RABIES SERO-SURVEY IN VACCINATED DOMESTIC DOGS AND KNOWLEDGE ASSESSMENT OF RABIES AMONG DOG OWNERS, OHANGWENA REGION, NAMIBIA

EMMANUEL HIKUFE HIKUFE

APRIL 2016
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KNOWLEDGE ASSESSMENT OF RABIES AMONG DOG OWNERS,
OHANGWENA REGION, NAMIBIA

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ABSTRACT

Rabies kills over 55,000 people worldwide annually of which about 97% die resulting from the bite(s) of rabid dogs. Despite the free annual vaccination of dogs in Namibia and Ohangwena region in particular, rabies is still on the increase in both animals and humans. We conducted this study to establish the level of protection against rabies in the vaccinated domestic dogs through antibody testing. Furthermore, the study assessed the level of people's knowledge on rabies.

A descriptive cross-sectional study design was used. A random sample of 170 sera was collected from the dogs after one year from the previous vaccination. We tested sera at the Central Veterinary Laboratory using the BioPro Rabies ELISA test kit and defined rabies protective antibody titre as titres ≥0.5IU/mL. Data were collected using a structured questionnaire and analysed using Epi info 7 and Microsoft excel.

Among the 170 dogs, 136 (80%) acquired protective antibody titres (95% CI: 73.2%-85.7%). The majority of samples came from dogs younger than 3 years 90(53%). However, dogs older than 3 years maintained rabies protective antibodies better than the younger dogs (87% versus 74%), Chi²= 4.2, df=1, P=0.04. About 88% of dogs that received repeated vaccinations (boosters) over the years maintained protective antibodies compared to only 74% of dogs that received a single vaccination a year ago without a booster (P= 0.03).

Eighty (80%) of the vaccinated dogs maintained protective rabies antibodies. High level of protective antibodies was observed more in older dogs and dogs that received booster vaccinations over the last three years. We recommend rabies vaccination to be conducted twice per year and forceful vaccination be instituted for
stray dogs and dogs that are difficult to handle during the campaigns. Cooperation among relevant stakeholders should be instituted to ensure effective rabies control.

Key words: rabies, dogs, protective, antibodies, vaccination,
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DEDICATION

I would like to dedicate this work to my family; first of all my lovely wife Patema and my two adorable boys Jesaya (19 years old) and Tangeni (3 years old) and to the rest of my family. My wife, your patience cannot be measured. You literally took care of our house alone for two years. I will always be grateful for that. Jesaya, my boy, your patience during my absence from home is highly appreciated. I hardly came home to spend time with you during the two years of my studies. I remember Jesaya telling me "dad I think I am not performing well at school just because you are far away from me, I just feel that your absence has affected my school performance negatively." I vividly remember Tangeni telling me on the phone that I should come home, sometimes while on the phone with my wife, the gate bell would ring and Tangeni would shout "Daddy! Daddy!" The little boy would rush to answer the bell, only to be disappointed again that it is not his daddy. This work is for you my family. Thank you very much.
DECLARATION

I, Emmanuel Hikufe Hikufe, hereby declare that the Rabies sero-survey in the vaccinated dog population and knowledge assessment of Rabies among dog owners, Ohangwena region, Namibia, is a true reflection of my own study, that it has not been submitted for any degree at any other university.

No part of this work may be reproduced, stored in any retrieval system or transmitted in any form or means (e.g. electronic, mechanical, photocopying, recording or otherwise) without the prior permission of the author or the University of Namibia on the author's behalf.

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Emmanuel Hikufe Hikufe                        Date
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Ab</th>
<th>Antibody</th>
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<tr>
<td>ABLV</td>
<td>Australian Bat Lyssavirus</td>
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<td>AHT</td>
<td>Animal Health Technician</td>
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<td>ARAV</td>
<td>Aravan Virus</td>
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<td>CBPP</td>
<td>Bovine Contagious Pleuropneumonia</td>
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<td>CI</td>
<td>Confidence Interval</td>
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<td>CVL</td>
<td>Central Veterinary Laboratory</td>
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<td>DUVV</td>
<td>Duvenhage Virus</td>
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<td>DVS</td>
<td>Directorate Of Veterinary Services</td>
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<tr>
<td>EBLV-1</td>
<td>European Bat Lyssavirus Type 1</td>
</tr>
<tr>
<td>EBLV-2</td>
<td>European Bat Lyssavirus Type 2</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immune-Sorbent Assays</td>
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<td>FAVN</td>
<td>Fluorescent Antibody Virus Neutralisation Test</td>
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<td>FMD</td>
<td>Food And Mouth Disease</td>
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<td>IRKV</td>
<td>Irkut Virus</td>
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<td>IU/ml</td>
<td>International Units / Millilitre</td>
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<td>KHUV</td>
<td>Khujand Virus</td>
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<td>KM</td>
<td>Kilometre</td>
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<td>LBV</td>
<td>Lagos Bat Virus</td>
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<td>MOKV</td>
<td>Mokola Bat Virus</td>
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<tr>
<td>NAMFELTP</td>
<td>Namibia Field Epidemiology and Laboratory Training Program</td>
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None
ODPC
ODsample
OIE
P_{75}
P_{90}
PB
PEP
P_{Risk}
RABV
RFFIT
SV
SVO
VNA

No Education
Optical Density Of The Positive Control
Optical Density Of The Sample
Office International Des Epizooties Or World Organisation For Animal Health
Population of dogs that received a sing dose of vaccination last year without a booster, where a proportion of 75% is expected to maintain protective antibodies
Population of dogs that were vaccinated every year or twice last year, where a proportion of 90% are expected to maintain protective antibodies
Percentage of Blocking
Post Exposure Prophylaxis
Population of dogs that were vaccinated only once within two or three years and regarded as proportion at risk of rabies infection
Classical Rabies Virus
Rapid Fluorescent Focus Inhibition Test
State Veterinarian
State Veterinary Office
Virus Neutralizing Antibodies
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>WCBV</td>
<td>West Caucasian Bat Virus</td>
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<td>WHO</td>
<td>World Health Organization</td>
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CHAPTER ONE

INTRODUCTION TO THE STUDY

1.1 INTRODUCTION AND BACKGROUND OF THE STUDY

Rabies, a fatal nervous system disease of warm blooded animals including man is caused by a virus, belonging to the family *Rhabdoviridae*, of the genus, lyssavirus. It has been associated with animal bites for more than 3000 years and it is the oldest infectious disease known to medical science (Atuman et al., 2014).

According to Bingman (2005), rabies virus is transmitted via saliva through the bite of an infected animal. After gaining entry to the central nervous system by peripheral nerves, it causes encephalitis, leading to fulminant, progressive neurologic disease, characterized by excitement, muscular paralysis, impaired responses to social and environmental signals, and other abnormal neurologic signs.

According to Blanton et al. (2012), rabies has the highest fatality ratio compared to any other infectious disease if post exposure prophylaxis (PEP) is not promptly initiated. In the United States, PEP consists of wound care (at a minimum, washing of the wound with soap and water), infiltration with rabies immune globulin (RIG), and administration of a series of 4 doses of rabies vaccine over 14 days (Blanton et al., 2011), however in Namibia, PEP consists of wound care (washing of the wound with running water and soap), infiltration with human rabies immunoglobulin and the administration of a series of 5 doses over 28 days. It is estimated that rabies kills more than 55,000 people worldwide annually of which about 95% deaths occur in Asia and Africa (Knobel et al., 2005 and Yang et al., 2014). The burden of rabies in Africa is second globally behind that in Asia, with around 24,000 human deaths
estimated each year despite the availability of effective vaccines (Dodet et al., 2008). Sudarshan et al. (2006) and Bourhy et al. (2008) reported that more than 97% of human deaths due to rabies resulted from the bite(s) of rabid dogs.

There are 7 major species within the genus: classical rabies virus (RABV), Lagos bat virus (LBV), Mokola bat virus (MOKV), Duvenhage virus (DUVV), European bat lyssavirus type 1 (EBLV-1), European bat lyssavirus type 2 (EBLV-2), and Australian bat lyssavirus (ABLV). Recently, 4 new members have been approved by the International Committee on Taxonomy of Viruses: Aravan virus (ARAV), Irkut virus (IRKV), Khujand virus (KHUV), and West Caucasian bat virus (WCBV) (Jin et al., 2011). Davis et al. (2007) reported that there are at least four genetic variants of RABV circulating in Africa. Two of these variants, or biotypes, circulate in Zimbabwe and South Africa. The canid variant primarily infects carnivores of the family Canidae such as dogs (Canis familiaris), black-backed and side-striped jackals (Canis mesomelas and Canis adustus, respectively) and bat-eared foxes (Otocyon megalotis). The mongoose variant is so-called because it is principally maintained by mongooses, particularly the yellow mongoose (Cynictis penicillata) and the slender mongoose (Galarella sanguinea), as well as viverrids, mustelids, felids and canids. According to Paweska et al. (2006), the canid, or dog biotype of RABV is the most widely distributed in the world.

The development of trans-oceanic travel during the 15th century is thought to be responsible for the transmission of rabies to all continents, resulting in the global dissemination of the so-called cosmopolitan dog RABV lineage (Bourhy et al., 2008). Talbi et al. (2009) revealed that RABV currently circulating in dogs in this region fell into a single lineage designated Africa 2. The same authors further
reported that dogs (*Canis familiaris* L.) have always been the principal host species of rabies throughout Africa. The development of a vaccine for rabies virus (RABV) by Louis Pasteur in 1886 led to the development of prevention strategies against the disease in the developed world, and this fatal encephalitis is now preventable (Bourhy et al., 2008). Traditional rabies control measures in dogs have included mass vaccination, movement restriction and control of stray dogs (Atuman et al., 2014). The measures have been effectively applied in most of the developed world since the 1940s, resulting in relatively effective control and in some cases elimination of dog and human rabies. Serological testing for rabies is widely used to allow pets animals (especially cats and dogs) from rabies endemic territories to enter rabies-free countries which strictly regulate animal trade (Servat, 2007).

Kennedy et al. (2007) reported that there are a number of companies who manufacture rabies vaccines. These researches indicated that serological tests have been developed principally to measure post-vaccination antibody titres in people and animals and not to detect antibodies induced by natural infections. They further indicated that a serum titre of 0.5 IU/mL and above of rabies virus-specific antibodies is considered adequate protection against rabies. A titre below this level is therefore considered a vaccination "failure", leaving the dog less likely to be protected from the rabies virus. The serological survey (sero-survey) of this study was done in accordance with the standard procedures as found in several published articles on similar studies.

The aim of this sero-survey was to assess the rabies vaccination campaigns as well as establish the knowledge level of the disease among dog owners in the region in order to institute appropriate measures. The findings of this study will result in informed
control measures being put in place for the elimination of the disease among humans and animals in the region as well as the whole country at large. Due to the similarities in culture and practices of most communities in the northern regions, the results of this study may be generalized.

1.2 STATEMENT OF THE PROBLEM

Despite the free annual vaccination of dogs and cats against rabies in Namibia, the disease is on the increase in both animals and humans especially in the northern part of the country and in Ohangwena region in particular (Bishi and Marais, 2005-2012). According to the Ohangwena region’s state veterinary annual vaccination reports (unpublished) by Hedimbi et al. (2011-2013), over the last three years an average of 74% vaccination coverage was recorded in the region, 81% (2011), 71% (2012) and 70% (2013) based on the estimated dog population of 11 000. Knobel et al. (2008) and Atuman et al. (2014) indicated that vaccination coverage of 70% in a dog population is sufficient to eliminate rabies by stopping the circulation of rabies virus among susceptible animals. However, secondary data analysis by Hikufe et al. (2014) (unpublished) indicates an increasing trend of the disease over the last three years (2011-2013) which caused 112 deaths of animals and 13 humans respectively, in Ohangwena region. Given the increasing number of rabies cases in the last three years, it is therefore not clear whether the current vaccination campaigns result in adequate immunity in the vaccinated dogs in the region. Furthermore, it is also not clear if the vaccinated dog population represents the actual dog population in the region. Questions also arise as to whether there exists other animal species in the region that may also be involved in the transmission of the disease to other animals apart from the dogs. It is further assumed that the Ohangwena region’s community
lacks knowledge and understanding of rabies disease, given the increasing trends of rabies cases in animals and humans over the years, in that region.

1.3 THE AIM OF THE STUDY

This study was aimed at assessing the level of protection against rabies in the vaccinated domestic dog population in Ohangwena region through antibody testing. Furthermore, the study also aimed at assessing the level of people’s knowledge on rabies in the region, specifically dog owners.

1.4 OBJECTIVES OF THE STUDY

Objectives of the study were to:

- Establish the demographic information of the vaccinated domestic dogs and their owners;
- Establish the level of rabies protective antibodies in the vaccinated domestic dogs of Ohangwena region;
- Describe the relationship between the demographic characteristics of the dogs and their owners and the level of rabies antibodies in the vaccinated domestic dogs; and
- Assess the level of rabies knowledge in the dog owners.

1.5 SIGNIFICANCE OF THE STUDY

The results of this study will be instrumental in establishing the level of protection against rabies in the vaccinated dog population in order to assess the current rabies control measures particularly the annual vaccination campaigns. Assessing the level of understanding of the disease among dog owners will trigger appropriate awareness strategies by the veterinary officials to the community. The findings of this study will result in the design and implementation of improved rabies control measures and
policies or be adjusted accordingly. The improved rabies control measures will result in the elimination of rabies in both human and animal populations or at least lead to a significant reduction in the prevalence thereof, resulting in more human lives being saved and the prevention of severe economic loss due to the death of both humans and animals.

1.6 DEFINITIONS OF CONCEPTS
The concepts to be defined in this study are derived from the title "Rabies Serosurvey in Vaccinated Dogs and knowledge assessment of rabies among dog owners, Ohangwena region". The concepts are defined as follows:

1.6.1 Rabies Sero-survey (serological survey)
Rabies Sero-survey is described as an investigation in which serological information on rabies is collected systematically and usually carried out on a sample of a defined population group, within a defined time period (OIE surveillance, 2014). In this study, rabies sero-survey is regarded as the collection of serum from the vaccinated dogs during the study period in order to determine and analyse the antibodies acquired from the vaccination campaigns.

1.6.2 Vaccinated dogs
Vaccination means the successful immunization of susceptible animals through the administration, according to the manufacturer's instructions and Office International Des Epizooties (OIE) (also known as World Organization for Animal Health) Terrestrial Manual, where relevant, of a vaccine comprising antigens appropriate to the disease to be controlled (OIE, 2014). In this study, vaccinated dogs are referred to as all domestic dogs in Ohangwena region that were administered with
the rabies vaccine during the vaccination campaigns at least once in the last three years.

1.6.3 Knowledge assessment about rabies is the process of establishing the understanding of dog owners about rabies disease by finding out their knowledge about the disease’s dynamics, prevention and control measures.

1.6.4 Dog owners

When a person takes on the ownership of a dog, there should be an immediate acceptance of responsibility for that dog, and for any offspring it may produce, for the duration of its life or until a subsequent owner is found (OIE, 2014). For the purposes of this study, dog owners are people whose dogs were recruited and participated in this study.

1.6.5 Domestic dogs

Dogs that are bred and kept by owners as described in 1.6.4.

1.6.6 Ohangwena region

Ohangwena region as defined in the Act of the Namibian Parliament, is one of the fourteen (14) magisterial units each headed by a governor. The region is further divided into constituencies which are headed by regional councillors.

1.7 OUTLINE OF CHAPTERS

This thesis is presented in the following chapters:

Chapter 1: This chapter consists of the introduction and background to the study. The rationale for the researcher to conduct the study as well as the objectives and benefits of the study are outlined in this chapter.

Chapter 2: This chapter deals with the literature review.
Chapter 3: In this chapter, the research methods which include the research design are described as well as ethical considerations, data collection methods and the procedure followed. The research tools including the BioPro test kit is also described in this chapter.

Chapter 4. In this chapter, the results are analysed and presented. The results for both objectives are presented in the form of figures, tables as well as the narrative.

Chapter 5. In this chapter the findings of the study are discussed. Various similar findings by other researchers in Namibia and abroad are reflected upon in this chapter and literature is integrated into the discussions to serve as additional evidence to the findings. The major conclusions, recommendations as well as study limitations are also found in this study.

1.8 SUMMARY

In this chapter, the background and the location of the study was described. A picture of the global and local public health impact of rabies was also given. Moreover, the problem statement, aims and objectives, were also discussed. The benefits of the study were also discussed and finally the outline of all the study chapters was given.
CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter discusses rabies in domestic dogs and humans and the measures used by different countries in fighting the disease. Furthermore, the different factors that affect rabies immunogenicity in domestic dogs are discussed. The chapter also focuses on the literature review based on similar studies conducted by previous researchers.

2.2 OVERVIEW OF DOG MEDIATED RABIES AND ITS CONTROL

Rabies is one of the most virulent diseases of humans and animals and may have been reported in the Old World before 2300 BC (Bourhy et al., 2008). It is a viral zoonosis transmitted to man, mainly by rabid dogs, although infection is also possible from bats and other mammals (Kennedy et al., 2007). According to Sudarshan et al. (2006), there are an estimated 55,000 human deaths annually worldwide from rabies and more than 97% of these are transmitted by rabid dogs. The burden of rabies in Asia is about 31,000 human deaths annually and about 20,000 of these deaths are from India (Verma et al., 2011). According to Yousaf et al. (2012), the disease causes at least 24,000 deaths per year in Africa. Southern African countries like Angola, Namibia, Mozambique, and Zimbabwe are also considered high risk areas. However, human rabies deaths are now extremely rare in Western Europe and North America, where mass vaccination successfully eliminated the disease from domestic dog populations (Hampson et al., 2009).
Rabies is a disease that infects the central nervous system of mammals and is caused by lyssaviruses (within family *Rhabdoviridae*), a genus of RNA viruses with single-stranded negative sense genome of approximately 12 kb (Davis et al., 2007). Verma et al., (2011) reported that the virus can infect all warm-blooded animals and is transmitted to humans by the bite/ lick/ scratch of an infected animal. Traditional rabies control measures in dogs have included mass vaccination (Hampson et al., 2007), as well as movement restriction and control of stray dogs (Atuman et al., 2014). The measures have been effectively applied in most of the developed world since the 1940s, resulting in relatively effective control and in some cases elimination of dog and human rabies. As guided by WHO (2007), human deaths can be prevented through timely and appropriate Post Exposure Prophylaxis (PEP), which consists of rapid and thorough washing of the wound, completion of post-exposure vaccination schedules plus inoculation with rabies immunoglobulin (RIG) for severely exposed bite-victims. According to Sambo et al. (2013), despite the effectiveness of PEP, many thousands of people still die from rabies especially in rural areas of Asia and Africa where canine rabies is endemic. These deaths are affected by the accessibility and affordability of PEP, which are key factors in determining human disease risk.

According to Hatam et al. (2014), Japan is the first country to have successfully removed rabies by implementing the collective vaccination of dogs in 1956. These authors further reported that Malaysia could also control rabies by obligatory vaccination and killing stray dogs. Other countries, such as Singapore, showed that rabies could be controlled by removing dog rabies intermediates. These countries
demonstrated that the disease could be controlled even in less developed countries. Theoretical and experimental analyses show that vaccinating 70% of the dogs is enough for prophylaxis of the disease outbreak and eradicating it (Caceres 2011).

Haimbodi et al. (2014) stated that rabies has been reported since 1887 in Namibia, occurring mainly in the north where 70% of the human population resides, affecting domestic and wild animals, while confirmed rabies cases in humans have ranged between five and 26 cases per year for decades. In Namibia a rabies control strategy has been developed to control the disease. However, the strategy requires all relevant stakeholders to be involved for its successful implementation such as the Ministry of Health and Social Services (MoHSS), Local Authorities, Traditional Authorities and others.

2.3 OVERVIEW OF THE RELATIONSHIP BETWEEN THE DOGS’ DEMOGRAPHICS AND THEIR RABIES ANTIBODY LEVELS

Knobel et al. (2008) indicated that reasons for keeping or tolerating dogs vary across societies and may involve aspects of security (guarding), companionship, transport, food acquisition (hunting) or religious belief. Knobel et al. (2008) further urged that understanding the demographics and predictors of dog ownership at household level may be of importance in fields such as public health, social psychology, or of commercial interest in the provision and marketing of veterinary services and products. The dogs’ demographics characteristics were included in this study to describe their relationship with rabies vaccination and eventually antibody development.
Bahloul et al. (2005) reported that the antibody response from dog to dog varies as a result of several interfering factors, such as the vaccine brand, the route of vaccination, the age at vaccination, as well as the health and breeding status of dogs. According to Hidano et al. (2012), a single dose vaccination without boosters would result only in 75% of the animals retaining the internationally accepted threshold antibody titre of 0.5 IU/mL or higher one year after vaccination; however, 90% of the animals would retain the accepted antibody threshold if administered a booster vaccination every year or vaccinated at least twice within one year (the previous year). It is therefore essential to obtain information on past vaccination history to estimate immune status among dogs. Hence, the inclusion of the vaccination status of dogs as one of the variables in this study. However, Bahloul et al. (2005) reported that according to several studies based on the evaluation of rabies antibody responses after mass vaccination of dogs, the results obtained were contradictory as demonstrated further below. In Peru, 12 months after mass vaccination of dogs under field conditions with inactivated cell culture-derived rabies vaccine, 97% of dogs had rabies-neutralizing antibody titres of ≥ 0.5 IU/mL. Bahloul et al. (2005) further revealed that, in contrast, a study conducted in Thailand concluded that the administration of a single dose of tissue culture-derived rabies vaccine was not able to maintain significant rabies antibody titres over a period of one year.

According to Kennedy et al. (2008), the relationship between antibody response and animal (dog) age is an important consideration as far as vaccine failure is concerned. Kennedy et al. (2008) further revealed that young dogs (younger than one year of age) make a poorer immune response to rabies vaccination than the adult dogs,
which could be due to their immune systems being less mature. In addition, Mansfield et al. (2004) reported that the interference by maternal antibodies may explain the slightly increased risk of vaccine failure in such young animals. Mansfield et al. (2004) further indicated that the ability of dogs to develop an adequate antibody titre after being vaccinated against rabies also declines steadily with age, presumably as the immune system becomes less efficient in older dogs.

Day et al. (2010) reported that poor immunogenicity could be caused by a range of human factors from the stage of vaccine manufacturing to its administration to the animal as, for example, production errors in the manufacture of a particular batch of product. Day et al. (2010) further stated that post-manufacture factors such as incorrect storage or transportation (interrupted cold chain) also may reduce the immunogenicity of the vaccine. Furthermore, other contributing factors such as incorrect administration of the vaccine to the animal for example, incorrect dosage and route of administration (subcutaneous or intramuscular routes) could result in a poor antibody response in the vaccinated animal.

As far as dog size is concerned, Kennedy et al. (2008) reported that a general relationship between animal size and level of antibody response clearly exists. These researchers further gave two possible explanations for the above findings, the first one being a vaccine dose-effect, as it is unclear whether any standard policy exists between veterinarians for adjusting vaccine dose by body weight and whether small and large dogs receive the same volume of injection. However, Kennedy et al. (2007) ruled out this explanation as an unlikely reason: so long as the immune system of an animal encounters sufficient antigen to make a response, larger doses of antigen are not a major factor in increasing antibody production in primary responses. Secondly,
the alternative explanation given by Kennedy et al. (2007) could be that larger dogs are more likely to have deeper subcutaneous fat at popular sites for injection, and deposition and sequestration of antigen in fat are known to reduce the level of immune response.

It is often assumed that rabies incidences in vaccinating countries are due to feral dogs. However, in Sub-Saharan Africa the results of several recent studies suggest that the proportion of unowned feral dogs is low: The majority of dogs are accessible for vaccination through households which claim responsibility for them (Gsell, 2006). In order to assess the presence of stray/feral dogs in the different parts of the region, the participating dog owners were asked if they do see stray/feral dogs in the surroundings of their homesteads

2.4 RABIES VACCINE BRAND

The World Health Organization (WHO) initiated a number of programmes aimed at controlling canine rabies by mass vaccination. According to Chaudhuri (2015), many of the developed countries like the United States have eliminated canine rabies while South and Central American countries reduced the incidence of canine rabies by mass vaccination of dogs and prophylactic treatment of the exposed person.

There exist several rabies manufacturing companies; however, in Ohangwena region Rabisin by Merial South Africa (Pty) is being offered for free by the Namibian government for the vaccination of dogs and cats. The vaccine is briefly described below as derived from the manufacturer’s manual/vaccine pamphlet:

- Rabisin is an inactivated rabies vaccine for the immunization of dogs, cats, horses, cattle, sheep and goats against rabies by subcutaneous or intramuscular injection;
The vaccine is produced on cultures of NIL cells, inactivated with betapropiolactone and adsorbed onto the adjuvant, aluminium hydroxide; According to the manufacturer's instruction the vaccine should be used as per the following schedule for the protection of dogs and cats:

- All dogs and cats should be vaccinated at the age of three months followed by a booster not less than one month later and not more than nine months later and then every three years;
- Puppies may be vaccinated at any age after birth but must thereafter be vaccinated at three months followed by a booster administered between one and nine months later and again every three years;
- Dose is 1ml irrespective of species;

Although it is required by legislation that dogs and cats be vaccinated every three years in rabies control areas, it is advisable to vaccinate annually.

2.5 RABIES SEROLOGICAL SURVEY, A GLOBAL PERSPECTIVE

According to Fachin et al. (2005), post-assessment of the efficacy of rabies vaccination campaigns requires blood sampling of vaccinated animals to measure the respective immune response. According to Servat et al. (2007), the only method approved by World Organisation for Animal Health (Office International des Epizooties or OIE) to perform serological testing for rabies are the Fluorescent Antibody Neutralization test and the Rapid Fluorescent Focus Inhibition test. However, Servat et al. (2007) reported that several enzyme-linked immune-sorbent assays (ELISA) are inherently and more easily standardized in laboratories than neutralization tests and allow simple and more rapid large scale processing of sera,
thus recommending their use as a reference technique for the detection of rabies antibodies in domestic dogs. Wasniewski and Cliquet (2012) have therefore recommended the BioPro ELISA kit to be considered as a valuable method for assessing the levels of rabies antibodies in domestic vaccinated carnivores following their satisfactory results after their evaluation of this kit. The researcher of this study therefore made a decision to use the BioPro test kit in this study.

**2.6 POSSIBLE PRESENCE OF WILD ANIMALS SPREADING RABIES IN THE REGION TO OTHER ANIMALS**

Rabies primarily affects jackals, kudus and cattle in the commercial farming central regions of Namibia (Haimbodi et al., 2014). However, dogs have been assumed to be responsible for most cases of rabies in humans and other animals in the northern regions. This study also sought to establish by interviewing the participating dog owners, whether there are wild animals that may be transmitting the disease to other animals in the region apart from the dogs.

**2.7 RABIES AWARENESS AMONG DOG OWNERS IN THE REGION**

Despite the fact that Haimbodi et al. (2014) established that the majority of dog owners in the two northern towns of Oshana region have basic knowledge and awareness of rabies, it was also discovered in the same study that educated participants were more likely to have a basic knowledge and awareness of rabies, as well as a good understanding of other animals involved in the local rabies epizootiology. This study included a few questions on rabies knowledge, attitudes and practices of dog owners living in the villages of Ohangwena region and also by putting their level of education into consideration.
2.8 CONCLUSION

In the previous chapter an overview of dog rabies was given as well as the commonly used control methods. The different methods used in a few countries which have successfully eliminated the disease were also referred to in the chapter. Furthermore, different theories on rabies sero-survey were discussed. It was indicated here that it has been accepted globally that canine rabies can only be effectively controlled through mass vaccination. However, multiple factors such as internal parasites, health and nutritional status of the dogs and others are reported to impede immunity production. It was further reported that through serological tests, one can successfully assess the immune status induced by vaccination. In this chapter the rabies vaccine (Rabisin) which is currently being used in the region was clearly described as per the manufacturer’s instructions. This study is expected to be crucial for veterinary services to put in place relevant rabies control measures for the better control of the disease.
CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

In this chapter the research design and methods will be described. The chapter will describe in detail the design, data collected, instruments used to collect the data, the methods used to manage and analyze the data, and how the ethical requirements were addressed. The study was carried out in all the five (5) Animal Health Technician (AHT) areas (An Animal Health Technician Area is referred to as the area under the responsibility of the Animal Health Technician as demarcated by the Veterinary Services' regional management), namely: Omafo, Eenhana, Omundaungilo, Okongo and Omauni. However, due to the low number of dogs and the close proximity of Omauni and Okongo Animal Health Technician areas, these two areas were joined/merged as one for sampling purposes. The AHT areas in this study will therefore be reported as only four.

3.2 RESEARCH DESIGN

The study design is a quantitative, cross-sectional, descriptive study of the level of individual protective immunity in the vaccinated dog population. The designs are described as follow:

3.2.1 Quantitative

A quantitative research design was used as a systematic approach to describe the level of rabies antibodies in the vaccinated dog population of Ohangwena region. According to Burns and Groves (2009), the quantitative design is a formal, objective and systematic process which illustrates and tests relationship between variables.
This study tested the relationships between the demographic characteristics of the dogs and their owners with the level of rabies antibody development in the vaccinated dogs. The study further tested the relationship between the demographic characteristics of the owners with their knowledge on rabies.

3.2.2 Cross-sectional

According to Aschengrau and Seage (2014), cross-sectional studies typically take a snapshot of a population at a single point in time and so usually measure the disease prevalence in relation to the exposure prevalence. In this study the serum samples were collected within three weeks to measure the point prevalence of protective antibody levels in the vaccinated dogs of Ohangwena region.

3.2.3 Descriptive

A descriptive epidemiologic approach was employed in this study. Maree et al. (2007) defined the term descriptive design/statistics as a collective name for a number of statistical methods that are used to organize and summarise data in a meaningful way which serves to enhance the understanding or the properties of the data. According to Aschengrau and Seage (2014), descriptive epidemiology involves the analysis of disease patterns according to the characteristics of person, place and time. This study described the dogs’ protective antibody levels in terms of the demographical information of the dogs and their owners. Furthermore, the study described the knowledge of the disease based on the owners’ demography.

3.3 STUDY AREA / SETTING

The study was carried out in Ohangwena region, situated in the northern part of Namibia bordering with the neighboring country Angola. The size of the region is
estimated to be 10,706 km² and with a population of 245,446 inhabitants according to the 2011 census. The estimated animal population is: cattle 312,562, goats 16,474, sheep 940, horses 2030, donkeys 2549, dogs 11,000, and cats 1,100. The regional headquarters of Ohangwena is Eenhana town which is also serving as the political capital town for that region. The regional veterinary office is headed by two state veterinarians. The region is further subdivided into five veterinary areas each under the responsibility of an Animal Health Technician. These five veterinary areas are called Animal Health Technician Areas (AHT areas) and they are as follows: Omafo, Eenhana, Omundaungilo, Okongo and Omauni AHT areas. However, due to the small population of dogs in Omauni and Okongo AHT areas, these two areas were combined to become one (Omauni-Okongo) for sampling purposes of this study and therefore only one sample represents both AHT areas. Omauni and Okongo AHT areas are geographically adjacent to one another and located in one constituency (Okongo constituency).

3.4 POPULATION

Brink et al. (2010) define population as “the entire group of persons or objects that meets the criteria which the researcher is interested in studying”. The population in this study are all vaccinated dogs in Ohangwena region. The population of this study was estimated based on the vaccination figures for the previous three years. The vaccinated dog population is therefore an average of all vaccinated dogs for the last three years, i.e. 2012, 2013 and 2014 in Ohangwena region which is estimated at 11,000 dogs.

3.4 SAMPLE AND SAMPLING METHODS

Sample and sampling methods are defined as follows:
3.4.1 Sample

Brink (2010) defines a sample as part or fraction of a whole, or a subset of a larger set, selected by the researcher to participate in a research study. He further explained that a sample is representative of a population. It consists of a selected group of elements or units of analysis from a defined population but it has the same characteristics as the population. In this study, a probability stratified purposive sampling technique was used. Samples were collected from the four AHT areas (strata) as per the sampling method in 3.4.2.

3.4.2 Sampling method

Sampling is the process of choosing a group of people, events, behaviour and other components to be included in a study (Burns and Groves, 2009). It involves selecting some parts representative of a population to the study, thereby estimating something about the entire population.

In this study a sample of 170 dogs was selected using stratified random sampling, where the strata were the four AHT areas that were represented in the sample obtained. Sampling was conducted at the vaccination crush-pens (vaccination kraals) during the annual vaccination of cattle against Food and Mouth Disease (FMD) and Bovine Contagious Pleuropneumonia (CBPP), as dogs are also being vaccinated against rabies annually at the same time with cattle during these campaigns. This allows a large number of dogs to be vaccinated because cattle owners find it easy to have them vaccinated together with cattle. Sampling was planned to be conducted one year from the previous vaccination and was therefore conducted from the 21st of May 2015 until the 13th of June 2015. The previous year, vaccination was conducted
from 08 April till 29 July 2015. This means that the samples were collected on time, i.e. one year from the last vaccination. Samples were taken from vaccinated dogs in all five areas under the Animal Health Technicians (AHT): Omafo, Eenhana, Omundaungilo and Okongo-Omauni. The sample size from each AHT area was calculated to be proportional to the vaccinated population from that respective area. At the vaccination point, all the dogs that qualified for the study were isolated from the rest and counted. If the number of qualified dogs present at the vaccination point was less than the required sample needed from that vaccination point, then all the dogs were sampled. If the dogs were more than the required sample, then a simple random sample was generated using Microsoft excel.

**Inclusion criteria**

The dogs that qualified for the study were those that had the following characteristics:

- Had been vaccinated at least once in the last three years, i.e. 2012, 2013 and 2014;
- The dogs had to be presented with their vaccination certificates as proof of vaccination;
- The dogs should have been five months old, as passive immunity would have waned by 8–12 weeks of age to a level that allows active immunization (Day et al., 2010).

**Exclusion criteria**

The dogs that were excluded from the study were as follows:
• All dogs that were younger than five months old were excluded from the study as the purpose of this study was to assess the presence of antibodies which resulted from an active immunisation;

• All unvaccinated dogs irrespective of their age;

• Dogs that were not vaccinated in the last four years;

• Dogs whose owners failed to present their vaccination certificates as proof of vaccination were also excluded from the study.

3.4.3 Sample size

Three (3) conventional methods of sample size calculation were used and they all yielded the same sample size of 162 serum samples. The first two methods used were: \( N = Z^2 \times (PQ)/d^2 \) and Epi info 7 with StatCal. The 95% confidence interval was used i.e. \( Z = 1.96 \). According to Knobel et al. (2008) and Atuman et al. (2014), the expected proportion of protective antibody titre in the vaccinated population is 70%, \( P = 0.7 \), \( Q = 1 - P \), \( d^2 = \) confidence limits 7%. However, since the researcher had used the less sensitive BioPro ELISA kit with the sensitivity 86%, the third method of sample size calculation, the AusVet’s Epitools which puts the sensitivity of the test into account - was also used, and the obtained sample size was still 162 dogs as it was obtained by using the first two methods of sample size calculation. However, to give more statistical power to the study, more samples were collected, amounting to 170 dogs. According to Aschengrau and Seage III (2014) the term ‘statistical power’ refers to ‘the ability of the study to demonstrate the association if one exists’. With a bigger sample size, a value approximate to the true value of rabies protective antibodies in the vaccinated dog population will be produced.
Table 3.1. Research’s sampling frame of the vaccinated dogs

<table>
<thead>
<tr>
<th>AHT area</th>
<th>Estimated dog population</th>
<th>Number of samples collected (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omafo</td>
<td>5110</td>
<td>79</td>
<td>46.50</td>
</tr>
<tr>
<td>Eenhana</td>
<td>2780</td>
<td>43</td>
<td>25.30</td>
</tr>
<tr>
<td>Omundaungilo</td>
<td>1880</td>
<td>29</td>
<td>17.00</td>
</tr>
<tr>
<td>Okongo-Omauni</td>
<td>1230</td>
<td>19</td>
<td>11.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11,000</strong></td>
<td><strong>170</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

3.5 DATA COLLECTION

Lowrence (2012) defined data as the records of observations, actions or patterns of symbols that stands for observed values or actions. Data collection was defined by Connaway and Powell (2010) as an essential component in the production of useful data for analysis which involves selecting or designing specific techniques to be used to collect the necessary data. In this study data collection methods and procedures were designed in a way that guaranteed data quality, reliability and acceptance from the participating dog owners. The data collection procedures are described as follows:

3.5.1 Development of the research instruments

A structured questionnaire for data collection was created using Epi info 7 and divided into two sections, namely section A and B. About 98% of the time, the researcher completed this questionnaire due to the illiteracy of the owners of the participating dogs. The two sections and the respective contents of the used questionnaire are given below.
3.5.1.1 Section A: Demographic data of dogs and their owners

This section consisted of the demographic information of the sampled dogs, e.g. AHT area of the dog, type of surrounding where the dog lived, age group, sex, and size of the dog. Other information under this section is the age group of the dog owners and their educational status. Furthermore, this section consists of material such as the frequency of previous vaccinations of the dog, nutritional status of the dog, health status of the dog and the reason (usage) of the dog (pet, hunting, guarding, shepherding, etc.).

The dog breeds were not determined in this study; however, their sizes were determined based on three categories: small (< 40 cm in height), medium (40 < 50 cm in height), and large (≥ 50 cm in height) (Hidano et al., 2012).

3.5.1.2 Section B: Establish knowledge about rabies among dog owners

Under this section, a few questions were asked with the intention of assessing the dog owners’ knowledge of rabies. In this section dog owners were also asked to mention the type of wild animals seen around the surroundings of their residence.

3.5.2 Description of the research tools

In this study, the following tools were used during data collection namely: blood collection tools such as needles, blood tubes, protective/sampling gloves, methylated spirit, a table, dog handling tools such as dog mouth muzzles and dog catching ropes and others. Cool boxes were also available and adequate ice blocks/packs were used as well in order to keep the samples cool. Additionally, files and writing pens were purchased for the purpose of this study. A centrifuge and pipettes were used when separating serum from the red blood cells of the samples.
3.5.2.1 The BioPro Rabies ELISA test kit as a research tool

Blocking ELISA for detection of rabies virus antibodies in serum or plasma was used. The kit was manufactured in the Czech Republic by O.K. SERVIS BioPro, www.biopro.cz. According to the test manual that came with the kit, the manufacturer indicated that this ELISA Rabies kit was developed for the detection of antibodies against Rabies in domesticated and wild carnivores and was validated with fox and dog serum samples. The manufacturer further indicated that this method is rapid and simple. In comparison with golden standard methods like fluorescent antibody virus neutralisation (FAVN), or rapid fluorescent focus inhibition test (RFFIT), the BioPro ELISA Rabies kit is more convenient because very often the quality of fox serum samples may be decreased by bacterial contamination or autolysis.

Wasniewski and Cliquet, (2012) conducted an evaluation on the BioPro ELISA kit by comparing it with FAVN test and found this kit to have a specificity of 100% and an overall agreement reaching 86%. Consequently, they recommended that the BioPro ELISA kit should be considered as a valuable method for assessing the levels of rabies antibodies in domestic vaccinated carnivores.

The conditions of validation described by the manufacturer were used to interpret the results of this study by using the methods provided by the manufacturer together with the kit. According to the manufacturer's instructions, the percentage of blocking (%PB) was calculated for each sample according to the manufacturer's specifications (i.e. %PB = ((ODNC − ODsample)/(ODNC − ODPC)) ×100), where ODNC is the optical density of the negative control and ODPC is the optical density of the positive
control and OD\textsubscript{sample} is the optical density of the sample. In the context of international trade, for this ELISA kit a positive titre for rabies neutralising antibodies was indicated by a signal equal to or greater than 70% PB, whereas a negative titre is indicated by neutralising antibody levels of less than 70% PB (The manufacturer's specification indicate that serum samples with PB equal to or higher than 70% are considered as serum samples with antibody levels equal to or higher than 0.5 IU/mL based on the FAVN test). The manufacturer’s information was further used to interpreted the results of the rabies BioPro ELISA kit as follows: dogs with Percentage Blocking (PB) from 0 - 39% were regarded as negative for rabies antibodies, and PB equals to 40% but less than 70 % was regarded as positive to rabies antibodies. However, these dogs had not acquired, and respectively maintained, protective antibody titres, whilst dogs whose PB was 70% or more were regarded to have acquired rabies antibody titres equal to or higher than 0.5IU/mL (which is the acceptable threshold level).

### 3.5.3 Procedure for data collection

On the day of data collection, the researcher prepared the transport, data collection equipment (questionnaires), all the necessary tools needed for blood collection and travelled with the assistants to the vaccination point which was randomly selected for sampling. At the vaccination point the researcher first explained to the dog owners the purpose and the benefits of the study, after which they signed the consent letter allowing the researcher to collect blood samples from their dogs. The dog owners were further interviewed according to the questionnaire.

The process of blood sampling started with manually restraining the dogs using minimal force. Furthermore, mouth muzzles were placed on the dogs' mouths to
prevent them from biting. Very few dogs that were difficult to handle were restrained using the dog-catching ropes. The age of the dogs was estimated by assessing the development and wear of the teeth, using the birth month/year from the vaccination certificates as well as the dog owners’ statement about the age of their dogs. Blood samples were collected from the cephalic or tarsal veins. The dog’s height was established using a one metre (1m) measuring tape in order to determine their size category. The three categories of body sizes used in this study were: small (< 40 cm in height), medium (40 <50 cm in height) and large (≥50 cm in height).

Each blood tube was marked with a number. This identity number was the same as the questionnaire number, and by default the identification of that specific dog. Blood samples were then allowed to clot and centrifuged at the Regional Veterinary Office. Thereafter the sera were separated and frozen at -10 degree Celsius, before they were delivered on dry ice to the Central Veterinary Laboratory (CVL) for testing.

### 3.5.4 Validity and Reliability

#### 3.5.4.1 Validity

Also known as accuracy, validity is the ability of a measuring instrument to give a true measure (i.e., how well it measures what it purports to measure) (Friis and Sellers, 2014).

The two supervisors for the researcher of this study and one Veterinary Epidemiologist agreed with the format and content of the questionnaire used. These experienced researchers evaluated the questionnaire to determine its content validity.
3.5.4.2 Reliability

According to Friis and Sellers (2014), reliability, also known as precision, is the ability of a measuring instrument to give consistent results on repeated trials. The data collection instrument (the questionnaire) used in this study can be considered reliable as the questions used were consistent with other similar researches done before (e.g., Hidano et al. (2012) in Japan, (Haimbodi et al. (2014) in Namibia, Lourenson et al. (1997) in Tsumkwe, Namibia. The pilot study was first conducted on 10 dog owners in order to pre-test the questionnaire. A few changes to the questionnaire were made after the pilot study such as: to re-categorise the age groups of the dogs from five to four age groups, as well as categorizing the dog owners' ages into age groups as the researcher found it difficult in obtaining the actual ages from most of the dog owners. The same questionnaire (after incorporating the changes from the pilot study) was used to collect data from all the 170 participating dog owners.

3.6 DATA ANALYSIS

After receiving the laboratory results, they were entered in the respective questionnaires. Data were eventually captured in Epi Info 7. The data were then cleaned and analysed accordingly by using Epi Info 7. The level of antibody titres obtained was compared to the international standards for protective antibody titres. The international level of rabies antibodies considered to be protective is ≥0.5 IU/ml (Kennedy et al., 2007). Microsoft Excel was used to display data using charts. Tables were also used in analysis to depict the results. Descriptive statistics such as frequencies and percentages were calculated using Epi info and Microsoft excel.
Where necessary, categorical variables were compared using Chi-square test. The 95% confidence interval and p-value were calculated to demonstrate statistical significance.

3.7 RESEARCH ETHICS

Research ethics provide guidelines for the selection of the study purpose, design, methods of measurement, the collection and analysis of data, the interpretation of results, and the presentation and publication of results (Burns & and Grove, 2009). It is a tool that guides and ensures that the research subjects are respected in a humane manner. In Namibia, animals are protected through an Animal Protection Act (Animals Protection Act, 1962). This research has not in any way contravened the above act.

3.7.1 Permission to conduct the research

Permission to conduct the research was granted by the University of Namibia’s research committee. Additional permission was obtained from Namibia’s Directorate of Veterinary Services (DVS). Furthermore, during the execution of the project, each dog owner was thoroughly informed about the aim and scope of the ongoing research and its benefits for the community.

3.7.2 Confidentiality and anonymity

Confidentiality was highly respected in this study as the dog owners’ names were not required in the questionnaire. The sampled dogs were not identified by their names or any device, but were given individual study numbers, i.e. from 1-170, corresponding to the respective questionnaire number. The consent letters were not
stapled to the questionnaires. Therefore no linkage could be made of owners and their dogs to the information in the filled-in questionnaires.

3.7.3 Informed Consent

The researcher explained the research process to all dog owners (participants) and ensured that the process was clear and understandable to them. Thereafter, the participants were asked to sign the informed consent letters permitting their dogs’ participation in the study. The dog owners that were minors were represented by adults from the same homestead. The adults then signed the consent letters on the minors’ behalf. The dog owners were given the opportunity to accept participating in the study at their own free will.

3.8 CONCLUSION

In this chapter, the methodology of the study was thoroughly described. A descriptive cross-sectional study design was used to describe the level of individual protective immunity in the vaccinated domestic dogs in the five animal health technician areas of Ohangwena region. The average of the number of vaccinated dogs in the last three years (2012, 2013 and 2014) made up the population from where a sample was derived. Sampling of dogs was conducted during the annual vaccination of cattle against FMD and CBPP. The samples were tested by using a highly internationally recommended rabies ELISA kit (BioPro ELISA kit) purchased from the Czech Republic. The methods and interpretations of the BioPro ELISA kit were described in this chapter. The structured questionnaire was used to collect data. Consent from the dog owners was obtained and the confidentiality of the obtained information was assured.
CHAPTER FOUR

STUDY RESULTS

4.1 INTRODUCTION

In this chapter the analysed results will be presented. The research design and methods of the study were described in chapter three. The purpose of this study was to assess the level of protection against rabies in the vaccinated dog population through antibody testing. Moreover, the study was aimed at assessing the level of people’s knowledge of rabies in the region, specifically dog owners. The results of this study are based on the data collected by means of a structured questionnaire used by the researcher. Firstly the demographic description of the dogs and their owners were presented. This was then followed by a presentation of the analyzed data describing the rabies protective antibody level of the vaccinated domestic dogs in the region as well as a description of the relationship between demographic characteristics of the dogs and their owners and the level of rabies antibodies in the sampled dogs. The chapter will further describe an assessment of the level of rabies knowledge in the dog owners. The analyzed data were presented in the form of frequencies, tables, percentages and graphs. In this chapter, findings for all four objectives are presented in order to address the main purpose of the study.

4.2 DATA MANAGEMENT AND ANALYSIS

The study methods and procedures followed during data collection, data capturing and analysis thereof are described below.
4.2.1 Data collection and management of data

After the researcher was granted ethical approval from the University of Namibia’s Research Committee, the researcher further went ahead to request and successfully obtained an additional but compulsory permission to conduct the study from the Chief Veterinary Officer/Director of Veterinary Services under the Ministry of Agriculture, Water and Forestry. The collection of data started after the sampling frame was established per the five AHT areas as prescribed in chapter 3. The total number of samples collected was 170 blood samples from vaccinated dogs. Data collection started from Okongo-Omauni AHT area due to the fact that cattle vaccination campaign against FMD and CBPP was first started in this area.

During data collection only dogs meeting the inclusion criteria qualified, as described in chapter 3. The questions were easy to answer and not leading in any way. Every question in the questionnaire had alternative answers. The completion of each questionnaire took less than 10 minutes, which was important because during vaccination campaigns of dogs, prolonged overcrowding of these animals should be avoided as dogs often start fighting with one another, which can complicate sampling and even rabies vaccination. After completion, the questionnaires were filled and stored in the office where access was only granted to the researcher. Data were kept confidential and anonymous as no owners’ names were entered in the questionnaires and the consent letters were not attached to the questionnaires. In this way owners could never be linked to the information of their dogs.
4.2.2 Data analysis

All 170 dog sera were tested at the Central Veterinary Laboratory. After completing the test, the laboratory first sent the results to the manufacturer of the test kit for the manufacturer's experts to comment on the results before analysis could start. The manufacturer's experts were very satisfied with the results and recommended the data analysis. Upon receiving the results from the testing laboratory, the researcher entered them in the respective questionnaires according to their identification numbers (i.e. 1-170). After entering the laboratory results in each questionnaire, the researcher then captured all questionnaires into Epi Info 7. The data were later exported from Epi Info 7 to Microsoft Excel. Data were cleaned and analysed accordingly using Epi Info 7 and Microsoft Excel. Some variables were re-coded and dichotomized where appropriate on Epi Info 7. Categorical and the dichotomized variables were compared using Chi-square test, and a p-value < 0.05 was taken as a cut off point for statistical significance. Descriptive statistics such as frequencies and percentages were calculated using Epi Info 7 and Microsoft Excel. The association of the demographic characteristics of dogs and their owners with the level of protective antibody titres in the dogs was described using frequencies, percentages and Chi-square test when necessary. The association of rabies knowledge of the dog owners with demographic characteristics was also described accordingly. The level of antibody titres obtained was compared to the international standards for protective antibody titres of ≥0.5 IU/mL. Microsoft Excel was used to display data using bar charts and pie charts. Tables were also used in analysis to depict the results.
4.3 DEMOGRAPHIC DATA OF THE VACCINATED DOMESTIC DOGS AND THEIR OWNERS

4.3.1 Name of the AHT area

The majority of the sampled dogs came from Omafo AHT area while the least samples were from Omauni/Okongo AHT area. The samples obtained in each AHT area are presented in the table below.

Table 4.1: Numbers of samples per AHT area

<table>
<thead>
<tr>
<th>AHT area</th>
<th>Estimated dog population</th>
<th>Number of samples collected (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omafo</td>
<td>5,110</td>
<td>79</td>
<td>46.50</td>
</tr>
<tr>
<td>Eenhana</td>
<td>2,780</td>
<td>43</td>
<td>25.30</td>
</tr>
<tr>
<td>Omundaungilo</td>
<td>1,880</td>
<td>29</td>
<td>17.00</td>
</tr>
<tr>
<td>Okongo-Omauni</td>
<td>1,230</td>
<td>19</td>
<td>11.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>11,000</strong></td>
<td><strong>170</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

4.3.2 Type of surrounding of the sampled dogs

In order to describe the findings in terms of animal place, the three types of surroundings (town, village and settlement) were given as options, not forgetting that the study was meant for the village dogs. However, sampling took place as follows: 168 (98.8%) of the sampled dogs lived in the village set up and only 2 (1.2%) dogs lived in towns.

4.3.3 Sex of the dog owners

In this, the majority of the dog owners 121 (71%) were males in comparison to only 49 (29%) female owners.
4.3.4 Age group of the dog owners

Figure 4.1 below illustrates the number of the participating dog owners by their age groups. The dog owners older than 25 years were in the majority: 103 (61%).

![Bar chart showing age groups of dog owners](image)

Figure 4.1: Number of dog owners per their age groups

4.3.5 Educational level of dog owners

Table 4.2 presents the number of dog owners according to educational levels. People with primary education were the majority: 87 (51%) followed by those with no education: 44 (26%), while people with tertiary education were only 2 (1%).

Table 4.2: Number of dog owners per their educational level

<table>
<thead>
<tr>
<th>Educational level</th>
<th>Number dog owners</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>44</td>
<td>26</td>
</tr>
<tr>
<td>Primary</td>
<td>87</td>
<td>51</td>
</tr>
<tr>
<td>Secondary</td>
<td>37</td>
<td>22</td>
</tr>
<tr>
<td>Tertiary</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
4.3.6 Distribution of dogs’ sex

95 (56%) of the sampled dogs were males, the remaining 75 (44%) were females, giving a male: female ratio of 1.3:1.

4.3.7 Estimated age of the dogs

According to literature the effect of antibody development in different dog age groups varies. It was therefore important to consider each dog’s age in this study in order to establish its effect on rabies antibody development. The majority of the samples (90 = 53%) came from the young dogs in the age group of 1-3 years, followed by dogs in the age group of >3-5 years with 60 (35%), the older dogs from the age group of >5 years contributed 19 (11 %), and only one dog was younger than one year (1%) in the age group of 5-11 months. Figure 4.2 illustrates the number of dogs by their age groups.

![Figure 4.2 Number of dogs by their age groups](image)

4.3.8 Number of dogs per their body sizes
Literature revealed that the dog’s body size may have an effect on rabies antibody development. The three body sizes that were devised in this study were: small (< 40 cm in height), medium (40 < 50 cm in height), and large (Ô 50 cm in height). The number of dogs by their body sizes is presented in Table 4.3 below. The majority of the dogs 117 (68.8%) were of medium size, followed by large size 38 (22.2%), and small size 15 (8.8%).

Table 4.3. Number of dogs per their body sizes

<table>
<thead>
<tr>
<th>Body size of the dog</th>
<th>Number of dogs</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
<td>15</td>
<td>8.80</td>
</tr>
<tr>
<td>Large</td>
<td>38</td>
<td>22.40</td>
</tr>
<tr>
<td>Medium</td>
<td>117</td>
<td>68.80</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100.00</td>
</tr>
</tbody>
</table>

4.3.9 Vaccination status of the dogs

Table 4.4 illustrates the number of dogs per their vaccination status. The dogs that received vaccinations every year for the last three years or $P_{90}$ were 101 (59%), those that received only a single vaccination the previous year or $P_{75}$ were 46 (27%), and those that did not receive booster vaccinations within the last three years or $P_{Risk}$ were 23 (14%).

An interesting finding was observed at most of the vaccination points. A significant number of dogs (mostly young dogs) were presented for their first vaccination (although not counted for the purpose of this study), they were more than the dogs that were presented for their booster vaccination. At 88% (18/22) of the vaccination points, we could not collect the adequate numbers of blood samples intended for these specific vaccination points. The number of vaccination points had to be
increased to 22 in order to obtain the required number of samples for the study (=170).

Table 4.4. Number of dogs per vaccination status

<table>
<thead>
<tr>
<th>Vaccination status of dogs</th>
<th>Number of dogs</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twice last year</td>
<td>2</td>
<td>1.20</td>
</tr>
<tr>
<td>Annually for last 3 years</td>
<td>42</td>
<td>24.70</td>
</tr>
<tr>
<td>Annually for last 2 years</td>
<td>57</td>
<td>33.50</td>
</tr>
<tr>
<td>Once last year</td>
<td>46</td>
<td>27.10</td>
</tr>
<tr>
<td>Once 2 years ago</td>
<td>16</td>
<td>9.40</td>
</tr>
<tr>
<td>Once 3 years ago</td>
<td>7</td>
<td>4.10</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

4.3.12 Usage of the dogs

The aim for including this variable in the study was to establish whether there exists any association between the usage of a dog and its antibody development, and a possible effect of this variable on the dogs’ vaccination status. The study found that the majority of owners kept dogs for guarding (147 = 86%), followed by herding (12 = 7%), as a pet (7 = 4%), while only 4 (2%) owners reported to keep dogs for hunting purposes.
4.4 RABIES ANTIBODIES (Ab) LEVEL IN THE VACCINATED DOMESTIC DOGS OF OHANGWENA REGION

Using the information by Wasniewski and Cliquet (2012) as well as the testing manual of rabies BioPro ELISA kit, the results were interpreted as follows:

- Dogs with percentage blocking (PB) from 0-39% are regarded as negative for rabies antibodies (these results are consistent and often associated with unvaccinated dogs);
- Dogs with a PB equal to 40% but less than 70% are regarded as positive to rabies antibodies, however, these dogs have not acquired or maintained the internationally accepted protective antibody titres of 0.5 IU/mL;
- Dogs whose PB was equal to 70% or more were regarded to have acquired/maintained rabies antibody titres equal to or higher than 0.5 IU/mL which is an internationally acceptable threshold protective rabies titre.

4.4.1 Overall description of rabies antibody titres in the study sample

The overall description of rabies antibodies in the 170 dog samples, interpreted as per the manufacturer’s specifications is given in Table 4.5 below.

<table>
<thead>
<tr>
<th>Antibody Level (IU/mL)</th>
<th>Number of dogs</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab ≥ 0.5 (PB ≥ 70%)</td>
<td>136</td>
<td>80.00</td>
</tr>
<tr>
<td>Ab &lt; 0.5 (PB &lt; 40%)</td>
<td>18</td>
<td>10.60</td>
</tr>
<tr>
<td>Ab &lt; 0.5 (40% ≥ PB &lt; 70%)</td>
<td>16</td>
<td>9.40</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>
Among the 170 dogs, 136 (80%) of them maintained the required antibody titres ≥ 0.5 IU/mL (95% CI: 73.2% to 85.7%), 18 (10.6%) dogs were negative for rabies antibodies (Ab < 0.5 IU/mL, 95% CI: 6.4% to 16.2%), and the remaining 16 dogs (9.4%) were positive to rabies antibodies but this level of positivity was also not protective against the disease (95% CI: 5.5% to 14.8%).

4.5.1 Description of rabies antibody level by AHT area

The table below illustrates the description of protective antibodies in percentage per each AHT area.

![Figure 4.3. Distribution of Rabies protective antibodies per AHT area](image)

Apart from Omauni-Okongo, all the other three Animal Health Technician Areas were found to have protective antibodies above 70% with Omundaungilo area being the highest.

4.5.2 Antibody level by body size of the dogs

The level of antibody titres per the dog's body size is described in Table 4.6 below.
Table 4.6. Rabies antibody titres (IU/mL) in each category of dog sizes

<table>
<thead>
<tr>
<th>Dog size</th>
<th>Level of Antibody (Ab) titre IU/mL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 &lt;Ab (%)</td>
<td>Ab ≥ 0.5 (%)</td>
</tr>
<tr>
<td>Small</td>
<td>3 (20%)</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>Medium</td>
<td>23 (20%)</td>
<td>94 (80%)</td>
</tr>
<tr>
<td>Large</td>
<td>8 (21%)</td>
<td>30 (79%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>34 (20%)</td>
<td>136 (80%)</td>
</tr>
</tbody>
</table>

Table 4.6 indicates that a majority of the sampled dogs are of the medium size 117 (69%), followed by large dogs 38 (22%), and finally dogs in the small category 15 (9%). There is no difference in the level of protection in all size categories as about 80% of the dogs in all size categories acquired protective rabies antibody titres.

### 4.5.3 Antibody level per age group of the vaccinated dogs

The description of antibodies per age group is given in Table 4.7.

Table 4.7. Antibody level (IU/ ml) per age group of dogs

<table>
<thead>
<tr>
<th>Age groups of dogs</th>
<th>Antibody titre , IU/mL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab &lt; 0.5</td>
<td>Ab ≥ 0.5</td>
</tr>
<tr>
<td>5-11 months</td>
<td>1(100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>1–3 years</td>
<td>23 (26%)</td>
<td>67 (74%)</td>
</tr>
<tr>
<td>&gt;3–5 years</td>
<td>9 (15%)</td>
<td>51 (85%)</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>1 (5%)</td>
<td>18 (95%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>34 (20%)</td>
<td>136 (80%)</td>
</tr>
</tbody>
</table>

The only dog in the age group of dogs younger than one year (5-11 months) failed to maintain the required protective antibody titre. 74% (69) and 51 (85 %) of the dogs
in the age groups of 1-3 years and 3-5 years respectively, had protective antibody levels, while 18 (95%) of the dogs in the age group > 5 years acquired protective antibody titres.

Table 4.8. Comparison of antibody levels between the dichotomised age groups

<table>
<thead>
<tr>
<th>Dogs’ dichotomised age groups</th>
<th>Antibody level, IU/mL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab &lt; 0.5 (%)</td>
<td>Ab ≥ 0.5 (%)</td>
</tr>
<tr>
<td>≤ 3 years</td>
<td>24</td>
<td>67 (74)</td>
</tr>
<tr>
<td>&gt; 3 years</td>
<td>10</td>
<td>69 (87)</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>136</td>
</tr>
</tbody>
</table>

Despite the fact that dogs younger than 3 years made up the majority of this sample, 87% of the dogs in the age group older than 3 years maintained protective antibody titre compared to 74% of dogs younger than 3 years. Chi² = 4.2, df=1, P = 0.04.

4.5.4 Antibody level by vaccination status of the dogs

The effect of vaccination status on antibody development is described in Table 4.9 below.
Table 4.9. The relationship between the dogs’ vaccination status and the development of antibody titres.

<table>
<thead>
<tr>
<th>Vaccination status</th>
<th>Rabies Antibody titre, IU/ml</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab &lt; 0.5 (%)</td>
<td>Ab ≥ 0.5 (%)</td>
</tr>
<tr>
<td>Twice last year</td>
<td>0</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Annually for last 3 years</td>
<td>2 (5%)</td>
<td>40 (95)</td>
</tr>
<tr>
<td>Annually for last 2 years</td>
<td>10 (18%)</td>
<td>47 (82)</td>
</tr>
<tr>
<td>Once last year</td>
<td>12 (26%)</td>
<td>34 (74)</td>
</tr>
<tr>
<td>Once 2 years ago</td>
<td>6 (37%)</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Once 3 years ago</td>
<td>4 (57%)</td>
<td>3 (43)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>34 (20%)</strong></td>
<td><strong>136 (80%)</strong></td>
</tr>
</tbody>
</table>

About 88% of the dogs that received repeated vaccinations retained protective rabies antibodies. About 74% of the dogs that only had received a single vaccination without a booster a year ago also retained antibody titre equal to or greater than 0.5 IU/mL.

However, merely 63% of the dogs vaccinated only once in the last two years and 43% of the dogs having received a single vaccination three years ago retained the acceptable threshold of rabies antibodies.

4.5.4.1 Description of antibody level per vaccination status in terms of $P_{90}$ and $P_{75}$ categories of immune history

In Table 4.10, the vaccination history/status has been re-coded as follows:

**Category $P_{75}$**: Dogs which have had a single vaccination a year ago, where the proportion of 75% is expected to maintain threshold titres of $0.5$ IU/mL.
**Category P\(_{90}\):** Dogs which received one vaccination every year or twice or more within the past year, where a proportion of 90% was assumed to have threshold titres of 0.5IU/mL.

**Category P\(_{Risk}\):** Dogs that were vaccinated once in the past two years and only once in the past three years, i.e. the proportion at risk of rabies infection.

<table>
<thead>
<tr>
<th>Re-coded vaccination status</th>
<th>Antibody level , IU/mL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ab &lt; 0.5 (%)</td>
<td>Ab ≥ 0.5 (%)</td>
</tr>
<tr>
<td>P(_{75})</td>
<td>12 (26%)</td>
<td>34 (74)</td>
</tr>
<tr>
<td>P(_{90})</td>
<td>12 (12%)</td>
<td>89 (88)</td>
</tr>
<tr>
<td>P(_{Risk})</td>
<td>10 (43%)</td>
<td>13 (57)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34 (20%)</strong></td>
<td><strong>136 (80%)</strong></td>
</tr>
</tbody>
</table>

Only 74% of dogs in the category of P\(_{75}\) retained protective antibodies as compared to 88% of the dogs in category P\(_{90}\). In the population at risk only 57% of the dogs maintained protective antibodies. The association between P\(_{75}\) and P\(_{90}\) is Chi2 = 4.7, df=1, p= 0.03.

### 4.5.5 Description of the dog’s vaccination status by age groups

The association of age and the vaccination status of dogs is shown in Table 4.11 below.
Table 4.11. Vaccination status of sampled dogs by age groups

<table>
<thead>
<tr>
<th>Age group of dogs</th>
<th>Vaccination status</th>
<th>( \text{Total} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Annually for last 2 years</td>
<td>Anually for last 3 years</td>
</tr>
<tr>
<td>5-11mon</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1-3 years</td>
<td>34</td>
<td>7</td>
</tr>
<tr>
<td>&gt; 3-5 years</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>TOTAL</td>
<td>57</td>
<td>42</td>
</tr>
</tbody>
</table>

The young dogs (younger than 3 years) made up 53% of the sample followed by dogs in the age group of > 3-5 years with 35%. Furthermore, 80% (37/46) of the dogs being only vaccinated once the previous year were also younger than 3 years. 70% (42/60) of the dogs in the age group of >3-5 years received yearly vaccinations for the last two and three years. 84% (16/19) of the dogs older than 5 years received yearly vaccinations for the last two to three years.
4.6 LEVEL OF RABIES KNOWLEDGE IN DOG OWNERS

4.6.1 Presentation of rabies knowledge and practices among dog owners

4.6.1.1 Are stray dogs seen in the surroundings?

For purposes of establishing the possible sources of rabies infection in the region, dog owners were asked if they see stray dogs in their surroundings. The responses were as follows: about 107 (64%) dog owners responded to have seen stray dogs in their surroundings while 63 (37%) did not see any stray dog in their surroundings.

4.6.1.2 Which wild animals are seen around the surroundings?

The study also sought to establish whether the wild animals that potentially play a role in the transmission of rabies were seen by the dog owners in their surroundings. Only 82 dog owners responded to have seen wild animals in their surroundings. Squirrels and jackals constituted 44 (54%) and 38 (46%) respectively of the wild animals that were seen around the residences by the dog owners.

4.6.1.3 Are you aware of the disease your dog is being vaccinated against?

It is perceived that the people in the northern part of the country lack awareness of rabies hence the inclusion of this question and the subsequent two questions (4.5.1.4 and 4.5.1.5). Among the dog owners 138 (81%) responded with a "YES" while 32 (19%) of them were not aware of the disease their dog was being vaccinated against.

4.6.1.4 Where do you go for help when bitten by a dog?

Dog owners' responses are given in Table 4.14 below: about 99% of the dog owners would seek medical help and only about 1% would treat their wounds themselves.
Table 4.12. Number of dog owners by the type of source of help one would seek when bitten by a dog

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Number of dog owners</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td>168</td>
<td>98.80</td>
</tr>
<tr>
<td>Self-wound treatment</td>
<td>2</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>170</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

4.6.1.5 Do you know that rabies vaccine is available free of charge at government veterinary offices any time throughout the year?

146 (86%) dog owners responded with a "YES" while 24 (14%) of them were not aware of this fact.

4.6.2 Description of rabies knowledge in dog owners by their demographic characteristics
The relationship between rabies knowledge among the dog owners and their demographic characteristics is described as follows:

4.6.2.1 Description of awareness by dog owners’ level of education
The question of whether the dog owners are aware of the disease their animals are being vaccinated against and the level of their education is described in Table 4.15.

Table 4.13. Responses of dog owners on whether they are aware of the disease their animals are being vaccinated against by their level of education

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Owners’ level of education</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Primary</td>
</tr>
<tr>
<td>Yes</td>
<td>41</td>
<td>63</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>44</strong></td>
<td><strong>87</strong></td>
</tr>
</tbody>
</table>

About 81% (138) of the participants responded "YES", they are aware of the disease their dogs are being vaccinated against as compared to the 32 (19%) that responded
with a "NO". About 84% (27/32) of the dog owners who were not aware belonged to the "None" and Primary education levels.

**4.5.2.2 Dog owners who were not aware of the disease their dogs are vaccinated against by their age group**

The association between the age groups of dog owners who were not aware of the disease their dogs are vaccinated against has been illustrated in Figure 4.4.

![Bar chart showing the number of "NO" responses by age group.]

**Figure 4.4. The distribution of dog owners that responded with a “NO” on whether or not they are aware of the disease their animals are being vaccinated against**

The majority of dog owners who responded "NO" were found in the age group of 8-16 years 15 (47%), followed by the young adults in the age group of 17-25 years 10 (31%), and lastly the older group aged above 25 years 7 (22%).

**4.6.2.3 Description of awareness about free rabies vaccine by the owners’ level of education**

Awareness on free rabies vaccine and dog owners’ education level is described in Table 4.16 below.
Table 4.14. Dog owners’ awareness about free rabies vaccination of dogs and other pets at government veterinary offices throughout the year by the owners’ education level

<table>
<thead>
<tr>
<th>Type of response</th>
<th>Education level of the owners</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Primary</td>
</tr>
<tr>
<td>YES</td>
<td>42</td>
<td>70</td>
</tr>
<tr>
<td>NO</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>TOTAL</td>
<td>44</td>
<td>87</td>
</tr>
</tbody>
</table>

Apart from the annual vaccination of dogs at the crushpens 24 (14%) of the dog owners were not aware that rabies vaccine is offered at government veterinary offices free of charge throughout the year (95% CI: 80% to 91%). 79% (19/24) of the owners not being aware had none or primary education respectively (95% CI: 9% to 20%).

4.6.2.3 Awareness about the source of assistance in case of a dog bite by the dog owners’ level of education

The association between the awareness about the source of help in case of the dog bite and the education level has been described in Table 4.17.

Table 4.15 Response by dog owners on where one should seek help when bitten by a dog

<table>
<thead>
<tr>
<th>Source of help</th>
<th>Owners’ level of education</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Primary</td>
</tr>
<tr>
<td>Hospital</td>
<td>44</td>
<td>86</td>
</tr>
<tr>
<td>Self-wound treatment</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL</td>
<td>44</td>
<td>87</td>
</tr>
</tbody>
</table>
Almost all dog owners knew where one could go for help in case of a dog bite: 98.8% (95% CI: 95.8% to 99.8%). However, two persons responded to treating their own dog bite wounds or 1.2% (95% CI: 0.14% to 4.2%). The two respondents were of primary and secondary level of education respectively.

4.6.2.4 Description of wild animals seen per AHT area

Table 4.8 below describes the type of wild animals seen in the different AHT areas in the region.

4.16. Distribution of wild animals seen around the four AHT areas

<table>
<thead>
<tr>
<th>Type of wild animals</th>
<th>Name of AHT Area</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eenhana</td>
<td>Okongo/Omauni</td>
</tr>
<tr>
<td>Jackals</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Squirrels</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>28</td>
<td>19</td>
</tr>
</tbody>
</table>

Eighty two (82) dog owners reported to have seen only squirrels and jackals in their surroundings, 53 % of these have seen jackals. Squirrels were seen in almost every AHT area with Eenhana AHT area having the majority of 61%. No dog owner reported to have seen jackals in Omafo AHT area while only one dog owner reported to have seen a jackal in Eenhana AHT area. Generally, jackals were only seen in Okongo-Omauni and Omundaungilo AHT areas with the latter inhabiting 61% of the jackals seen.
CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5. PRESENTATION AND DISCUSSION OF THE RESULTS

5.1 INTRODUCTION

The analysed results of this study were presented in chapter four. The research design and methods of this study were described in chapter three. The main purpose of the study was to describe the level of protective immunity against rabies in the vaccinated dog population of Ohangwena region. These results are based on the data collected by means of a structured questionnaire and by interviewing the dog owners. In this chapter the results are interpreted and discussed to give meaning to the collected data. In addition, the findings of this research are supplemented by literature to re-contextualize the results with existing literature based on the findings of similar studies. Subsequently, conclusion and recommendations will be made to address the main purpose of the study based on all the four objectives of the study.

5.2 DISCUSSIONS OF THE RESULTS FOR OBJECTIVE ONE

The following discussions of the results were focused on the first objective of the study.

5.2.1 Discussion of the results based on the demographic information of the vaccinated domestic dogs and their owners

This discussion is focused on the questionnaire and the laboratory results of the 170 dogs’ samples from the five AHT areas of Ohangwena region. The first objective was to establish the demographic information of the vaccinated domestic dogs and their owners.
The majority of the samples came from Omafo AHT area, which may be due to the fact that this area is the largest of all, having a bigger proportion of the vaccinated dog population in the region and densely populated homesteads, whilst the least samples came from Okongo-Omauni AHT area which can be explained by the sparsely populated homesteads and thereby few vaccinated dogs.

The reason why almost all sampled dogs were found to be living in a village set up is due to the fact that sampling was done concurrently with the vaccination campaign of cattle against Food and Mouth Disease and Bovine Contagious Pleuropneumonia. Nevertheless, the majority of dogs that were sampled at Eenhana state veterinary office (regional office) also came from the nearby villages, and only two dogs were from Eenhana town.

The majority of dog owners (60 %) who brought their dogs for vaccination belonged to the age group of above 25 years. Due to their age, it is possible that they could have accumulated personal experience in rabies over the years and gained a better understanding of the disease. A significant number of pensioners were observed to have brought their dogs for vaccination. The explanation for people in the age group of 17-25 years occupying second place in bringing more dogs for vaccination can be attributed to the fact that most cattle herders are in this age group. Children in the age group of 8-16 years were the least in bringing dogs for vaccination because vaccination was done mostly during school days. However, a significant number of children (16%) in this age group also presented their animals for vaccination which could be explained by their fondness for their dogs coupled with the received awareness on rabies at school. Some school going children would miss school just to
bring their animals for vaccination; others were seen at the vaccination points in their school uniforms. An interesting observation at all sampling points was that young children and women handled their dogs much better during vaccination, more so than older men. The explanation for this behaviour could be that women and children grow closer to their dogs by giving them some sort of affection than older men would.

The educational level of those that brought their dogs for vaccination was easy to understand since only a few dog owners with secondary and tertiary education were observed, unlike the composition of the study by Haimbodi et al., (2014) in the two northern towns of Oshana region. This can be explained by the fact that only a few people in these educational categories live in villages because they were expected to be away with employment in towns, in tertiary institutions etc. However, people with no education (none) and those with primary education levels were expected to be school going children (primary school), animal herders and the elderly people who are readily available in the villages.

This study sample consisted of more male dogs, contrary to that of Laurenson et al. (1997) whose research contained more female dogs in Tsumkwe, another Namibian village located in the eastern part of the country. Based on these findings it is therefore not clear what the right ratio between dog sexes in Namibia is.

About 53% of the dogs were younger than 3 years and only 11% were older than 5 years. Very few dogs older than 10 years were observed. The fact that the dog population is skewed towards young dogs could be an indication that the dog population changes quickly (dynamic). At the majority of the vaccination points it
was observed that a significant number of dogs - if not the majority (although dogs were not counted specifically for this purpose), that were presented for vaccinations are the young ones that came for their first vaccination. A most likely explanation for this is that in these communities dogs do not live long as they are mainly slaughtered for meat, and a few die from diseases before their next vaccination which happens a year later. However, given the culture of these communities, these dogs are always immediately replaced with young pups. It is therefore likely that there will always be many unvaccinated young dogs in these communities before the next vaccination campaign a year later.

Pure dog breeding in the northern part of the country is practiced on a very small scale. Traditionally people keep cross-breed dogs (mixed breeds) and most of them happen to make up the 69% of the medium sized dogs observed in this study. This study found that only 101 (59%) of the dogs received repeated vaccination within the last three years (2012, 2013 and 2014). The findings suggest that some dogs may die as a result of diseases or being slaughtered for meat before they could be revaccinated the following year. Other possible explanation for dogs missing their vaccinations may be due to emigration of cattle herders with their dogs to places with better grazing in the neighboring Angola, whose arrival and stay in that country may not coincide with the vaccination program. Similar to the findings of Knobel et al. (2008), this study also found that the majority of dog owners keep dogs for guarding purposes (guarding from intruders). Furthermore, just as it was suggested by Laurenson et al. (1997), hunting was also probably under-reported in this study as a reason for keeping dogs, owing to its illegality in Namibia.
5.3 DISCUSSIONS OF THE RESULTS FOR OBJECTIVE TWO

5.3.1 Discussion of the level of protective antibodies in the vaccinated dogs

The proportion of dogs that retained international acceptable rabies antibody threshold in Ohangwena region is 0.8 or 80% which according to Hidano et al. (2012) is beyond the required protection coverage level of 70%. We can therefore conclude that the annual vaccination campaign is achieving the intended objective for the population of 11,000 dogs. However, given the constant incidences of dog rabies in the region, it is suggested that the actual dog population in the region may be higher than the vaccinated proportion. Other dogs that might have been unaccounted for are those owned but difficult to handle during vaccinations, stray dogs around the towns, and dogs that are owned by people who do not own cattle, as many non-cattle owners may not take their dogs to cattle vaccination points.

It has to be taken into consideration that the dog population in these communities can be rather dynamic (as discussed in objective one). Thus, the level of protection may be relatively small, should the study be directed at establishing the level of protection in the whole population, as the unvaccinated proportion of dogs may be large.

The proportion of dogs (20%) that did not retain the internationally acceptable antibody threshold could be due to different factors contributing to vaccine failure as discussed in the following sections.

5.4 DISCUSSIONS OF THE RESULTS FOR OBJECTIVE THREE

5.4.1 Discussion of the described relationship between the demographic characteristics of the dogs and their owners with the level of rabies protective antibodies in the vaccinated domestic dogs
Okongo-Omauni AHT area could not reach the required prevalence of dogs with protective antibody titres. This could be explained by the fact that most of the dwellings in these areas are rather cattle posts or households with only few persons. Normally only few people live at the cattle post, in some cases only one person. Hidano et al. (2012) found that a single person-household reduced the frequency of dogs’ vaccination. In this case the dog has fewer chances of being taken to the vaccination point if an individual stays alone, compared to households with many persons.

In contrast to the findings of Kennedy et al. (2008), this study found no difference as far as development of rabies antibodies is concerned among the three dog body sizes. This study came to the same conclusion with the findings by Laurenson et al. (1997), that the dog’s sex did not have any effect on the level of antibody development since both sexes acquired almost the same level of protective antibodies. Dogs older than 3 years maintained the protective antibody titres better than the younger dogs (p=0.04), because older dogs were likely to have received repeated vaccinations over the years. This study had gained similar findings to those of Hidano et al., (2012). In this study 88% of the dogs in category P90 (dogs that received repeated vaccinations every year or booster vaccination the previous year) retained the internationally accepted threshold antibody titre of 0.5 IU/ml and higher. However, 90% of dogs in this category were expected to retain the accepted threshold. Furthermore, 74% of the dogs in category P75 (dogs that received only a single dose without a booster a previous year) also retained the acceptable threshold of rabies antibody titres, 75% of dogs in this category were expected to maintain the acceptable antibody threshold.
This study found a statistical significant difference between $P_{75}$ and $P_{90}$ with regards to the maintenance of protective antibodies ($p=0.03$). Due to lack of boosters for dogs in category $P_{\text{Risk}}$ (dogs that were vaccinated only once two or three years ago and thereby at risk of rabies infection), only 57% of the dogs retained protective rabies antibodies.

5.4 DISCUSSIONS OF THE RESULTS FOR OBJECTIVE FOUR

5.4.1 Discussion of the demographic information based on objective three

In this study about 107 (64%) dog owners saw stray dogs in their surroundings while 63 (37%) responded not to have seen any stray dogs in their surroundings. These stray dogs may be a source of the rabies infections that is still being observed in the region since they are not accessible for vaccination.

The fact that only 82 of the dog owners reported to have seen wild animals in their surroundings, and among those merely ground squirrels and jackals, is an indication of how few these animals are in this part of the country. Particularly jackals are more frequently found in the commercial farms in the centre and southern parts of the country. There were almost no jackals seen in Omafo and Eenhana AHT areas since these areas are densely populated. Nonetheless, the forests of Omundaungilo and Okongo-Omauni AHT areas are suitable for the habitation of these animals.

In concordance with other studies (Haimbodi et. al., 2014), dog owners in the villages of Ohangwena region were found to have a basic knowledge of rabies: more than 80% of dog owners were aware of the disease their dogs were being vaccinated against, 98.8% would seek medical attention in case of a dog bite and 86% of them knew that the free rabies vaccine exists at government offices throughout the year.
5.4.2 Discussion of the results on the relationship between the demographic characteristics of dog owners and their knowledge of rabies

This study found that, the younger the dog owners, the less they knew about the disease their dogs are vaccinated against. Older people seemed to be more aware of rabies probably due to their experiences about the disease accumulated over the years. The study found that the education level of 84% of dog owners, who did not show awareness, had little or no education. This group was made up of mostly young school going children, animal herders and the elderly people of those villages. This was in agreement with the study by Haimbodi et al. (2014) who found that people with education had a better understanding of the disease than those without education.

It was further established in this study that only 14% of the dog owners were not aware that rabies vaccine for pets is available at every government veterinary offices free of charge throughout the year. This can also be explained by their education levels since 79% of these persons had little or no education. Only two dog owners would not seek medical assistance at the hospital in case of a dog bite. This exhibits lack of awareness of the disease.
5.5 CONCLUSIONS

5.5.1 Conclusions for objective one

- The study was dominated by dogs equal to or younger than 3 years as 53% of the participating dogs came from this age group;
- The majority of dog owners were those with primary and no education respectively;
- Only 59% of the participating dogs received repeated/booster vaccinations;
- Many young dogs (about one year old) were observed as they were presented for their first vaccination.

5.5.2 Conclusions for objective two

- It was established that about 80% of the vaccinated dogs in Ohangwena region have acquired or maintained an acceptable antibodies threshold of ≥0.5 IU/mL.

5.5.3 Conclusions for objective three

- With the exception of Okongo-Omauni AHT area, more than 70% of the dogs in three other AHT areas retained protective antibody titres;
- There was no difference in antibody retention in dogs of different sizes;
- Older dogs were found to retain better the rabies protective antibodies than the younger ones (younger than 3 years);
- Only about 74% of the dogs that received a single dose a year ago retained the internationally accepted rabies antibody threshold;
- About 88% of the animals that received repeated vaccination annually or vaccinated twice the past year retained the protective antibody threshold;
• Only 63% of the dogs that were vaccinated once two years ago retained rabies protective antibodies;

• Only 43% of the dogs that received their last vaccination three years ago managed to retain rabies protective antibodies.

5.5.3 Conclusions for objective four

• The study found that 107 (64%) of the participating dog owners reported to have seen stray dogs in their villages;

• It was established that rabies awareness is less in dog owners with low education levels;

• Furthermore, the study found that age was also a factor as children and young adults lagged behind in some knowledge aspects of rabies disease;

• More than 80% of the dog owners had a basic understanding of the disease, especially the adults.
5.6 RESEARCH RECOMMENDATIONS

5.6.1 Recommendations for objective one

- Rabies education should be intensified to the general public particularly among village communities and school going children;
- Cooperation among the relevant stakeholders should be instituted to ensure effective rabies control (awareness, vaccination, stray dog management etc).
- Rabies vaccination campaigns should be increased to two times per year for all dogs; the second vaccination will cater for young dogs that will be born after the first vaccination as well as those that might have missed it. Dedicated regional rabies team (s) need to be formed for this purpose.

5.6.2 Recommendations for objective two

- In order to eliminate rabies incidences in the region (despite the 80% protection in vaccinated dogs), mass vaccination should be extended to all other possible available dogs in the region including stray dogs. Other mechanisms that include forceful vaccination of stray and violent dogs can be used.
- The use of rabies oral vaccine should also be exploited to cater for stray dogs and those that are difficult to handle during vaccination;

5.6.3 Recommendations for objective three

- Experimental studies to establish the effects of dog size on the development of rabies antibodies should also be undertaken. This will ensure that relevant vaccination programmes for different dog sizes are put in place;
- In order to address the issue of young dogs retaining protective antibodies poorly as compared to the older dogs, it is recommended that the vaccination be
increased to twice per year to have these young dogs given a booster within one year which at the same time cater for the dogs in category $P_{\text{Risk}}$.

- We further recommends that studies be undertaken to establish dogs’ life expectancy in the northern part of the country;

- Although genotyping and geno-sequencing could not be conducted in this study due to financial constraints, it is crucial to understand the dynamics of rabies virus circulating in this part of the country in order to institute relevant control measures. It is thus recommended that further studies on geno-sequencing of the rabies virus in the region be conducted.

5.6.4 **Recommendations for objective four**

- Community members need to be sensitised about the danger stray dogs play in rabies transmission;

- Rabies awareness programmes should be developed, prioritised, intensified and conducted in remote villages and schools. People should be informed to take their dogs regularly for vaccination in order to ensure adequate protection against rabies.

5.7 **LIMITATIONS OF THE STUDY**

Financial constraint was the major limitation to the study, putting into consideration the scope of study activities. The budget for this study was not sufficient to pay for the expensive antibody neutralization test performed at the University of Pretoria. The researcher opted for the cheaper serological test kit, the BioPro Rabies ELISA test kit. Even though this test kit has a slightly lower sensitivity compared to the neutralization test (Wasniewski & Cliquet, 2012), it has produced satisfactory results
in several researches. Therefore this test method is highly recommended in projects aimed at the eradication of rabies. Another proposed component of the study "Rabies genotyping of all rabid animal species" could also not be conducted due to the lack of research funds.

The study was limited by other factors as well, such as the unwillingness of some dog owners to give their actual age, claiming that the study was about dogs and not their owners. However, the researcher’s decision to categorise the age groups of dog owners eventually worked better.

The performance of the used vaccine (Rabisin by Merial) could not be compared with other vaccine brands due to it being the only vaccine offered for free by government in the region. Other possible factors that influence immunogenicity such as the route of vaccination, batch number of vaccine, health and nutritional status were also not tested in this study.

5.8 CONCLUSIONS TO CHAPTER FIVE

This chapter dealt with the discussions of the study results, conclusions and recommendations based on all four objectives. The results were interpreted to give meaning to the collected data. The main findings were listed under study conclusions from where the main recommendations were made.
6. REFERENCES


Gsell. (2006). Demographic, sparcial and behavioural heterogeneities in an urban dog (canis familiaris) population, relevant in planning rabies control in Iringa,United Republic of Tanzania. . Swiss tropical Institute ,University of Basel.


Sambo, M; Cleaveland, S; Ferguson, H; Lembo,T; Simon, C; Urassa, H; Hampson. (2013). The Burden of Rabies in Tanzania and Its Impact on Local Communities. . PLOS Neglected Tropical Diseases | www.plosntds.org.


7. ANNEXES

Annexure A: Approval letter from UNAM PGSC

TO WHOM IT MAY CONCERN

RE: RESEARCH PERMISSION LETTER

1. This letter serves to inform that student, Immanuel Hikute (Student number: 200005211) is a registered student in the School of Nursing and Public Health at the University of Namibia. His research proposal was reviewed and successfully met the University of Namibia requirements.

2. The purpose of this letter is to kindly notify you that the student has been granted permission to carry out postgraduate studies research. The School of Postgraduate Studies has approved the research to be carried out by the student for purposes of fulfilling the requirements of the degree being pursued.

3. The proposal adheres to ethical principles.

Thank you so much in advance and many regards.

Yours truly,

[Name and Signature]

[Name and Signature]

[Name and Signature]

Director, School of Postgraduate Studies
Tel: 2063523
E-mail: cnahmemanya@unam.na
Annexure B: Ethical Clearance From UREC

UNAM UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: SONPH/6/2015 Date: 10 February, 2015

This Ethical Clearance Certificate is issued by the University of Namibia Research Ethics Committee (UREC) in accordance with the University of Namibia’s Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the Faculty/Centre/Campus Research & Publications Committee sitting with the Postgraduate Studies Committee.

Title of Project: Rabies Sero-survey in vaccinated dogs and virus Genotyping in all rabid animal species, Oshangevuma Region

Nature/Level of Project: Masters

Researcher: EMMANUEL HIKUFE

Student Number : 200005219

Host Department & Faculty: School of Nursing and Public Health

Supervisor : Dr H J Amukago ; (Main) | (Co) Dr B. Kahler

Take note of the following:
(a) Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the UREC. An application to make amendments may be necessary.
(b) Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the UREC.
(c) The Principal Researcher must report issues of ethical compliance to the UREC (through the Chairperson of the Faculty/Centre/Campus Research & Publications Committee) at the end of the Project or as may be requested by UREC.
(d) The UREC retains the right to:
(i) withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,
(ii) request for an ethical compliance report at any point during the course of the research.

UREC wishes you the best in your research.

Prof. L Mapare
UNAM Research Coordinator
ON BEHALF OF UREC
Annexure C: Permission letter from the Ministry of Agriculture, Water and Forestry

TO WHOM IT MAY CONCERN

RE: PERMISSION TO CONDUCT A RESEARCH ON – DR E HIKUFE

This letter serves to inform you that, in April 2015; I verbally granted permission to Dr Emmanuel Hikufe (an FELTP Resident) to conduct the research in Ohangwena region titled Rabies sero- survey in the vaccinated dogs and virus genotyping in all rabid animal species.

I am therefore, duly confirming such permission with this written communication. The Directorate of Veterinary Services is confident that this research will contribute positively to the fight against rabies in Namibia.

Regards

[Signature]

Dr A Maseke
CHIEF VETERINARY OFFICE
MINISTRY OF AGRICULTURE, WATER AND FORESTRY
Annexure D : Concern letter from the dog’s owners

I, the owner of the dog (name of the dog), hereby declare that I understand the objectives and benefits of the Rabies study as explained to me by the researcher Dr E. Hikufe and fully give the authorization for my dog to be sampled for the purposes of this study.

Signed at ...

Witness: at ...

Signature for the researcher at ...
Annexure E: Research Questionnaire to dog owners

Title: Rabies sero-survey of the vaccinated domestic dogs and assessment of knowledge of Rabies among dog owners, Ohangwena region, 2015

Compiled By: E.H. Hikufe

Student No: 200005219

Course: Master’s degree in Applied Epidemiology and Laboratory Management

Institution: University of Namibia (Unam)

Qualifications: (1) Bachelor Degree Veterinary Medicine (Russia)
(2) Certificate in Management Development Program (Stellenbosch)

Supervisor: Dr H J Amukugo (Senior Lecturer Nursing Department) UNAM

Dr B Kahler (Senior Lecturer at the school of Medicine) UNAM
Dear Participant:

The aim of this study is to establishing the level of protection against rabies in the vaccinated domestic dog population through antibody testing of the dogs as well as to assess the knowledge about rabies among dog owners.

**The Objectives of the questionnaire are as follows:**

**SECTION A:**
- To explore and describe the demographic information of the vaccinated dogs and their owners

With this information and after receiving the results of the dogs’ samples we would be able to establish the level of rabies protective antibodies in the vaccinated domestic dogs as well as to describe the relationship between the demographic characteristics of the dogs and their owners and the level of rabies antibodies in the vaccinated domestic dogs.

**SECTION B**
- To assess and establish the knowledge about rabies among the dog owners

**Instructions:**

(1) This questionnaire may be completed by the dog owner or Veterinary official.

(2) Please mark with an “X” in the applicable column when completing this questionnaire.

(3) Answer all questions both in Section A and B.

(4) Be open, honest and objective in your answers, as this will determine the recommendations resulting from this research study.

(5) Dog owner’s names should not be written on the questionnaire to guarantee confidentiality and anonymity.
(6) The results of this research study will be available towards the end of January 2016.

Your participation in this research study is highly appreciated.

Yours,

Emmanuel H. Hikufe (researcher)
**Questionnaire №……..**

**SECTION A**

1. **Name of the HTA area:**
   - Omafo
   - Eenhana
   - Omundaungilo
   - Epembe
   - Okongo/ Omauni

2. **Type of surrounding**
   - Village
   - town
   - settlement

3. **Sex of the dog owner:**
   - Male
   - Female

4. **Age group of the dog owner:**
   - 8-16 years
   - 17-25 years
   - >25 years

5. **Education level of the owner**
   - None
   - Primary
   - Secondary
   - Tertiary

6. **Sex of the dog**
   - Male
   - Female
7. Estimated age of the dog

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5 -11 months</td>
<td>1-3 years</td>
<td>&gt; 3-5 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;5 years</td>
</tr>
</tbody>
</table>

8. Body Size of the dog

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Small</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>Large</td>
</tr>
</tbody>
</table>

9. Vaccination status

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Once 2 years ago</td>
</tr>
<tr>
<td>Once 3 years ago</td>
</tr>
<tr>
<td>Once last year</td>
</tr>
<tr>
<td>Twice last year</td>
</tr>
<tr>
<td>Annually for the last 2 years</td>
</tr>
<tr>
<td>Annually for the last 3 years</td>
</tr>
</tbody>
</table>

10. Usage of the dog

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hunting</td>
</tr>
<tr>
<td>Guarding</td>
</tr>
<tr>
<td>Herding animals</td>
</tr>
<tr>
<td>As a pet</td>
</tr>
</tbody>
</table>

SECTION B

11. Are stray/feral dogs seen in the surrounding?

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

12. Which wild animals are seen around in the surrounding?

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackals</td>
</tr>
</tbody>
</table>


13. Are you aware of the disease your dog has been vaccinated against?

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

14. Where to go for help when bitten by a dog?

<table>
<thead>
<tr>
<th>Hospital</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional doctor</td>
<td></td>
</tr>
<tr>
<td>Self-wound treatment</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

15. Do you know that the rabies vaccine is available free of charge at Government veterinary offices any time throughout the year

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
</tr>
</thead>
</table>

16. Rabies antibody level, IU/ml (to be completed after receiving the laboratory results)

| Ab <0.5 IU/ml (PB <40%) | |
| Ab < 0.5 IU/ml (40 ≤ PB <70%) | |
| Ab ≥0.5 IU/ml (PB ≥70%) | |
Annexure F: Letter from the editor of the thesis

4th December, 2015

Dear Sir/Madam,

I hereby acknowledge that I have edited Dr. E.H Hikufe's thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Applied Field Epidemiology and Laboratory training Program, titled “Rabies Sero-Survey in vaccinated domestic dogs and knowledge assessment of rabies among dog owners, in Ohangwena region”. The editing focused on the following areas: grammar; sentence order; clarity of ideas; punctuations; repetition of ideas/findings; and the overall organisation of the paper.

I trust you will find this in order.

Yours Sincerely,

Saara Mupupa