tr>
<tr>
<td>No</td>
<td>29 (55.8)</td>
<td>23 (44.2)</td>
<td>2.57 (1.35, 4.88)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Report sick animals to DVS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65 (38.7)</td>
<td>103 (61.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13 (36.1)</td>
<td>23 (63.9)</td>
<td>0.9 (0.42, 1.89)</td>
<td>0.77</td>
</tr>
<tr>
<td>Variable</td>
<td>Positive farms (n = 80) n (%)</td>
<td>Negative farms (n = 127) n (%)</td>
<td>OR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------------------------</td>
<td>--------------------------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Salvage dying animals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>39 (35.5)</td>
<td>71 (64.5)</td>
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</tr>
<tr>
<td>No</td>
<td>39 (41.5)</td>
<td>55 (58.5)</td>
<td>1.29 (0.73, 2.27)</td>
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<td><strong>Carcasses left in grazing sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23 (36.5)</td>
<td>40 (63.5)</td>
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<tr>
<td>No</td>
<td>53 (38.4)</td>
<td>85 (61.6)</td>
<td>1.08 (0.59, 2.01)</td>
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<tr>
<td><strong>Dogs attack livestock</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27 (22.9)</td>
<td>91 (77.1)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>51 (58.6)</td>
<td>36 (41.4)</td>
<td>4.77 (2.61, 8.75)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td><strong>Livestock kraaled daily</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>48 (34.3)</td>
<td>92 (65.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (47.8)</td>
<td>35 (52.2)</td>
<td>1.75 (0.97, 3.17)</td>
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</tr>
<tr>
<td><strong>Animals arrive weeks later after release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>20 (28.2)</td>
<td>51 (71.8)</td>
<td>1.0</td>
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<tr>
<td>Yes</td>
<td>60 (44.1)</td>
<td>76 (55.9)</td>
<td>2.01 (1.09, 3.73)</td>
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</tr>
<tr>
<td><strong>Provide nutritional supplements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53 (38.7)</td>
<td>84 (61.3)</td>
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</tr>
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<td>Yes</td>
<td>27 (38.6)</td>
<td>43 (61.4)</td>
<td>1 (0.55, 1.8)</td>
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</tr>
<tr>
<td><strong>Had the interviewee heard of rabies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>77 (38.5)</td>
<td>123 (61.5)</td>
<td>1.25 (0.22, 7.0)</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>Heard of rabies from the DVS</strong></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (33.7)</td>
<td>67 (66.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>46 (43.9)</td>
<td>60 (56.1)</td>
<td>1.54 (0.37, 114)</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Knowledge of signs of rabies in cattle</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>26 (21.9)</td>
<td>93 (78.1)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (61.4)</td>
<td>34 (38.6)</td>
<td>5.68 (3.08, 10.46)</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Variable</td>
<td>Positive farms (n = 80)</td>
<td>Negative farms (n = 127)</td>
<td>OR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-------------------------</td>
<td>--------------------------</td>
<td>--------------</td>
<td>---------</td>
</tr>
<tr>
<td>Transmission occurs through biting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>14 (37.8)</td>
<td>23 (62.2)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>66 (38.8)</td>
<td>104 (61.2)</td>
<td>1.04 (0.5, 2.17)</td>
<td>0.91</td>
</tr>
<tr>
<td>Protection occurs through vaccination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>33 (32.7)</td>
<td>68 (67.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>47 (44.3)</td>
<td>59 (55.7)</td>
<td>1.64 (0.93, 2.89)</td>
<td>0.08</td>
</tr>
<tr>
<td>Know that rabies is a zoonosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27 (36.5)</td>
<td>47 (63.5)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>53 (39.9)</td>
<td>80 (60.1)</td>
<td>1.15 (0.64, 2.07)</td>
<td>0.63</td>
</tr>
<tr>
<td>DVS is well resourced</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5 (35.7)</td>
<td>9 (64.3)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>71 (38.4)</td>
<td>114 (61.6)</td>
<td>1.12 (0.36, 3.48)</td>
<td>0.84</td>
</tr>
<tr>
<td>DWNP does enough to control rabies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>31 (32.0)</td>
<td>66 (68)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29 (42.7)</td>
<td>39 (57.3)</td>
<td>1.58 (0.83, 3.01)</td>
<td>0.16</td>
</tr>
<tr>
<td>Has no reason not to vaccinate their dogs and cats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>55 (36)</td>
<td>98 (64)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25 (46.3)</td>
<td>29 (53.7)</td>
<td>1.54 (0.82, 2.88)</td>
<td>0.18</td>
</tr>
<tr>
<td>Kill wildlife that display clinical signs and don’t report the case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17 (30.3)</td>
<td>39 (69.7)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63 (41.7)</td>
<td>88 (58.3)</td>
<td>1.64 (0.85, 3.16)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Approximately 65% (95% CI 58.8, 72.1) of farmers indicated that their livestock returned to their kraals some week(s) after release (Table 5.2). Ruminants returning week(s) later were more likely to be owned by farmers with cases of rabies in their
stock than in farmers without a history of rabies (OR 2.01; 95% CI 1.09, 3.73). The difference in the risk of livestock developing rabies if they were kraaled daily or were provided with water or nutritional supplements daily were not significant. Similarly, when comparing leaving of carcases in the grazing areas, salvaging of freshly dead or dying ruminants and not reporting sick animals to the DVS were not significantly associated with a history of rabies (95% CI of OR contained the value 1.0).

Most farmers (n = 167, 87.9%; 95% CI 82.4, 92.2) reported vaccinating their dogs and cats for rabies each year. Forty-six farmers (23.4%; 95% CI 17.7, 29.9) reported using their dogs for herding goats and sheep. In the univariable analysis farms where herding dogs were used were 2.5 (95% CI 1.25, 4.81) times likely to have had a case of rabies in ruminants than farms who didn’t use herding dogs. However, there was no difference in the risk of infection between farms that owned dogs and those who didn’t (OR 1.29; 95% CI 0.46, 3.62).

Farmers who were part-time farmers were almost 3 times more likely to have rabies cases than full time farmers (OR 2.89; 95% CI 1.44, 7.32). Rabies was more likely on farms where owners had a secondary level education or higher than on farms owned by farmers with only a primary level of education (OR 2.04; 95% CI 1.13, 3.68).

Rabies was more likely on farms in communal grazing areas than on commercial farms, although the difference was not significant (OR 1.29; 95% CI 0.46, 3.58). Interestingly, ruminants on farms located in towns and villages were almost 13 times more likely to develop rabies than those located in the cattle posts (OR 12.98; 95% CI 5.77, 29.23).
Approximately half of the farmers surveyed (n = 97, 47.3%; 95% CI 40.3, 54.4) indicated that stray dogs were a problem in their areas and stray dogs were identified as a significant risk factor for rabies in this study (OR 4.79; 95% CI 2.59, 8.89). Similarly locations where dogs were observed by farmers to be attacking livestock were more likely to have cases of rabies than places where dog attacks were not observed (OR 4.77; 95% CI 2.61, 8.75).

Hyenas and jackals were the carnivores most frequently reported with 83.6% (95% CI 77.1, 88.9) and 80% (95% CI 73.1, 85.5) of farmers reporting these animals, respectively. These animals were observed to have attacked cattle, goats and sheep with significantly more farmers (90.6%; 95% CI 85.0, 94.6) reporting attacks in winter than summer (33.8%; 95% CI 26.5, 41.7). Surprisingly places where no wildlife predators were reported were more likely to have had cases of rabies than localities with predators (OR 2.57; 95% CI 1.35, 4.88) in the univariable analysis.

Approximately two thirds of the farmers (64.9%; 95% CI 57.9, 71.4) reported that rabies could be transmissible to humans and would result in death if not treated in time. Knowledge about the disease, its route of transmission and the effectiveness of vaccination as a control method were similar between the cases and control farmers (Table 5.2). In contrast farmers who could recall at least one clinical sign of rabies in ruminants were more likely to have had a case of rabies than those who did not know any clinical signs (OR 5.68; 95% CI 3.08, 10.46). Most farmers could recall some clinical signs of rabies in dogs with salivation (79.6%; 95% CI 73.5, 84.9) and aggression or change in behaviour (49%; 95% CI 42.0, 56.1) being the most common ones reported. Some farmers also reported aimless chasing and biting of objects and circling as signs
of rabies in dogs. For cattle the clinical signs of salivation, continuous bellowing and change in behaviour were identified by between 21 and 26% of the farmers; however over half (58%; 95% CI 51.0, 64.9) did not know any clinical signs in cattle. Farmers who provided reasons on why they did not vaccinate their dogs for rabies had a slight, but not significant, increased risk of rabies in their ruminants (OR 1.54; 95% CI 0.82, 2.88).

As far as who was supposed to report cases of sick wild animals, 33.8% of farmers indicated it was their responsibility, 27.2% stated it was the responsibility of anybody who saw such animals, 28.7% indicated they would kill, eat or bury such affected wild animals and 10.3% were unsure of what to do. Farmers who would kill the affected wild animals had slightly less risk than those who would report the disease (OR 1.64; 95% CI 0.85, 3.16).

**5.3.2: Multivariable logistic regression**

In the final multivariable logistic regression model (Table 5.3), livestock located in peri-urban areas (OR 10.6), livestock roaming unattended for weeks (OR 3.1), history of dogs attacking livestock (OR 3.1) and use of dogs for herding livestock (OR 4.5) were all significantly associated with a history of rabies diagnosed in ruminants. The logistic regression model exhibited a good overall fit (Hosmer and Lemeshow Chi-square value 8.7, \( P=0.191 \)), with 75.6% of farms (responders) being classified in the correct category.
Table 5.3: Final logistic regression model

<table>
<thead>
<tr>
<th>Description of variables</th>
<th>B</th>
<th>SE(^a)</th>
<th>Wald(^b)</th>
<th>Sig(^c)</th>
<th>OR(^d) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farms located in peri-urban areas</td>
<td>2.362</td>
<td>0.475</td>
<td>24.68</td>
<td>&lt; 0.0001</td>
<td>10.6 (4.2, 26.9)</td>
</tr>
<tr>
<td>Livestock roam for weeks unattended</td>
<td>1.132</td>
<td>0.413</td>
<td>7.511</td>
<td>0.006</td>
<td>3.1 (1.4, 7.0)</td>
</tr>
<tr>
<td>Dogs attack livestock</td>
<td>1.127</td>
<td>0.385</td>
<td>8.571</td>
<td>0.003</td>
<td>3.1 (1.5, 6.6)</td>
</tr>
<tr>
<td>Herding dogs used</td>
<td>1.495</td>
<td>0.450</td>
<td>11.055</td>
<td>0.001</td>
<td>4.5 (1.9, 10.8)</td>
</tr>
<tr>
<td>Constant</td>
<td>-3.861</td>
<td>0.612</td>
<td>39.758</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)SE=Standard error; \(^b\)Wald=a test that a coefficient is zero based on the Wald statistic; \(^c\)Sig=Significance for the Wald statistic; \(^d\)OR=Odds ratios

5.4 Discussion

The targeted survey plan of 227 farmers was not possible as some farmers who previously had positive cases of rabies in their ruminants no longer owned livestock due to destocking following the FMD outbreak of 2012. The rate of non-participation was higher among farmers with known positive cases because the FMD outbreak in 2012 was close to the city of Francistown where most of positive cases of rabies clustered and because of the psychological impact of destocking some of these farmers did not want to participate. In contrast farmers who had not had a positive case on their farms (controls) were easily replaceable during the interviews with alternatives. Also some farmers with cases had died and were lost from the study. It was important in this situation to be sensitive, sympathetic and avoid confrontation with the participating farmers and in this case it was not possible to recruit replacements for farmers with a history of rabies in their livestock. The sample size of 207 provides a 93% level of confidence that the data are representative of the current situation in the North East district. The dominance of males in the interviewed population was not surprising as males are responsible for rearing livestock, especially cattle, in Botswana (CSO, 2011). Similarly the older age represented (mean of 58 years)
indicates the demographics responsible for raising and keeping livestock in the country (CSO, 2011). This older cohort also grew up during the pre-independence era of Botswana when the education system was not fully developed and this would account for the high proportion of participants who only attained a primary level education. The mean ratio of cattle to goats to sheep in the north district was approximately 15:6:1, however the mean number of cattle kept varied dramatically between the production systems adopted and the location of the farms. The mean number of cattle raised in farms located at the cattle posts was higher than in farms located in towns and villages and these differences are likely to be associated with the availability of pasture. On average farmers kept three dogs per farm and few (7.7%) did not keep any dogs.

The most common predators reported in the North East district included hyenas, jackals, lions, leopards, caracal (Caracal caracal) and wild dogs and, according to most (74.9%) farmers, these presented a problem. Not surprisingly this problem was more significant in the cattle posts and areas further away from urbanised and populated areas. In contrast jackals have close and continual interaction with domestic dogs and are more often associated with human settlements than other predators (Sabeta et al., 2007).

This study revealed that the risk of ruminant rabies cases was higher in towns and peri-urban villages than in cattle posts (OR 10.6 in final logistic regression model). In the cattle posts and other remote locations of Nata the lack of rabies cases may be because in these locations there are few stray dogs and a lack of garbage sites which
provide potential food source for wild animals and stray dogs. The farmers also tend to spend more time looking after their cattle in the cattle posts and have a lower tolerance for canid wildlife due to known predatory behaviours of such animals (Gusset et al., 2009), than in farmers from peri-urban areas. It was evident from this study that currently there is a lack of a planned, structured and coordinated national surveillance system for rabies in wildlife species. There is a need for general education of the public, as well as livestock and animal owners, on their responsibilities if they suspect cases of disease in wildlife (WHO, 2013a). The killing of wildlife by farmers also impedes the surveillance for rabies and it is likely that the number of cases of rabies in wildlife is seriously under-estimated. In contrast farmers keeping livestock close to the towns and villages are reluctant to kill wildlife because of the close proximity of law enforcement agents, restricting the use of firearms in populated areas.

Although larger predators, such as lions and leopards, are occasionally diagnosed with rabies (Tremlett, 1993) their role in the transmission of rabies would be expected to be minimal as they would most likely kill their prey outright, in contrast to hyenas and jackals. According to farmers hyenas are notorious for biting chunks of muscle or the tails from live animals. The hyenas then run away, leaving the animals to die, or to recover with or without treatment from the farmers. Such a process is likely to foster the transmission of rabies. However hyenas are seldom found in the vicinity of human dwellings and do not have a close relationship with dogs, making this animal less important in the rabies cycle than jackals. Hyenas also tend to be involved in “lightning raids”, making interaction with livestock and dogs less likely. In the data summarised in Chapter 3, there were only three cases of rabies diagnosed in hyenas during the study period. Jackals are known to attack kids and lambs but in desperate times they can
attack larger prey (Brassine, 2012), including goats, sheep and cattle. The predatory attacks on livestock during the winter and summer seasons reported by the farmers interviewed in this survey, is supported by the findings reported in Chapter 2 where more cases are recorded in summer when abundant surface water and pastures mean that livestock do not need to return to their kraals in the evenings. The winter period is also the breeding season for jackals and is characterised by the movement of jackals which is in synchrony with the long distance travel of wild ruminants (WHO, 2013a). In addition in Botswana winter is a post-harvest time which coincides with minimal husbandry interactions for livestock which are allowed to graze freely on the remnants of the harvested crops. At this time there is also mobility of jackals to the agricultural crops in search of rodents and dead livestock (Kaunda, 2001). These features foster the interaction between jackals and dogs and domestic ruminants.

In this study the use of herding dogs was found to be significantly associated with the presence of rabies in the logistic regression model (OR 4.5). Farmers, in an attempt to deal with the predatory problems of jackals and hyenas, use herding dogs to protect their sheep and goats. This strategy may be detrimental if these animals are not protected against rabies. Some farmers reported that herding dogs were easily forgotten during rabies vaccination campaigns and even when they were immunized proper procedures were not in place to get them re-immunized when the need arises. This is important as the risks for rabies infection in these animals are potentially higher and continuous in the wild for such dogs. Because of their close association with livestock, a rabid dog on a farm can easily bite a large number of livestock before it dies. These dogs attacking livestock are often over-looked by farmers because of their close relationship with livestock (Jennens, 2002). This study found that the number of
dogs owned did not influence the likelihood of infection on a farm; however most farmers owned dogs (mean of 2.8 dogs owned per farmer). Almost half of the farmers (47.3%) indicated that there was a problem with stray dogs in their locality with the presence of stray dogs having a very significant positive influence on the number of cases of rabies in ruminants in the univariable analysis (p < 0.01). Importantly 42.0% of the respondents reported a history of dog attacks on livestock and this was significantly associated with rabies in ruminants in the multivariable analysis (OR 3.1; 95% CI 1.5, 6.6). Stray dogs in towns and peri-urban areas are likely to be or have originated from pets abandoned by owners who have transferred to other areas of Botswana for work. Population control or the use of oral vaccination against rabies is required to reduce or protect this population, respectively.

The normal practices adopted by farmers in the North East district also predispose their animals to an increased incidence of rabies. Over two-thirds of farmers (67.6%; 95% CI 60.8, 73.9) admitted not being able to account for the whereabouts of their livestock on a daily basis, with 30% indicating that they left carcases of dead livestock in the grazing areas, a potential food-source for carnivores. The roaming of livestock for periods longer than a week without returning to their kraal significantly increased the risk of rabies in the final logistic regression model (OR 3.1; 95% CI 1.4, 7.0). This roaming is likely to increase the opportunity for an interaction with a rabid animal, be it either a domestic canid or a wild animal (Gusset et al., 2009).

In this study more farmers were aware of the presenting clinical signs of rabies in dogs than in cattle. Of concern is that over half of the farmers interviewed (58%) did not
know of any clinical signs in cattle. Not surprisingly farmers who had cases of rabies were more likely to know the clinical signs in cattle than farmers who had not had cases of rabies diagnosed in their livestock. This highlights the potential for under-reporting of cases of rabies in ruminants. There is a need for the development and implementation of educational programmes focusing on the disease in non-carnivore species specifically targeting this group of the community. This educational material needs to be developed and offered in a way to maximise the educational impact on farmers.
Chapter 6: General discussion and recommendations

General discussion

The objectives of this research: to describe the distribution of rabies in domestic ruminants in Botswana in terms of both spatial and temporal distribution; to identify the risk factors associated with the disease in a high risk area; and to assess the knowledge, attitudes and practices of the farmers in a high risk area; were met in this study.

During the period from 2000 to 2010 an average of 35 cases of rabies were reported in ruminants in Botswana compared with 134 cases per year in the previous decade. This improvement was likely the result of the disease implementation programmes instituted by the DVS and improved infrastructure in the country facilitating contact with domestic livestock and dogs and their owners (Tremlett, 1993, Masupu, 1999). Furthermore the outbreak of FMD in the high risk area for rabies and the subsequent control measures implemented (OIE, 2013), as well as drought which affected the entire country (CSO, 2011), might have also contributed to the downward trend of the disease observed during this period. These achievements occurred at the same time as an improving economic status of Botswana from the expansion of the local mining industry. However this development has led to the growth of towns and peri-urban dwellings, resulting in a problem with excess waste and issues associated with its disposal. This has resulted in an environment that fosters the growth of some wildlife, in particular black backed jackals, as well as increased numbers of livestock in more densely populated regions where there is also an increased population of owned and stray dogs (Kariuki and Ngulo, 1985).
However, the decreasing number of cases in ruminants and dogs over the eleven year study period was offset by an increase in the number of cases in jackals. These animals could lead to subsequent outbreaks in ruminants (Tremlett, 1993), as a strong positive correlation was demonstrated between the number of cases in ruminants and the number in jackals ($r = 0.78$, $p < 0.005$). In contrast there was no significant correlation between the number of cases in ruminants and the number in dogs ($r = -0.066$, $p = 0.85$). However, there are a number of factors which could have affected these results including the disease control measures (e.g. baiting and killing of jackals and dogs) adopted in the previous decade and the killing of sick animals by farmers instead of reporting suspicious cases to the DVS. The number of cases in cattle and goats were also strongly correlated ($r = 0.96$), however surprisingly this was not the case for sheep and cattle or sheep and goats. The level of anti-predatory mechanism these animals (sheep) have against jackals and dogs, the flock size and their size may play a role in reducing their risk (Jennens, 2002).

Cases of rabies in ruminants were generally localized to the north-eastern part of Botswana. To evaluate risk factors which may be associated with the disease and to assess the KAP of farmers a more focused approach on the district of highest risk to ruminant rabies was used (North East District where the incidence of ruminant rabies over the 11 year period was 0.029%). In this district the majority of cases originated in the peri-urban villages clustered around Francistown. In contrast few cases were reported in Nata where the cattle posts are located (22.4%). Livestock from farms in towns and peri-urban villages were almost 11 times more likely to have rabies than stock from the cattle posts. This increased risk in the peri-urban environment appears
to be the contribution of stray and owned dogs attacking livestock in these locations. The key epidemiological features fostering stray dogs and owned dogs attacking livestock in these areas are believed to be the waste management problems encountered providing food for dogs and inadequate control of dogs by owners. These problems are often associated with growing towns and cities in the world and Botswana is not an exception (Hughes and Macdonald, 2013). In addition, annual rabies vaccination coverage of dogs and cats was inconsistent and it is likely that most stray animals would not have been vaccinated in a vaccination campaign.

Furthermore, the availability of plentiful surface water, which also fosters arable agriculture, in the Francistown and peri-urban areas encourages roaming of livestock and at the same time being an attractive site for jackals because of rodents and other pests associated with the agricultural fields (Kaunda, 2001). According to this study, the presence of jackals, free roaming livestock, the presence of stray dogs as well as dogs attacking livestock is a well-developed epidemiological complex situation resulting in increased disease in the Francistown area of the North East district in Botswana.

There was no obvious seasonal effect on the number of cases of rabies in ruminants, however increased roaming and uncontrolled livestock movement influenced by water and pasture availability are possible predisposing factors to these animals interacting with potentially rabid predators (Masupu, 1999). The long incubation period for rabies (Lojkic et al., 2013) may have minimised any seasonal effect, even though there are some features of the disease which have a seasonal component. For example winter is
the breeding season for jackals and is characterized by the movement of jackals in synchrony with the long distance travel of wild ruminants (WHO, 2013a). Winter is also post-harvest time in Botswana when there are minimal husbandry interactions for livestock which are allowed to graze freely the remnants of the harvested crops. This coincides with increased movement of jackals to the agricultural crops in search of rodents and dead livestock (Kaunda, 2001). These features would be expected to increase the likelihood of interaction between jackals and dogs and/or domestic ruminants.

Disease was less frequent at the cattle posts and this was due to a number of reasons. Firstly the tolerance for wildlife by farmers at the cattle posts is low due to their predatory behaviour as opposed to towns and villages where there are law enforcement agents restricting the use of firearms in populated areas. Secondly cattle posts are areas isolated from agricultural fields, towns and villages and underground water is utilised as a result of inadequate surface water. In addition farmers at cattle posts are often retired, older individuals who are full time farmers and therefore are able to supervise their livestock activities better than those in the town and peri-urban villages where farmers are usually younger, part-time farmers.

The normal practices adopted by farmers in the North East district also predispose their animals to an increased incidence of rabies. The use of herding dogs increased the risk of rabies in ruminants probably through exposure of these animals to wild canids and subsequently close contact with domestic ruminants. Lack of supervision of livestock activities and leaving carcases of dead livestock in the grazing areas resulting
in the attraction of wild carnivores and stray dogs also increases the opportunities for interactions between canids and ruminants and consequently raises the likelihood of an interaction with a rabid animal (Gusset et al., 2009).

In this study more farmers were aware of the presenting clinical signs of rabies in dogs than in cattle. Of concern is that over half of the farmers interviewed (58%) did not know of any clinical signs in cattle. Not surprisingly farmers who had cases of rabies in ruminants were more likely to know one or more clinical signs in cattle than farmers who had not had rabies diagnosed in their livestock. This highlights the potential for under-reporting of cases of rabies in ruminants. There is a need for the development and implementation of educational programmes focusing on the disease in non-carnivore species. Education, in conjunction with vaccination of domestic and potentially wild canids, should reduce the number of cases of rabies in ruminants in Botswana.

In this study the risk factors were investigated in one high-risk district. The findings from this study may not necessarily be extrapolated to other districts in Botswana, due to differences in populations of canids and management and husbandry practices adopted by farmers from other districts. More nation-wide studies are required to establish a better understanding of risk factors which may be associated with rabies in ruminants throughout Botswana.

Studies on dogs and jackals in Botswana are required to provide a better estimate of the population so that more informed disease control planning can be implemented. There is also a need for further work on understanding the interaction between
jackals, ruminants and dogs, as jackals seem to be a key feature in the disease in Botswana. More comprehensive national disease surveillance on rabies is required, adopting strategies that are more coordinated and involve input from the DVS, DWNP and MoH. Educational campaigns should be developed and implemented to promote the knowledge of clinical signs in wild animals, as well as in ruminants, with emphasis on the reporting systems/structures when animals displaying suspicious clinical signs are encountered. Extensive vaccination campaigns against rabies should be continued on a much more consistent basis and consideration to extend these to jackals through an oral vaccination programme should be evaluated. In addition population control in high risk areas through capture, neuter/spay, release programmes should be considered for stray dogs and population control evaluated for jackals. Dog owners should be held responsible for the management of their pets and implementation of a small dog tax levy may be prudent to fund the expanded surveillance, education and control programmes recommended. The congested peri-urban areas and towns should be declared “no stock zones” and farmers should consider moving their livestock to cattle posts where opportunities for exposure to rabid carnivores are less.

Although rabies is an ancient disease, this study has demonstrated that management and husbandry factors, along with environmental factors associated with the presence of canids, result in the disease still being a problem in Botswana.
References


Jennens, Garth. 2002. *Domestic dog attacks on sheep In the urban fringe areas of Perth Western Australia*. Veterinary and Biomedical Sciences, Murdoch University, Western Australia.


Appendix A

Survey questionnaire for farmers in the high risk areas for ruminant rabies in Botswana

1. Personal Information

<table>
<thead>
<tr>
<th>Name of the respondent:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>District/village/extension area:</td>
<td></td>
</tr>
<tr>
<td>Crush/Farm Name:</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
</tr>
<tr>
<td>Type of farmer</td>
<td>Full time</td>
</tr>
<tr>
<td>Production type</td>
<td>Commercial</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
</tr>
<tr>
<td>Level of education</td>
<td>Tertiary</td>
</tr>
<tr>
<td>Number of people living on the farm</td>
<td></td>
</tr>
<tr>
<td>Number of animals owned</td>
<td>Cattle</td>
</tr>
<tr>
<td>How long you have been rearing livestock on this farm (years)</td>
<td></td>
</tr>
</tbody>
</table>


For the following questions state whether you strongly agree, somewhat agree, somewhat disagree, strongly disagree or don’t know for each question read out.

<table>
<thead>
<tr>
<th>My livestock are kraaled every day.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly Agree</td>
</tr>
<tr>
<td>Sometimes my livestock return to their own kraals weeks after</td>
</tr>
<tr>
<td>Strongly Agree</td>
</tr>
</tbody>
</table>
being away.

My livestock are provided with licks or water every day on the farm.

In my area wildlife attacking livestock is a problem?

Dogs attacking farm animals are a problem in my local area?

How many animals do you lose from any natural causes per year?

<table>
<thead>
<tr>
<th>Cattle</th>
<th>Goats</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rank the following using a scale from 1 to 6 where 1 is the most likely cause of livestock losses and 6 is the least likely cause in your local area.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Wild carnivores</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drought</td>
<td>Stealing</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

List the top 4 wild carnivores common in your area which are known to attack livestock.

<table>
<thead>
<tr>
<th>1.</th>
<th>2.</th>
<th>3.</th>
<th>4.</th>
</tr>
</thead>
</table>

In which season do your animals experience most attacks?

<table>
<thead>
<tr>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Knowledge on rabies, practices on suspect animals.

Which disease(s) transmissible from animals to humans always results in death if not treated on time?

<table>
<thead>
<tr>
<th>1.</th>
<th>2.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Have you heard of rabies? Yes No

Where did you hear about it from?

What signs do you know of that rabies could cause in cattle and in dogs?

<table>
<thead>
<tr>
<th>Dogs</th>
<th>1.</th>
<th>2.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cattle</th>
<th>1.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. For the following questions state whether you strongly agree, somewhat agree, somewhat disagree, strongly disagree or don’t know for each question below.

<table>
<thead>
<tr>
<th>Strongly Agree</th>
<th>Somewhat Agree</th>
<th>Somewhat Disagree</th>
<th>Strongly Disagree</th>
<th>Don’t Know</th>
</tr>
</thead>
<tbody>
<tr>
<td>In my local area rabies is one of the diseases killing our ruminants?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freshly dead or dying ruminants are often salvaged for meat?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freshly dead or dying ruminants are often not reported to the animal health officials?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead carcasses left in the open grazing area are usually eaten by wild animals and dogs in the area?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. **Attitude and practices towards dog vaccination against rabies in farms**

For the following questions state whether you strongly agree, somewhat agree, somewhat disagree, strongly disagree or don’t know for each question below.

<table>
<thead>
<tr>
<th>Strongly Agree</th>
<th>Somewhat Agree</th>
<th>Somewhat Disagree</th>
<th>Strongly Disagree</th>
<th>Don’t Know</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs on my farm are used for herding the cattle/sheep/goats?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs on my farm are used for hunting wild animals?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In general farm dogs are not well cared for compared to those in cities or villages?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs on my farm are vaccinated against rabies? If vaccinated when was the last vaccination done?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In my local area there are too</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
many stray dogs which are not vaccinated?

Why do you vaccinate your dogs against rabies?

Are there reasons why you would not vaccinate your dogs against rabies? If so what are they?

5. **Knowledge on transmission and control methods and attitude towards Veterinary Authorities.**

<table>
<thead>
<tr>
<th>Between dogs and wildlife- Where do you think cattle, goats/sheep get rabies from?</th>
<th>Dogs</th>
<th>Wildlife</th>
</tr>
</thead>
</table>

Do you know how rabies is transmitted to livestock?

Have you ever had any of your cattle or goats or sheep diagnosed positive for rabies by Animal Health Officers?

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

Who is responsible for reporting sick or rabid wild animals?

To whom should a sick or rabid animal be reported to?

For the following questions state whether you strongly agree, somewhat agree, somewhat disagree, strongly disagree or don’t know for each question below.

<table>
<thead>
<tr>
<th>Vaccination of wildlife, as well as dogs and cats, would protect farm animals against rabies?</th>
<th>Strongly Agree</th>
<th>Somewhat Agree</th>
<th>Somewhat Disagree</th>
<th>Strongly Disagree</th>
<th>Don’t Know</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Killing unvaccinated dogs, as well as stray dogs/cats, in my areas would help reduce the spread of rabies to our livestock?</th>
<th>Strongly Agree</th>
<th>Somewhat Agree</th>
<th>Somewhat Disagree</th>
<th>Strongly Disagree</th>
<th>Don’t Know</th>
</tr>
</thead>
</table>
Do you think over the years you have gained enough knowledge needed to help control rabies in your area?

Do you think your local animal health office is well resourced to deal with rabies in your area?

Do you think the Department of Wildlife and Tourism is doing enough to control rabies?

If No what do you think could be done to improve the rabies situation in your area?

How is your family likely to get rabies?

What do you do to protect your family from rabies?

Thank you very much for answering the questions. Feedback concerning the study and the recommendations will be communicated to you through the existing extension networks. We expect to reduce the frequency and the number of rabies cases in cattle, sheep and goats and to protect your health by coming up with effective rabies control program.
Appendix B

Consent Form for the Questionnaire Survey

“A survey to determine the knowledge, attitudes and practices (KAP) of farmers in regards to rabies in Botswana”

1. I understand the explanatory notes provided by the researcher, which explains the nature of the research and the possible risks. The information has been explained to me and all my questions have been satisfactorily answered.

2. I agree voluntarily to take part in this study.

3. I understand I am free to withdraw from the study at any time without needing to give any reason.

4. I understand I will not be identified in any publication arising from this study.

5. I understand that my name and identity will be stored separately from the data, and these are accessible only to the investigators. All data provided by me will be analysed anonymously using code numbers.

6. I understand that all information provided by me is treated as confidential and will not be released by the researcher to a third party unless required to do so by law.

7. (For animal health officials only) I understand that the results of this study will have no bearing on my employment with the Ministry of Agriculture, Department of Veterinary Services or any other related department.

Signature of Participant: __________________________ Date: ....../....../.......

Signature of Investigator: __________________________ Date: ....../....../.......