PREVALENCE OF BACTERIAL ZOONOSES IN SELECTED TROPHY HUNTED SPECIES, AND THE POTENTIAL OF HUMAN HEALTH RISK IN BWABWATA NATIONAL PARK, NAMIBIA

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ABSTRACT

Zoonotic diseases are infections acquired from vertebrate animals (wild or domesticated) animals to humans through direct or indirect contact with live animals, their derivatives or contaminated surroundings. The aim of this study is to determine the prevalence of potential bacterial zoonoses in selected trophy hunted species *Loxodonta Africana* (African Elephant), *Syncerus caffer* (African Buffalo), *Tragelaphus strepsiceros* (Kudu), *Hippopotamus amphibious* (Hippopotamus), *Hippotragus niger* (Sable antelope), and *Hippotragus equinus* (Roan antelope), and the potential human health risk in Bwabwata National Park, North East Namibia. The Park covers an area size of 6 274 km^2. It is divided in three Core Areas designated for wildlife conservation and controlled tourism namely: Kwando, Buffalo and Mahango core area and a large Multiple Use Area zoned for community-based tourism, trophy hunting, human settlement and development by the resident community. Forty-four tissue samples (kidney, heart, liver, spleen, lungs, and lymph nodes) were drawn from freshly shot carcasses of kudu (3), buffalo (2), hippo (1), roan (1), sable (1) and elephant (1). Blood Agar (BA), MacConkey Agar (McC) and Eosin Methylene Blue-agar (EMB) were used as culture media. A total of 16 isolates were obtained and identified using the Biochemical methods and the amplification of the 16S rRNA gene. All bacteria isolated are potential human pathogens. The prevalence 50% (8 of 16) of potential bacterial zoonoses was highest in Kudu followed by Sable 37.5% (6 of 16) and Hippo 31.25 % (5 of 16). While it was low in Buffalo 25% (4 of 16), Roan 18.75% (3 of 16) and Elephant 12.5% (2 of 16). Kidney tissues had the highest prevalence 68.8% (11 of 16), followed by liver 32.5% (10 of 16), heart 37.5% (6 of 16), lung 31.25% (5 of 16), spleen 31.25% (5 of 16) and lymph nodes 18.75% (3 of 16). *Klebsiella pneumonia* dominated the tissue organs 18.9% (recovered 8 times from 44 tissues), *Micrococcus caseolyticus* dominated the animals 66.7% (4 times from 6 animals). Phylogenetic relationship formed four clusters with 58-100% bootstrap values. This study demonstrated the prevalence of potential bacterial zoonoses in trophy hunted animals in BNP which might pose potential human health risk if transmitted. Trophy hunters and the BNP community should therefore be informed about the risks associated with trophy hunted animals and their derivatives.

Keywords: *Bacteria, Zoonoses, Trophy hunted animals, Bwabwata National Park*

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LIST OF ABBREVIATIONS

°C	degree Celsius
BA	Blood Agar
BLAST	Basic Local Alignment Search Tool
BNP	Bwabwata National Park
CVL	Central Veterinary Laboratory
DNA	Deoxyribonucleic acid
EMB agar	Eosin Methylene Blue-agar
IMViC	Indole Methyl red Voges-Proskauer Citrate
KAZA TFCA	Kavango-Zambezi Transfrontier Conservation Area
McC	MacConkey Agar
MP	Maximum Parsimony
MR	Methyl Red
MR-VP	Methyl red-Voges Proskauer
MUA	Multiple Use Areas
NCBI	National Center for Biotechnology Information
OF	Oxygen Fermentation test
PA's	Protected Areas

PCR	Polymerase Chain Reaction
PI	Primary Isolate
RNA	Ribonucleic acid
TB	Tuberculosis
TSI	Triple Sugar Iron agar

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DEDICATION

To my beloved family and friends

DECLARATIONS

I, Matheus-Auwa Ameya, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

Matheus-Auwa Ameya Date

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CHAPTER 1: INTRODUCTION

1.1 Orientation of the study

Zoonotic diseases are infections acquired from vertebrate animals (wild or domesticated) (WHO, 2011; Messenger, Barnes and Gray, 2014; Helmy, El-Adawy and Abdelwhab, 2017; CDC, 2018b) to humans through direct or indirect contact with live animals, their derivatives or contaminated surroundings (WHO, 2011). Despite significant attention given to zoonotic infections in the last half-decade, about 60% of all human infectious diseases are zoonotic and 75% of all emerging infectious diseases affecting humans are of animal origin (CDC, 2018). Consequently, zoonotic infections can cause major threats with global effects to public health, animal welfare and to wildlife conservation (Gebreyes *et al.*, 2014). In recent years, Southern Africa and particularly Namibia has seen more interactions between wildlife, domestic animals and human, thereby increasing the risk of diseases spreading between species. These interactions pose a threat to the livelihoods, health and incomes of communities living close to national parks and conservancies. In addition, Namibia has been known to have an ever-increasing number of conservancies in the last half decade (Turpie *et al.*, 2010; MET, 2015; Birch, 2017).

A conservancy is an area of land or areas of communal land or registered associations (with Local Authority or the State) who co-operatively manage natural resources for the purpose of conserving and using wildlife sustainably (Baker, 2010). The advent of conservancies, though of economic importance, has reduced the interface space between wild animals and humans, hence favouring the spread of zoonoses. In these conservancies, the effective scrutiny, monitoring of zoonotic diseases constitute a

significant challenge with direct human health implications on the population and professionals who are directly involved in trophy hunting (Cantlay, Ingram and Meredith, 2017). Similarly, with the increase in conservancy settings, the resulting movement of wildlife, people and livestock across conservancies and park boundaries may increase the potential for diseases transmission between species (Ferguson *et al.*, 2013).

In conservancies, trophy hunting and ecotourism are important forms of sustainable wildlife utilization. Trophy hunting is an organized activity conducted by professional hunters whereby wild animals are killed for certain body parts such as horns and the skin but not mainly for food (Frssaf, 2008; Barnes *et al.*, 2009). Lindsey, Havemann, *et al.*, (2013) reported that about 75% of the farmers in Namibia hunt wild animals for meat consumption, whereby the extracted meat is usually used to feed a great number workers on commercial farms and the remaining 15-25% of private farmland concentrates on making profit from game production (safari hunting, wildlife viewing, live wildlife sales, game capture and release) occasionally combined with domestic livestock.

Protected Areas henceforth referred to as PAs play an important role in the global conservation of biological diversity which is both ecologically and economically related (Turpie *et al.*, 2010; Launcher, 2011). PAs are defined as terrestrial and aquatic areas devoted to the management, conservation and protection for biodiversity and cultural values to achieve the long-term conservation of nature, and they are officially designated and regulated by legal means or other effective means (Goh and Yusoff, 2010).

Despite the long concern of wildlife being a potential source of zoonotic diseases, trophy hunting and ecotourism has drawn attention especially when they are considered a threat to the public health and to agricultural ecosystems (Azoh, 2014). At times wildlife-based activities may come in direct conflict with management and conservation principles.

According to Cooney *et al.*, (2017), trophy hunting is coupled with several forms of problems such as underlying biological, social and ethical problems which hinder the degree to which the sector contributes to conservation. However, while the presence of trophy hunting activities in PAs is acknowledged, biodiversity conservation remains key priority (Blanco and Fedreheim, 2011). In the same vein, even though trophy hunting is part of the wildlife-based activities, the livelihood of the rural communities living in conservancies in Namibia and Southern Africa largely depend on wildlife resources as a source of food and income. This includes ecotourism and domestic animal production (van Schalkwyk *et al.*, 2010; Barnes *et al.*, 2013; de Vos *et al.*, 2016).

Moreover, irrespective of the possibilities of zoonotic transmissions by aerosols, PAs in North East of Namibia are not fenced off. As a result, this allows for transboundary movement of wild animals resulting in a high risk of disease exchange across species (Ferguson and Hanks, 2012; European Commission, 2013). In addition, North East Namibia is representative of many conservation settings, including (Bwabwata National Park, Mudumu National Park, Salambala Conservancy, Kansa Ruparo National Park, Mangeti National Park, Kaudum National Park) with close wildlife-human-livestock interactions (MET, 2010). Although, the control of zoonoses in domestic animals may seem to be achievable, historically, evidence are showing a trend rising of certain diseases (Wiethoelter *et al.*, 2015).

Historically, bacterial zoonoses have been the cause of some of the major fatal diseases with a wide extend of occurrence of epidemics of human civilization (Taylor, Latham and Woolhouse, 2001). Some of these zoonoses have caused 26.2% of all life lost in 2012 accounting for 17% of all global deaths (WHO, 2016). Approximately, more than 25 human infectious disease outbreaks of zoonotic origin over a period of 10-years (1990-2000) were highlighted in a previous report by Bender and Shulman, (2004). The outbreaks were associated with visits to animal settings (Bender and Shulman, 2004).

Endemic zoonotic infections such as brucellosis (*Brucella spp*.), Q fever (*Coxiella burnetii*), leptospirosis (*Leptospira spp*.), rickettsioses (*Rickettsia spp*.), bartonellosis (*Bartonella spp*.) and plague (*Yersinia pestis*) are among others that considerably pose challenges to health workers and clinicians in both human and animal health settings (WHO, 2005). Recent research in Tanzania has revealed that several bacterial zoonoses including brucellosis, leptospirosis, and Q fever as a result of human-wildlife-livestock interface are common causes of human diseases (Grace, 2015; Zhang *et al.*, 2016). These diseases and several others are also known to affect herbivores wildlife species (Turner *et al.*, 2013).

Among herbivorous wildlife species that may die as a result of zoonotic infections such as bovine tuberculosis (TB) and *Bacillus anthracis* (Anthrax) include plains zebra (*Equus quagga*), blue wildebeest (*Connocheates taurinus*), Greater Kudu (*Tragelaphus strepsiceros*), African elephant (*Loxodonta africana*), springbok (*Antidorcas marsupialis*) and wild boar (*Sus scrofa*) (Gortázar *et al.*, 2007; Turner *et al.*, 2013). A recent study by Katakweba *et al.*, (2015) which aimed at determining whether the practice of co-grazing with cattle and wildlife (buffalo, wildebeest and zebra) in

Tanzania constitutes a risk of transmission of antibiotic resistant bacteria to wild ungulates reported that wildlife generally harbour higher number of resistant *Escherichia coli* and *Enterococci* than cattle. It was concluded that increased human activities within the animal's habitats resulted in a notable increase in interspecies' resistance bacteria gene transmission (Katakweba *et al.*, 2015). Therefore, extra observance and perfection in diagnostic techniques are of extreme importance. Moreover, there is a necessity to develop novel scrutiny methods in wildlife for some diseases may not be clinically or pathologically noticeable but serologically positive (Magwedere *et al.*, 2012).

This study investigated the prevalence of potential bacterial zoonoses in selected trophy hunted species and the potential human health risk in Bwabwata National Park (BNP).

1.2 Statement of the problem

The ever shrinking wildlife-human and domestic animal interface coupled with the consumption of game meat has become as of late a great human health concern and challenge (Alexander *et al.*, 2012; Hoffman, Swanepoel and Leslie, 2017). Namibia has a number of regulations that apply to the sustainable use of game which are applicable when harvesting game for meat production (van Schalkwyk and Hoffman, 2010). Despite the regulations, poor and rural communities in North East Namibia often obtain game meat from uncontrolled and unregistered facilities. These facilities include local markets, rural slaughter facilities and homemade meat butcheries operating without or no valid permits. In addition, the poor and rural communities consume meat from trophy carcasses with less control or no meat inspection by veterinarians. As a result, the utilization of uninspected game meat may contribute to the spread of potential zoonotic

diseases to rural communities, meat consumers, domestic animals and the surrounding populations through contact with infected or carrier animals or their derivatives. Moreover, there are insufficient documentations and publications on bacterial zoonoses (especially in wildlife and humans) in Namibia. Furthermore, there are no phylogenetic studies of zoonotic bacterial communities in BNP. It is also not known which trophy hunted animals' harbour a significantly more phylogenetic diversity of zoonotic pathogens of public concern in BNP. This is an ecologically important way to classify bacteria, as closely related bacteria often have similar ecological characteristics, functional traits and effects on their host or ecosystems.

1.3 Main objectives of the study

The overall aim of this study was to detect potential zoonotic infectious pathogens in selected trophy hunted species and the potential human health risk in BNP.

1.3.1 Specific objectives

a) To determine whether trophy hunted elephant, buffalo, kudu, roan, sable and hippo carry potential zoonotic bacteria.
b) To determine the prevalence of potential bacterial zoonoses in selected trophy hunted elephant, buffalo, kudu, roan, sable and hippo species.
c) To document the phylogenetic relationship of potential pathogenic zoonotic bacteria in trophy hunted elephants, buffalo, kudu, roan, sable and hippo in Bwabwata National Park.

1.4 Significance of the study

Understanding the prevalence of potential bacterial zoonoses in wildlife animal species in BNP may serve as a beneficial model for exploring the occurrence, spread and transmission of other infectious zoonotic diseases, both between animals, and from animals to other taxa including human. An inventory of organisms known to be associated with a particular species is considered critical baseline information for species management and conservation priorities. In addition, this information can be used as an important starting point for further studies to predict the spread of particular infectious diseases to new areas that are in close proximity with BNP.

1.5 Limitations of the study

Only two core areas namely; Mahango and Buffalo core area out of four core areas of the Bwabwata National Park were covered in this study. Furthermore, taking into account the terms of Namibia's hunting laws and regulations, this study did not have control over the hunting location within the park, date, species, size and the number of animals that were hunted. In particular, the samples of obtained in this study were depended on the trophy hunted animals. Moreover, the culturing method (selective media) used in this study, could not capture the full spectrum of bacterial microbial diversity. As a result, some bacterial microbes are uncultured organisms in nature and could not be detected. Furthermore, the study was limited by time.

1.6 Delimitations of the study

Results from this study may not be extrapolated to other PA's or conservancies in Namibia.

CHAPTER 2: LITERATURE REVIEW

2.1 Historical context of wildlife zoonotic diseases

Historically, infectious zoonotic diseases and human beings share a long history. This history is tied to the effects that these diseases had on wildlife and public health for thousands of years (Shanko, Kemal and Kenea, 2014; CDC, 2016; Han, Kramer and Drake, 2016). For decades, zoonotic diseases have been constraints affecting public health and wellbeing through impacts on livelihoods and animal health to support food security (FAO, 2012; Halliday *et al.*, 2015). For example, before the 20th century, the common known zoonoses amongst others were, plague, rabies, anthrax and tuberculosis (Shanko, Kemal and Kenea, 2014). Most of these diseases affected the people's capacity to efficiently manage their livestock and decreased food insecurity (Mangesho *et al.*, 2017).

Among these diseases, it is believed that tuberculosis was introduced into Southern Africa through imported cattle during the colonial era and thereafter spread and became endemic in wildlife, and later spread to human beings causing public health concerns (CDC, 2018). Furthermore, Anthrax has historically been responsible for causing mortalities among domestic and wild animals in sub-Saharan Africa, with an approximate 30,000 to 60,000 death of animals in South Africa (Hoffman, Swanepoel and Leslie, 2017). Anthrax is endemic in Northern parts of Namibia, where it affects mainly herbivorous mammals, both wild and domestic (Beyer *et al.*, 2012). Anthrax has caused devastating outbreaks in Southern Africa in 2013 and 2014, in Namibia, Zimbabwe and Lesotho (NICD, 2016).

2.2 The economic importance of the wildlife trophy hunting industry in Namibia

Despite the invaluable social, economic, environmental and conservation benefits that the wildlife industry played in Southern Africa, in Namibia, the wildlife trophy hunting industry has gained enormous importance (Birch, 2017).

Namibia is one of the major African destinations preferred by hunters, with one of the recognized, well managed and organized trophy hunting sector in Africa (van Schalkwyk *et al.*, 2010; Chiringa, 2016) with a current estimated number of 4,000 to 6,000 safari hunters visiting Namibia each year (Mcnamara, Descubes and Claasen, 2016). Currently, Namibia has over 400 registered professional hunters (Venter, 2015; NAPHA, 2018). An estimated 75% of hunting takes place on communal conservancies whereas the remaining 25% takes place on private lands (Lindsey *et al.*, 2012).

On the other hand, the Namibian Community based Natural Resources Management (CBNRM) which is an association of decentralizing conservation and management rights to local communities estimated that income generated from communal conservancies, trophy hunting and meat derived from trophies rose from 36% in 2014 (N$31.5 million) to 50% in 2017 (N$ 57 million) in-kind benefits (Venter, 2015; Birch, 2017; Matthys, 2017). In addition to this, at the end of 2015 there were 52 conservation hunting concessions in Namibia utilized in conservancies (NACSO, 2015).

During 2017, the value of the trophy hunting industry in Namibia generated a revenue about N$450 million from hunting on commercial conservancies (private game farms) (NACSO, 2015; Venter, 2015; Nakale, 2017). Likewise, between 2012 - 2013 and 2015 - 2016, trophy hunting generated more than N$39 million for the Game Products Trust Fund (GPTF) (Birch, 2017). In 2018, the Ministry of Environment and Tourism

revealed that trophy hunting generates revenue of about N$30 million annually for Namibia (NBC, 2018).

Returns from wildlife generated through community conservation including hunting has been a very important element to the rural people's livelihood and improving food security. In 2017, 15,000 jobs were created through hunting in various categories, including professional hunters, hunting guides, skinners and trackers (Nakale, 2017). Meanwhile, there are currently 42 joint venture lodges established in Namibian conservancies playing an important role in providing community benefits such as employment and household income including food (MET, 2018).

Table 1: The number of the major wildlife species hunted by trophy hunters in Namibia in 2008 and 2009 (van Schalkwyk *et al.*, 2010).

Species	Number 2008	Number 2009
1. Oryx	5845	3417
2. Warthog	4230	2517
3. Springbok	3704	2043
4. Kudu	3193	1835
5. Red hartebeest	2679	1586
6. Hartmann's zebra	1820	1064
7. Blue wildebeest	1532	895
8. Blesbok	1204	744
9. Black wildebeest	1163	705
10. Steenbok	1229	702
11. Common impala	1127	670
12. Eland	1002	580
13. Burchell's zebra	732	387

Considering the low populations of trophy hunted species on hunting farms and ranches in Namibia, Mostert and Hoffman (2007) reported that trophy hunting contributes lowest financial return per unit area. Furthermore, in 2010, trophy hunting generated N$ 9.9 million of which concession fees comprised 83%, whereas, the distribution and sales of meat comprised the remaining 17 % (van Schalkwyk *et al.*, 2010). A recent publication on the multi-sectoral analysis by Barnes *et al.*, (2013), indicated that trophy hunting contributed 19 % to the Gross National Product in 2004.

In realizing the importance of trophy hunting in Namibia, the Ministry of Environment (MET) has passed a Protected Areas and Wildlife Management Bill (MET, 2017) on wildlife-based activities which has also emphasized on hunting in Protected Areas (PA's) in order to ensure that these activities are environmentally, economically and socially sustainable to meet the wildlife conservation targets and the needs of the rural communities as stipulated in the National Development Plans (NDPs) and Vision 2030.

In addition to wildlife-based activities, currently, Namibia has about 659 different registered hunting farms, with over 20 state run protected areas and 82 registered communal conservancies, with the majority of protected land located along the coast, benefiting a large number of rural Namibians making it one of the world's successful conservation Region (Turpie *et al.*, 2010; MET, 2015; Birch, 2017).

In Namibia, trophy hunting is estimated to only eliminate about 1% of the country's wildlife population per annum (van Schalkwyk *et al.*, 2010). The major trophy hunted species during 2008 and 2009 in Namibia are shown in table 1. Furthermore, in Namibia, all PAs are declared under the Nature Conservation Ordinance No 4 of 1975 and are managed solely for wildlife management (MET, 2010).

2.3 The wildlife - human-livestock continuum and its consequences

The wildlife-human-livestock interface is recognized by the complex interactions involving humans, domestic animals, and wildlife, creating environments favourable to zoonotic infections (Martin *et al.*, 2011; Siembieda *et al.*, 2011). These interactions includes the growth in human population which has resulted in greater land use, thereby bringing human and livestock close to wildlife populations (Martin *et al.*, 2011; Phukon, 2015).

Notably, all wild animals, livestock including companion animals, possess a unique set of factors which may act as potential risk for transmission of zoonotic infections through their specific paths with human contact (Pedersen and Davies, 2009). The contact may involve, direct contact with animal mucus, water, faeces, soil, ingestion or inhalation of pathogenic spores.

In Zambia, a previous study has shown that *Kobus leche kafuensis* (Kafue lechwe antelope) presents a prevalence of 27.7% of *Bovine tuberculosis* (Munyeme *et al.*, 2010). This prevalence level indicates a potential risk of *Bovine tuberculosis* transmission to humans. Moreover, trophy hunters often hunt the *Kobus leche kafuensis* (Kafue lechwe) for meat and trophies. And yet, an estimated 80% *of K. leche kafuensis* carcasses are used for meat consumption (Siamudaala *et al.*, 2005; Lindsey, Barnes, *et al.*, 2013). Consequently, poachers, trophy hunters and meat consumers are at a high risk of infectious *Mycrobacterium bovis*.

Pavlin, Schloegel and Daszak (2009) further indicated that the simplest way to reduce the risk of zoonotic diseases is through the reduction of opportunities for transmission of

diseases from wildlife to humans. This is well recognized in previous systematic literature reviews providing extensive information, through prevalence surveys such as cases of 78% brucellosis in patients with fever (Dean *et al.*, 2012), 7.8% leptospirosis in humans (Saif, 2013) and around 75% zoonoses overall (Grace *et al.*, 2012; CDC, 2018). Furthermore, Cantlay, Ingram and Meredith (2017) determined the potential risks of transmission of zoonotic infections to humans from trade hunted animals, butchering and consumption of wildlife in Malaysia. Their study isolated 19 zoonotic bacterial species. The risk of transmission was associated with hunting (during capturing and handling of the animals, butchering, skinning and cutting of carcasses) and through the consumption of animals.

In East Africa, human and animals residing in pastoral communities make up the highest prevalence rates of brucellosis compared to those living in isolated small-holder systems (Ducrotoy *et al.*, 2015). Other specific bacterial zoonoses detected in pastoral communities in Tanzania include 3.5% brucellosis in inpatients, 8.8% leptospirosis, and 5.0% Q fever (Crump *et al.*, 2013).

Among the major bacterial zoonoses that are of public health concern is *Bacillus anthracis*, (Anthrax) which mainly affects all endothermic animals, including, human, livestock and wildlife (Friedman and Yakubu, 2013). Anthrax was recently reported to have killed more than 100 hippos and over 50 buffalo in the Bwabwata National Park, Kavango River in 2017 (Maseke, 2017). However, neither human cases nor livestock cases were reported during the time of the outbreak. Other bacterial zoonotic agents include *Mycobacterium bovis* (Smith *et al.*, 2006); *Campylobacter spp*, *Brucella spp.*, *Escherichia coli*, *Rickettsia spp.*, and *Shigella* (Cantas and Suer, 2014). In Southern

Africa, some of these bacteria are mainly harbored by wild animals serving as reservoirs with an increasing spill over to rural communities (Zinsstag *et al.*, 2008).

2.4 The physical continuum and the transmission of zoonotic bacterial infections

Defining the continuum in a physical sense is important to understanding the infection transmission dynamics between human-livestock and wildlife animals. All zoonotic diseases can be categorized based on the exposure of the infectious agent from animals to humans (Cantas and Suer, 2014). Several reports documented and classified bacterial infections according to the mechanism of transmission such as wildlife population density, and the relationship between wildlife and humans (Grace *et al.*, 2012; Cantas and Suer, 2014; Shanko, Kemal and Kenea, 2014; Helmy, El-Adawy and Abdelwhab, 2017).

2.4.1 Mechanisms of disease transmission

Several studies have demonstrated ways in which animals can transmit zoonotic infectious agents to humans and vice-versa (Cascio *et al.*, 2011; Scallan *et al.*, 2011; Havelaar *et al.*, 2013; Cantas and Suer, 2014; Fong, 2017). These include infections with direct transmission (e.g. Rabies, through bites) through contaminated environments (e.g. inhalation of Anthrax spores from contaminated soil) Barandongo (2015) and through food (e.g. *Campylobacteriosis*) (Grace *et al.*, 2012). Infections may also be through indirect transmission (e.g. via vectors, such as mosquitoes or ticks, e.g. Lyme disease). It is important to understand that zoonotic agents of the family *Enterobacteriaceae* can be transmitted from both sick and healthy animals (Cantas and Suer, 2014). Figure 1 present's potential routes of transmission of infectious zoonotic diseases from animal to

human and vice-versa. Moreover, for zoonotic bacterial species to persist, the infectious agents must infect humans or animals (Friend, 2006).

2.4.2 Infections of direct contact and their prevalence

Friend (2006) highlighted that most zoonotic infectious diseases are acquired through contact rather than bites from vectors such as ticks or fleas. These infectious diseases are transmitted from an infected animal host to a susceptible host (Sham, 2005). Of relevance is a study by Bender and Shulman (2004) and Angulo *et al.*, (2006) where several zoonotic outbreaks including *E. coli* O157:H7, *Salmonella* and *Coxiella burnetii* were acquired by humans through petting zoos, feeding wild animals and domestic animals in captivity.

Furthermore, hunters, wildlife professionals, animal keepers and butchers are also exposed to occupational infectious diseases through direct contact with live animals or carcasses and animal tissues such as kidneys, heart, lungs, liver, lymph nodes and spleen. Kumar *et al.*, (2015) reported that bacterial pathogens go through these tissues during their life cycles. Other routes of direct contact with zoonotic infections includes body fluids and excretions, and consumption of contaminated inadequately cooked meat or unpasteurized milk (Grace *et al.*, 2012).

Direct transmission is also a common situation of contact with bacterial foodborne zoonoses, such as *Campylobacter spp.* and *Salmonella spp.* which are reported as leading causes of bacterial foodborne zoonoses acquired from domestic animals (Scallan *et al.*, 2011; Havelaar *et al.*, 2013; Chlebicz and Śliżewska, 2018). Similarly, Yan *et al.*, (2010) isolated 6.28% of *Listeria monocytogenes* from raw meat and 4% from various

prepared food samples. According to Grace *et al.*, (2012) food is believed to be the utmost source of zoonotic infections in the United Kingdom. Whereas, meat (particular wild or game meat) as a protein source is believed to be the most source of zoonotic diseases in the African Region (Hoffman and Cawthorn, 2012; Hoffman, Swanepoel and Leslie, 2017).

Despite this, the role of wildlife and wildlife reservoirs in the transmission of many bacterial pathogens to human has been long recognized and it is now a known fact, until recently, been largely given attention (Azoh, 2014; Gebreyes *et al.*, 2014).

Throughout many regions around the world, wild boars (*Sus scrofa*) have been increasingly detected with the most important zoonotic bacteria including *Mycobacterium bovis, Brucella suis, Brucella melitensis* and *Brucella abortus, Coxiella burnetti*, *Yersinia pestis* and *Leptospira spp.*, among others (Meng, Lindsay and Sriranganathan, 2009). In some parts of the world, human-wildlife based activities such as recreational hunting and consumption of meat from wild boar has resulted in direct contact between humans and wildlife species whereby this interface has enhanced opportunities favourable for bacterial zoonotic disease transmission between wild boars and human (Navarro-Gonzalez *et al.*, 2015).

Similar relationships have been documented in Africa and elsewhere in the world where hunters run a higher risk of contamination as well as meat consumers (Cantlay, Ingram and Meredith, 2017). Persons associated with hunting, butchering and meat consumption risk the exposure and transmission of infectious agents through close-contact (e.g. contact with fluid during skinning) with carcasses or live animals (Cantlay, Ingram and

Meredith, 2017). Additionally, many hunted species of free-roaming wild ungulate such as the African buffalo, impala, kudu and giraffe have been reported to be brucellosis seropositive (Gomo *et al.*, 2011). Numerous studies provide evidence that contact between human, wildlife and their products may frequently cause transmission of zoonotic infections (Cumming and Atkinson, 2012; WHO, 2012). For example, butchering and skinning carcasses in the field without personal protective equipment have resulted in brucellosis cases in hunters in North American (Starnes *et al.*, 2004; Giurgiutiu *et al.*, 2009) and in Australia (Irwin *et al.*, 2009; Eales, Norton and Ketheesan, 2010).

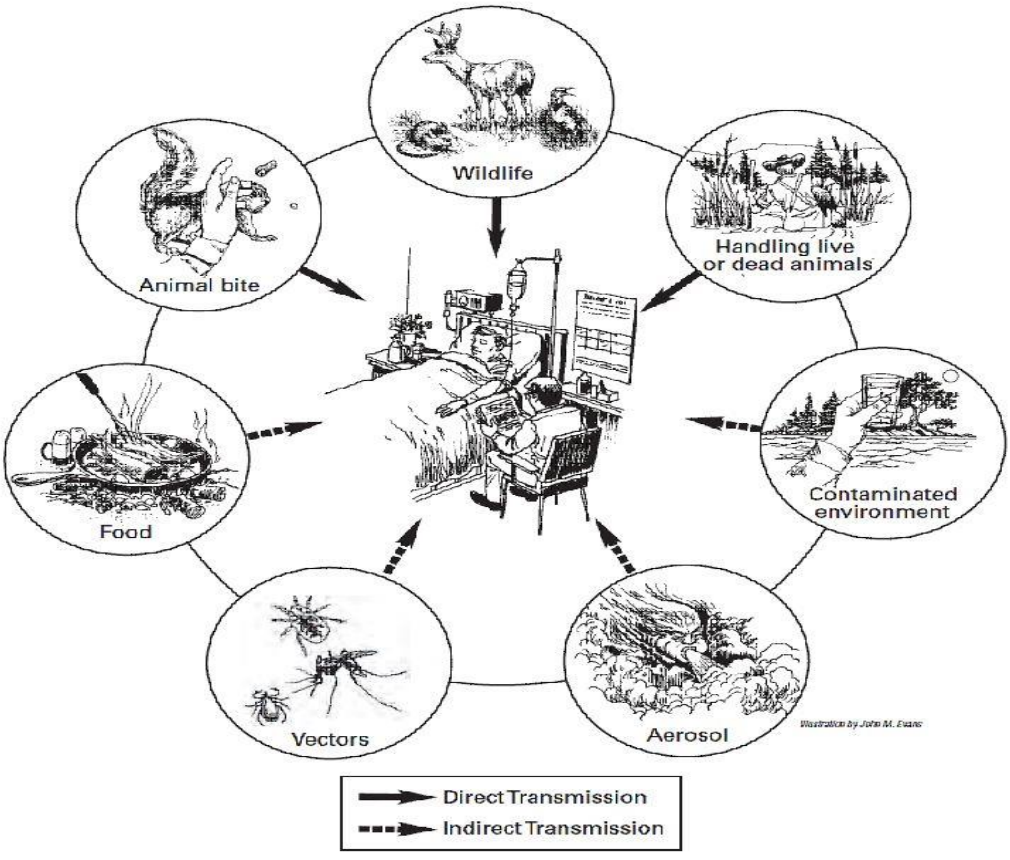

Figure 1: Potential common routes of transmission of bacteria zoonoses and how they are transferred from animals to human and vice versa (Friend, 2006)

As shown in Figure 1 above, human can be contaminated by handling live or dead animals or by wildlife found in human settings. Infected wildlife or livestock entering into human settings such as agriculture areas can be a potential threat to public health as well as other animal in the interface (Friend, 2006). In general, the exposure of human to zoonotic infections occurs in different routes, including contact with the contaminated physical environment. Therefore, understanding infectious diseases beyond the scale of individual cases requires intensive surveillance and assessment.

2.4.3 Infections of indirect contact and their prevalence

Friend (2006) demonstrated that infectious bacterial agents that fall in this category must go through a development phase outside the infected host before they are transmitted to the susceptible host (animal or human). These are infectious diseases that can be transmitted indirectly (e.g. consumption of animal products such as milk) (Newell *et al.*, 2010). Of relevance is study by Srivastava *et al.*, (2008) which isolated *M. bovis* and *M. tuberculosis* from cattle milk in north India. This however suggests the possible spillover of *M. tuberculosis* to humans from livestock through the consumption of unpasteurized milk. Similarly, zoonotic infections can be locally acquired from feral pigs which are confirmed as reservoirs of brucellosis and its acquisition has been associated with hunting and dressing of carcasses of feral pigs such as in a case were six hunters contracted brucellosis from wild swine in Florida MMWR (2009) and in Australian hunters from wild boar (Eales, Norton and Ketheesan, 2010). Furthermore, Brucellosis is known as a common human disease in Australia (Irwin *et al.*, 2009).

Moreover, Gadaga *et al.*, (2016) conducted a cross-sectional study in Zimbabwe in Malipati, Chikombedzi and Chiredzi, known as domestic animal-wildlife interface areas.

Their study focused on cattle owners, commodity chain and health-workers in order to assess the public health risk to food-borne zoonoses in these areas. Their study found that all the assessed respondents mentioned anthrax (69.2 %), tuberculosis (41.5 %) and brucellosis (23.9 %) as important zoonotic diseases. High proportions 98.4 % and 96.4 % of the respondents specified that they frequently consume game meat. This suggests that there could be a high means of zoonotic transmission with an increased risk of human exposure to zoonoses. Seemingly, these areas are located in the Great Limpopo Transfrontier Conservation Area (GLTFCA), an area considered with an increase in human-wildlife-livestock interface because of the shift in cattle by rural households into the game park in search of greener pasture.

Ernest (2015) investigated the microbial quality of fresh beef sold in retail markets in Ghana and found that the beef samples were contaminated with Staphylococcus spp, *Escherichia coli* and Salmonella spp. Of these, the prevalence of *Salmonella spp.* was found low (6 %) compared to Staphylococcus spp and *Escherichia coli* which recorded 47% each. The contamination of beef was associated with the slaughtering equipment in the cutting and processing of meat and inappropriate means of transporting carcasses to sale points by butchers. In the same way, human involved in these activities are at risk of zoonoses. In addition, Fong (2017) and Woodford (2009) reported that animal hides that are contaminated with Anthrax can cause infections. Besides animal meat and hides being a source of zoonotic infections, Goldberg *et al.*, (2008) investigated the genetic similarity of *Escherichia coli* from primates and humans and reported that sharing open water resources lead to infectious bacteria affecting both human and animal populations.

2.4.4 Risk factors influencing potential exposure of bacterial zoonoses

Bekker, Hoffman and Jooste (2012) outlined a number of risk factors influencing the prevalence of zoonotic infectious diseases in wild free-living animal species in Southern Africa. This includes the translocation and movement of domestic or wild animals and animal products and the global changing dynamics of agricultural practices shifting to wildlife farming (Rabozzi *et al.*, 2012; Grace, 2015; Phukon, 2015). The expansion in the host and vector, the wildlife-domestic interface, the ecological changes by human interventions, such as conservation measures and environmental alteration, and human population and their interactions with wildlife are among the factors facilitating the risk of bacterial zoonoses in wild free-living animal species and human (Martin *et al.*, 2011; Lindahl and Grace, 2015; Phukon, 2015). The risk may also result from contact with wildlife populations and wildlife products, such as the case of Leptospirosis in rice field workers, Anthrax during carpet weaving, Listeriosis in agricultural systems, Q-fever in abattoirs and trade in animal products in countries which import animal hides (Van der Merwe, Jooste and Hoffman, 2011).

In 2012, a study on behalf of the Meat Board of Namibia was conducted in order to assess the risk of animal disease hazards associated with import of animals and animal products (Thomson and Venter, 2012). The risk analysis concluded that the main animal disease threats to Namibia's livestock industries result from illegal entry of live animals that are either smuggled across the country's borders or grazed across the fencing system that protects Namibia's export zone against movements from the north and north-east. Contagious Bovine Pleuropneumonia was one the transboundary animal diseases reported to pause danger to Namibia (Thomson and Venter, 2012).

Several reports documented the risk of zoonoses from wild hunted animals in the developed world (Cantas and Suer, 2014; Helmy, El-Adawy and Abdelwhab, 2017). However, less attention has been focused on Africa and Southern Africa in particular (Asokan, Asokan and Tharyan, 2011). Regarding Southern Africa, Bekker, Hoffman and Jooste (2012) reported comprehensively on wildlife-associated zoonotic diseases in some southern African countries in relation to game meat safety which include Angola, Botswana, Namibia, Zambia and Zimbabwe and of which, Botswana, Namibia and Zimbabwe are neighbouring countries of South Africa. In addition to this, in South Africa, the common warthog (*Phacochoerus africanus*) are popular for recreational and trophy hunting and have traditionally been hunted and consumed as game meat (Hoffman, Swanepoel and Leslie, 2017). However, this species is associated with a number of important zoonotic diseases, such as the African swine fever and bovine tuberculosis (Hoffman, Swanepoel and Leslie, 2017). In Namibia, anthrax which has the second highest number of cases and widest geographical, domestic and wildlife species contributed 97% of the buffalo cases in 2012 (Magwedere *et al.*, 2012).

Meanwhile, the interdisciplinary method used by Atlas *et al.*, (2010); Cantlay, Ingram and Meredith (2017) and Daszak *et al.*, (2007) is essential to enhance our understanding of spill over dynamics of zoonotic diseases and the relationship between human-wildlife activities, such as wildlife hunting. On the other hand, it is the demand and increase for trophy and trade of wildlife products that increases the possibility of spread of zoonotic pathogens, therefore presenting potential human health risks in the region (Bekker, Hoffman and Jooste, 2012; van Schalkwyk, 2012; Magwedere, 2013).

2.5 Major bacterial zoonotic diseases of health concern in Southern Africa

In general, diseases with major zoonotic potential are the most pathogenic bacterial infections (e.g. Anthrax, Brucellosis, Tuberculosis) and these may have significant impact on wildlife and livestock populations as well agriculture-based activities (Sham, 2005).

Table 2: Classification of some zoonotic bacterial pathogens based on their causative agent, mode transmission and intermediate host

	Disease	Causative agent	Vector/host	Animals affected	Mode of transmission	Reference
Animal - born bacterial zoonoses	Meningitis, Pneumonia	*Escherichia coli*	Pets, domestic and wild Animals	Human, pets, domestic and wild animals	Faecal oral routes, Contaminated water or food	(Lu *et al.*, 2016)
	Among others; Pasteurellosis, Pneumonia or meningitis	*Pasteurella sp.*	Notably cats and dogs	Human, domestic and wild animals	Animal bite, scratch or nasal secretions	(Wilson and Ho, 2013; Cantas and Suer, 2014)
Farm animal-born bacterial zoonoses	Brucellosis	*Brucella spp.* notably *Brucella abortus*	Notably goats and sheep	Human, domestic and wild animals	Ingestion, contaminated pastures, food, uterine discharge, vaginal mucus, milk or semen plasma	(Beauvais, Musallam and Guitian, 2016)
	Rickettsioses, Q fever	*Rickettsia spp. Coxiella burnetii*	Ticks, lice, fleas, mites, domestic animals	Human, domestic and wild animals	Inhalation, ingestion, contaminated food, milk, faeces, urine, vaginal mucus	(Porter *et al.*, 2011; Yoshimizu and Billeter, 2018)
	Salmonellosis	*Salmonella sp.*	Pigs, cattle, cats, dogs, birds, reptiles	Human, domestic and wild animals	Faecal oral routes Contaminated water or food	(WHO, 2018)
	Campylobacteriosis	*Campylobacter sp.*	Notably poultry	Human, domestic and wild animals	Contaminated water, food, faecal oral routes	(Leedom and Spickler, 2013; Chlebicz and Śliżewska, 2018)
	Meningitis, Pneumonia	*Klebsiella pneumoniae*	Ubiquitous, Human, wide range of animals	Human, domestic and wild animals	Water, pasture, soil, food, notably faeces	(Martin and Bachman, 2018)
Wild animal-born bacterial zoonoses	Bovine tuberculosis (bTB)	*Mycobacterium bovis*	Cattle and buffalo	Human, domestic and wild animals	Ingestion, urine, saliva, milk, faeces, contaminated pastures, uterine discharges, handling contaminated animal products	(Fitzgerald and Kaneene, 2013)
	Anthrax	*Bacillus anthracis*	Human, domestic and wild Animals	Human, domestic and wild animals	Through air, inhalation of spores, contaminated feeds, water or soil, animal bite and scratches, handling contaminated animal products	(CDC, 2016)

2.5.1 Brucellosis

Brucellosis is a widespread zoonotic infection caused by bacteria of the genus *Brucella* (Moreno and Moriyón, 2006). Among the six *Brucella* species known to cause diseases in a significant number of animal species includes; *Brucella melitensis, Brucella abortus* and *Brucella suis*.

Bacteria of the genus *Brucella* can cause disease primarily in domestic animals including sheep, cattle, camels, goats, pigs, horses, moose, deer and dogs (Table 2) that are known to serve as a source of human infections (Moreno and Moriyón, 2006). The bacteria can be transmitted to human through direct contact with infected domestic livestock and wild animals, consumption of unpasteurized dairy products and inhalation and accidental inoculation (Cash-Goldwasser *et al.*, 2018). Other typical routes of transmission of brucellosis to human is by consuming bone marrow, lymph nodes or muscle tissue of infected carcasses (Alexander *et al.*, 2012; Mohammed, 2015). Hence, brucellosis should be considered significant to human health especially in developing countries where effective disease control measure and integrated zoonotic disease surveillance system are still at their infancy stage (Moreno and Moriyón, 2006). According to Godfroid *et al.*, (2011); Bekker, Hoffman and Jooste, (2012) and Godfroid *et al.*, (2013) the epidemiology of this disease in African wildlife, human and livestock is not well understood and recognized.

In Namibia, Brucellosis outbreaks in sheep have the potential to disturb Namibia's foreign income as the sheep industry contributes significantly to the economy of the country (Madzingira, 2013). In 2008, 2009 and 2010 an outbreak of brucellosis occurred

in Namibia, affecting sheep, goats and humans on a farm in Hardap Region with a sheep prevalence of 0.19% (Madzingira, 2013). In dairy cows on a dairy farm from 2011 to 2014 the prevalence showed 0.05% (Madzingira and Sezuni, 2017). Elsewhere in Namibia, the disease has been reported in sheep (Magwedere *et al.*, 2011) and springbok (Madzingira and McCrindle, 2014, 2016). Furthermore, a recent confirmed case of human brucellosis in Namibia motivated an investigation into the potential source of the infection (Magwedere et al., 2011). Magwedere *et al.*, (2011) have further shown that raw goat milk, home-made goat cheese and coffee served with raw goat milk, as well as contact with goats could serve as a source of human cases of brucellosis in Namibia.

In Botswana, a prevalence of 6% and 11% of brucellosis has been recorded in buffalo and giraffe respectively (Alexander *et al.*, 2012). It was suggested that the occurrence of the disease is still in its endemic status in the two species although 247 individuals were confirmed seropositive and acting as a potential source of bacterial zoonotic infections with low cases reported in humans with a prevalence of 46% in children. The seropositive buffalo were extensively distributed across areas with low cattle densities (Alexander *et al.*, 2012). Cases of brucellosis infections may also arise from butchering. In Nigeria, detection rates of brucellosis seropositivity among butchers range between 30% - 60% (WHO, 2010).

Water sources such as wells contaminated by contact with carcasses or infected animal excretions such as aborted foetuses, placentas, urine, manure may also cause a large amount of human infected cases (Arenas-Gamboa *et al.*, 2016; Saxena, Singh and Saxena, 2018). Common symptoms of human brucellosis are undulant fever,

manifestation with weakness, back pain headache, aches, night sweats and rarely coughing and chest pain (Arenas-Gamboa *et al.*, 2016).

Assenga *et al.*, (2015) reported the prevalence of brucellosis in humans, livestock, and wildlife in the Katavi-Rukwa ecosystem in Western Tanzania, in human the prevalence was 0.6 % and buffalo was 7.9 %. Their study indicated that the prevalence of brucellosis in buffalo is higher than that of the general human population in the area. They concluded that owing to the increasing human activities between human and wildlife interfaces, the transmission of the infection between wildlife, livestock and humans is expected to increase. Equally, Cash-Goldwasser *et al.*, (2018) conducted a study of patients presenting with fever to evaluate risk factors for brucellosis in northern Tanzania. Of 562 participants in the analysis, 50 (8.9%) were confirmed to have brucellosis. The risk of transmission was associated with the wildlife, livestock-human interface. Similarly, Bouley *et al.*, (2012) also investigated brucellosis among hospitalized patients in Tanzania and found (18.8 %) of the patients with confirmed acute brucellosis.

Furthermore, in many African sub-Saharan countries, brucellosis is endemic in cattle, it is known as a significant disease of economic and public health (Tanner *et al.*, 2015). During the period between 2009 and 2012, Motsi *et al.*, (2013) carried out a serological survey of brucellosis in wild ungulate species from five game parks. Samples were drawn from four different species, the African buffalo (*Syncerus caffer*), impala (*Aepyceros melampus*), black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) and were tested for brucellosis which showed (17.0%) seropositive brucellosis in buffalo and (1.4%) in impala (Motsi *et al.*, 2013).

Brucellosis induces abortion which is considered as an economic loss particularly in developing countries due to the loss in livestock (Madzingira, 2013). Brucellosis should therefore be considered a serious human health problem considering the variety of animal species it infects. To this end, (Ducrotoy *et al.*, 2015) stressed that as a global problem, the impact and distribution of brucellosis requires significant attention because of its public health concern.

2.5.2 Bovine tuberculosis or Tuberculosis (TB)

Mycobacterium bovis is the causative agent of bovine tuberculosis that is known to have established itself in different regions of the world and a well-known problematic disease in Southern Africa posing threats to human and animal health causing mortality, illness and economic losses (Miller, 2015). The disease affects buffalo populations and a variety of other wild animal species, including lions, leopard, spotted hyena (Table 2) (Fitzgerald and Kaneene, 2013; Miller, 2015). There is therefore a potential risk of transmission of *Mycobacterium bovis* from infected wild animals to livestock and humans in areas where they coexist (Michel, Müller and van Helden, 2010; Berg *et al.*, 2011).

Moreover, little is known about the proportion that *M. bovis* contributes to the global tuberculosis epidemic although in 2016, only 57% of the pulmonary cases of human Tuberculosis (TB) reported to WHO were bacteriologically confirmed (WHO, 2017). In 2016, an estimated 10.4 million people fell ill with TB with an estimated 1.3 million TB human deaths (WHO, 2017). Equally, a study in China on human tuberculosis identified 3,306 (99.5%) from 3,321 patient. Only 15 (0.5%) were *M. bovis* (Jou, Huang and Chiang, 2008).

Furthermore, the World Health Organisation (WHO) rated Namibia in the top 30 high TB burden countries in the world in 2017, with estimates of 446 cases per 100 000 population between 2016 and 2017 (WHO, 2017). Despite this high number of cases, the World Health Organisation (WHO) estimates that approximately 30% of patients with TB in Namibia remain undiagnosed, untreated or unreported (WHO, 2017). As a result, this has made Namibia one the worst affected countries in the world with the highest infection rates of human TB (MOHSS, 2016). According to Kahler, (2015), Namibia has not reported cases of bovine TB in cattle since 1995, this is because many cattle including livestock with bovine TB appear clinically healthy and that the disease may stay undiscovered until these animals present signs of continuous weakness.

Kahler, (2015), further stressed that the consumption of unpasteurized milk, milk products and undercooked meat are poor husbandry practices that might increase the risk of human tuberculosis infection in Namibia. Kahler, (2015), concluded that undiagnosed, and unreported cases of bovine TB are likely to occur in animals, particularly in remote rural areas close to the wildlife-interface, and represent a zoonotic hazard for the community.

In 2013, De Garine-Wichatitsky *et al.*, (2013) assessed and emphasized the zoonotic possibility about the main risk factors of bovine TB spill over at the wildlife-livestock-human interface outside the Kruger National Park in Sub Saharan Africa. Their study isolated *M. bovis* from 17 wild mammal species, although only four animals were suspected to play a role as maintenance host.

A review from 2013 assessed bovine tuberculosis in cattle and buffalo populations at the livestock-wildlife interface in two protected areas (Okavango Delta and Chobe National Park) of Northern Botswana (Jori *et al.*, 2013). The bovine TB prevalence in buffalo was 0 in Chobe National Park and 0.7% in the Okavango Delta, while the bovine TB prevalence in cattle was 0.7% in the Okavango Delta and 2.4% Chobe National Park. This was considered to be a serious public health concern, since the communities live in close contact with their animals, and are likely to consume untreated meat and milk.

Furthermore, *M. bovis* is widely spread among host animals causing diseases in its susceptible hosts which include cattle, goats, free-ranging wildlife such as buffalo, antelopes and humans (Al-Faris *et al.*, 2002). The outbreak of *M. bovis* in Kruger National Park is an example of where the disease originated from infected cattle as reported by authors in previous studies (de Lisle, Mackintosh and Bengis, 2001; de Garine-Wichatitsky *et al.*, 2010). Several recent reports demonstrated that *M. bovis* is still considered as a widespread infectious problem in cattle farming industries, as shown by the 37 cases which were reported between the period 2006-2008 in Lombardy (Italy) (Rabozzi *et al.*, 2012).

Despite the global recognition and contribution of zoonotic tuberculosis to human tuberculosis epidemic, the burdens caused by this disease are still unknown (Marcotty *et al.*, 2009). The disease is transmitted to human and wild animals through respiratory by aerogenous route or through consumption of contaminated daily products such as milk and other cattle products or secretions of infected animals as reported by Hamilton, Round and Sharp, (2002) and Ameni *et al.*, (2013). In fact, the transmission route of the infection to cattle is by inhaling or ingesting the bacterium as demonstrated by several

reported outbreaks observed in Lombardy (Italy) with 37 cases between 2006 and 2008 (Rabozzi *et al.*, 2012).

Furthermore, a study by Ameni *et al.*, (2011) indicated that 27% of the bacterial isolates from grazing cattle in Ethiopia were *Mycobacterium tuberculosis*. Of relevance is a study by Angkawanish *et al.*, (2010) on *Mycobacterium tuberculosis* infection of domesticated Asian elephants in Thailand. Their study isolated *Mycobacterium tuberculosis* from four Asian elephants. It was believed that the bacteria was transmitted to these elephants by human. *Mycobacterium tuberculosis* has also been isolated from livestock and wild animals in many countries worldwide (Jenkins *et al.*, 2011; Zachariah *et al.*, 2017).

The transmission of *Mycobacterium bovis* to human associated with hunting infected animals is well evidenced in a study by Wilkins *et al.*, (2008). They suggested that *Mycobacterium bovis* was acquired by human through an infected knife used on a *Mycobacterium bovis* positive wild deer. Further, the occurrence of *Mycobacterium bovis* in Nigeria was previously reported ranging between 2.5% to 14% and it has been detected in sour and raw milk at resident market places (Abubakar, 2007). Moreover, (Cadmus *et al.*, 2006) investigated molecular analysis of human and bovine tuberculosis from a local area in Nigeria, where 13% of *M. bovis* and *M. africanum* were isolated from human tuberculosis cases. A similar study conducted by Kazwala *et al.*, (2001) in rural areas of Tanzania isolated *M. bovis* in 4% of the culture positive pulmonary cases. Equally, 10% *M. bovis* has also been isolated from the same rural area in human (Cleaveland *et al.*, 2007). In a rural community of Uganda *M. bovis* was isolated from 6.9% of tuberculosis patients (Oloya *et al.*, 2008).

It is estimated that there is approximately 1.3% total burden of *M. bovis* in Tanzania (Coleman, 2002). The cause of this estimate was due to a large population of wild animals that are sometimes in close proximity with Masai population that use National parks as grazing areas. Therefore, more effort and assessments are needed for sensible estimations of the true status of the diseases in Namibia. Further investigations are necessary to better understand and contribute to the essential potential factors in the potential transmission and prevalence of *M. bovis* to human and animals in Namibia.

2.5.3 Anthrax

Anthrax is an acute infectious zoonotic disease caused by the bacterium *Bacillus anthracis,* with long lasting spores in the environment (Higgins and Dworkin, 2012). Equally, it is this persistence that represents a potential health concern to the public and livestock (CDC, 2016). Herbivores are known to be more susceptible to anthrax, both wild and domestic animals (Table 2) (WHO, 2014). The disease is well known for occasionally affecting human beings (Friedman and Yakubu, 2013). Anthrax has been reported in animal products, particularly food and in people who work closely with animals or handling animal products (Turnbull, 2008).

Anthrax affects African buffalo, Greater kudu, and waterbuck in many endemic areas in sub-Saharan Africa (de Vos and Turnbull, 2004; WHO, 2008). Buffalo are one of the species in demand for trophy hunting; they spend most of their time at water points which may be contaminated with anthrax thereby increasing their risk of exposure to anthrax consequently posing potential risk to human beings and their livestock (Ryan, Knechtel and Getz, 2006).

A recent study has shown that the African elephant (*Loxodonta africana*) may be a host of anthrax in Etosha National Park (Barandongo, 2015). Its occurrence has also been confirmed in buffalo herds in South Africa's Kruger National Park (Chaparro *et al.*, 1990), in Mozambique (Tanner *et al.*, 2015), in Impala (*Aepyceros melampus*) in Zimbabwe and Botswana (Alexander *et al.*, 2012), in Eland (*Taurotragus oryx*), Giraffe (*Giraffa camelopardalis*) in Tanzania (Fyumagwa *et al.*, 2009) and in Kafue lechwe (*Kobus leche Kafuensis*) in Zambia (Muma *et al.*, 2010).

Although anthrax is decreasing in many parts of the world, the disease remains persistent in many national parks such as those in Southern Africa and North America (Kruse, Kirkemo and Handeland, 2004). In Zambia, 44 cases of Anthrax were isolated from people in areas neighboring North Luangwa National Park (NLNP), evidently after the consumption of meat from Hippos (*Hippopotamus amphibious*) (Hang'ombe *et al.*, 2012).

Moreover, there is lack of information regarding the degree of Anthrax in human beings although it has been ranked as a high priority disease regarding animal health and poverty (Friedman and Yakubu, 2013). The disease affects many animals through the ingestion of contaminated soil, water, while feeding or by entry of spores through inhalation (Goel, 2015). The clinical sign in animals is rapid death. Humans can be infected through exposure by eating infected meat, contact with infected material, insect bites or inhalation of spores (Goel, 2015).

Table 3 presents a summary of the occurrence of bacterial zoonoses in wildlife species in Botswana, Namibia, South Africa, Mozambique and Zimbabwe.

Table 3: Summary of the occurrence of bacterial zoonoses reported in game animals in Botswana, Namibia, South Africa, Mozambique and Zimbabwe (Bekker, Hoffman and Jooste, 2012; Magwedere *et al.*, 2012; Magwedere, 2013).

Zoonotic disease	Species	Country
Anthrax: *Bacillus anthraces*	Buffalo (*Syncerus caffer*)	Botswana
	Buffalo (*Syncerus caffer*)	Mozambique
	Buffalo (*Syncerus caffer*)	Namibia
	Buffalo (*Syncerus caffer*)	South Africa
	Buffalo (*Syncerus caffer*)	Zimbabwe
	Elephant (*Loxodonta africana*)	Botswana
	Elephant (*Loxodonta africana*)	Mozambique
	Elephant (*Loxodonta africana*)	Namibia
	Elephant (*Loxodonta africana*)	Zimbabwe
	Hippo (Hippopotamus amphibious)	Botswana
	Hippo (Hippopotamus amphibious)	Zimbabwe
	Kudu(*Tragelaphus strepsiceros*)	Botswana
	Kudu(*Tragelaphus strepsiceros*)	Namibia
	Kudu(*Tragelaphus strepsiceros*)	South Africa
	Kudu(*Tragelaphus strepsiceros*)	Zimbabwe
	Roan antelope (*Hippotragus equinus*)	Botswana
	Roan antelope (*Hippotragus equinus*)	Zimbabwe
	Sable (*Hippotragus niger*)	Botswana
	Sable (*Hippotragus niger*)	Zimbabwe
Brucellosis: Brucella spp.	Buffalo (*Syncerus caffer*)	Zimbabwe
	Buffalo (*Syncerus caffer*)	South Africa
	Hippo (*Hippopotamus amphibious*)	South Africa
	Sable antelope (*Hippotragus niger*)	South Africa
Tuberculosis: *Mycobacterium bovis*	Buffalo (*Syncerus caffer*)	South Africa
	Kudu (*tragelaphus strepsiceros*)	South Africa

CHAPTER 3: MATERIALS AND METHOD

3.1 Description of the study area and study population

The study was conducted in Bwabwata National Park (BNP), North East part of Namibia as shown in Figure 2. The BNP covers an area size of 6,274 km^2, located at 17.8863° S and 22.7152° E (Pricope and Binford, 2012). The Park is located 200 km East of Rundu town and 517 km West of Katima Mulilo town. BNP is neighboured by four savannah countries namely: Angola in the North, Botswana in the South, Zambia and Zimbabwe in the North and East (Humphrey, 2015).

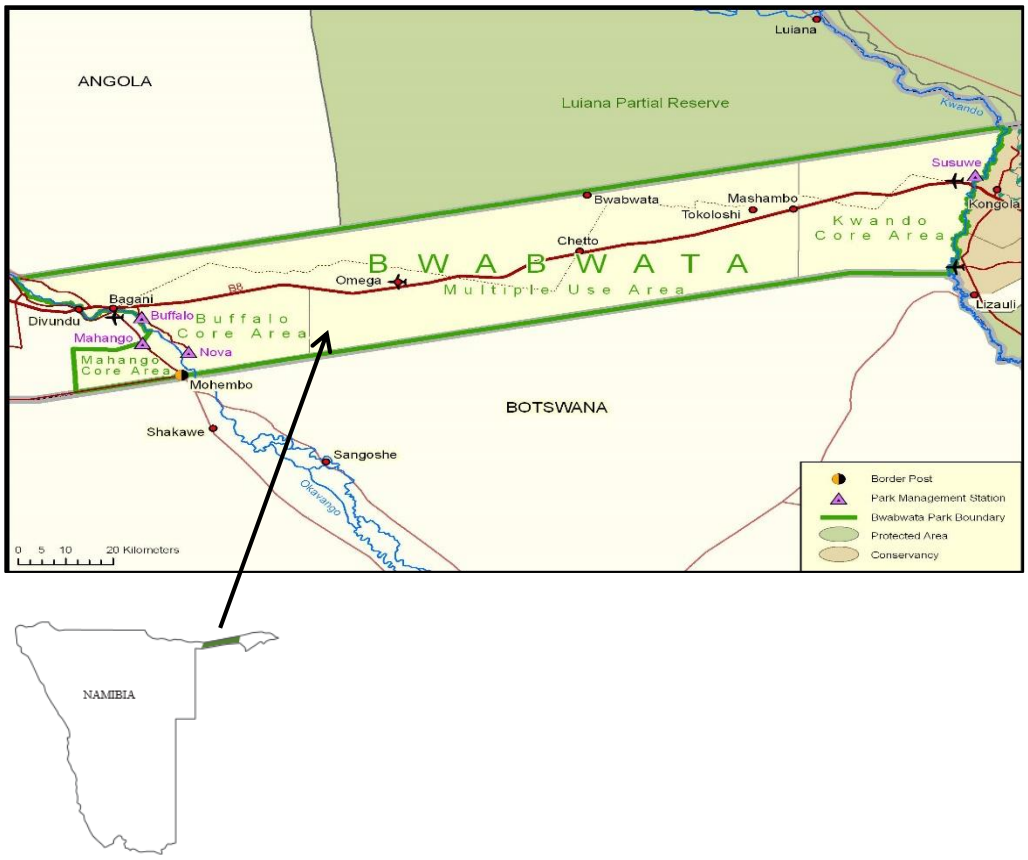

Figure 2: Geographic location of Bwabwata National Park (BNP) showing the boundaries, core areas and the road network within the Kavango East and Zambezi Regions (MET, 2013; Humphrey, 2015).

As part of the Kavango-Zambezi Transfrontier Conservation Area (KAZA TFCA), the BNP forms a corridor for large carnivore and wildlife population's migrations between Angola, Botswana and Zambia (Humphrey, 2015) and holds the largest territory of the world's migratory elephant (*Loxondata Africana*) populations (Taylor, 2007). The park is surrounded by floodplains of the Kwando-Linyanti, Kavango and Zambezi Rivers. The park is also home to numerous biodiversity hotspots because of its richness in bird species diversity and largest wetland ecosystem in Namibia (Taylor, 2007).

BNP management is viewed as the greatest achievement in Namibia, as both humans and wildlife co-exist in harmony which offers benefits to both biodiversity conservation and rural communities (MET, 2015). The park is located in an area which has a human population of about 220 000 (Immanuel, 2013). Most of the area is communal land, therefore, the livelihood of the rural communities residing in BNP and surrounding conservancies largely rely on subsistence farming especially, livestock and crop farming, and direct dependence on wildlife resources as a source of income (Beatty, 2011). Local level practices, including the animals they prefer to hunt for food, either through poaching or legally include but not limited to, elephants, kudu, hippopotamus, buffalo, zebra, crocodile, impala and roan antelope (MET, 2010; MET, 2015).

The park has been divided into three wildlife conservation core areas namely: Buffalo, Mahango and Kwando core area and a Multiple-Use-Area (MUA) to support the country's objectives towards the conservation biological diversity. The livelihoods of the

BNP's residents and surrounding communities residing in the MUA are involved in a variety of livelihood activities such as community-based tourism, trophy hunting, and human settlement. The Buffalo and Kwando core areas are mostly used for harvesting of natural resources such as wild food (Mulonga, 2003).

BNP is inhabited by people of the Kyaramacan Association, a representative legal body managed on democratic principles. The Association operates like a conservancy and largely depends on natural resources and wildlife for income and the livelihoods of the people in the Park (NACSO, 2015). In addition, the park's rich biodiversity attracts a great number of tourists which then realized the need for trophy hunting (MET, 2010).

3.2 Research design

During hunting, an opportunistic sampling was carried out. The tissue samples were drawn from the kills made by the trophy hunters. A purposive sampling method was used to select the group of animals for this study. The animals were solely identified and selected by the trophy hunters during the hunt. The group consisted of 6 species that are considered key trophy animals. The animals consisted of 9 individuals: 1 Elephant, 2 Buffalo, 3 Kudu, 1 Sable, 1 Roan antelope and 1 hippo.

3.3 Study species, sample collection, preparation and processing

The study population were animals that are considered key trophy animals. The target wildlife species for this study were: African elephant (*Loxodonta africana*), African buffalo (*Syncerus caffer*), Greater kudu (*Tragelaphus strepsiceros*), Sable antelope (*Hippotragus niger*), Roan antelope (*Hippotragus equinus*) and Hippopotamus (*Hippopotamus amphibious*). Tissue samples of spleen, liver, lungs, lymph nodes, heart and kidneys were drawn from freshly shot animals during slaughtering. A total of 44

tissue samples comprising of (13) Kudu, (11) Buffalo, (6) Elephant, (5) Roan, (5) Hippo and (4) Sable were collected respectively. The samples consisted of Kidney (n=12), Liver (n=7), Heart (n=4), Lung (n=6), Spleen (n=6) and Lymph node (n=3). The samples were collected at any time of the day depending on the hunting and slaughtering time. A Bio-safety plan (Appendix 1) was followed during the sampling. The plan was intended to provide instructions for all personnel involved during the field and laboratory experiments and the public from being exposed unnecessarily to equipment's and associated areas or contaminants that may contain potential infectious hazards during the study. Fresh tissue samples were collected from carcasses of the six selected trophy hunted species. Tissue samples were collected between May 2017 and August 2017. Tissue samples were collected by carefully cutting them immediately off from the carcasses using different sterile scalpels during slaughtering.

The collected tissues were packed in 3 (layers) of sterile plastics in forms of tissue blocks, approximately 500 cm^3. The plastics were completely sealed and were surrounded by enough absorbent material to completely contain a spill. The plastics were clearly labelled (sample identity, animal identification, area of collection, date of collection, principle investigator, and contact details) and placed in an ice insulated box in contact with a cooling agent and immediately transported to the Clinical Microbiology Laboratory at the Central Veterinary Laboratory (CVL) for analysis.

3.4 Bacteria culture and isolation

Bacterial isolation was performed in the Clinical Microbiology Laboratory Section at the Central Veterinary Laboratory (CVL) Windhoek, Namibia, following the CVL Biological Standard Operation test Procedures (BIO-SOP). Three selective agar media

were used in this study namely; Blood agar (BA), MacConkey (McC), Eosin Methylene Blue-agar (EMB) were used to culture bacteria. The procedures that were used to inoculate and incubate the agar plates were adapted from (Markey *et al.*, 2013).

From each tissue sample, a smaller size piece about 2.5g specimen was cut off using sterile scalpels and forceps. Each 2.5g specimen was first directly smeared individually on Blood agar (BA), MacConkey (McC) and Eosin Methylene Blue-agar (EMB) plates in duplicates using sterile forceps. BA was used to isolate gram-negative bacteria and detect and differentiate bacteria based on their haemolytic characteristics. Whereas, McC and EMB agar were used as a selective media for gram negative and differential for coliform (Ifeanyi, *et al.*, 2014; Nordmann, Jayol and Poirel, 2016).

Plates were incubated in duplicates both aerobically and anaerobically) at 37℃. Anaerobic media were incubated using a GasPak EZ Anaerobe Container System and aerobic plates were incubated and read after 24 hours for bacterial colonial or growth. From each plate, the streak-plate procedure was used to isolate pure cultures of suspicious bacteria from mixed colonies.

3.5 Morphology identification

The shape of the bacteria isolated in this study were observed under a light microscope as described by (Markey *et al.*, 2013).

3.6 Biochemical characterization

To identify and characterize bacteria isolated in this study, biochemical tests were used as described by (Markey *et al.*, 2013) (Figure 3).

1. Oxydase test

A small quantity of pure bacterial culture was transferred onto a clean slide with the aid of sterile inoculating loop. Two drops of oxidase reagent was then added on the slide. Oxidase (+) colour changes to dark purple within 5 to 10 seconds. Oxidase (-) if the colour does not change.

2. Catalyse test

A small quantity of pure bacterial colony was transferred into a drop of 3% Hydrogen peroxide solution on a clean microscopic slide with the aid of sterile non-metallic applicator. Gas seen as white foam indicated the presence of catalase enzyme.

3. Oxygen Fermentation (OF) test

Two tubes of OF test medium were inoculated with a pure bacterial colony using a sterile straight wire half way to the bottom of the tube. One tube of each pair was covered with 1 cm layer of sterile mineral oil to avoid the diffusion of oxygen and the other pair was left open. The tubes where then incubated at 37℃ for 24 to 48 hours. Fermentation results colour change from green to yellow. Oxidation results in acid production in the open tube.

4. Gram reaction test (Gram staining)

A thin smear of each of the pure bacterial colony was prepared on clean microscopic slides, and passed over a Bunsen flame. Gram staining procedure was performed following (Markey *et al.*, 2013). Gram negative bacteria appeared pink or red while gram positive bacteria appeared purple.

5. Triple Sugar Iron agar (TSI) tests: A 4 *ml* broth of Triple Sugar Iron agar (TSI) was prepared and inoculated with a bacterial colony from a pure culture using a sterile loop and incubated for 24 hours at 37°C. Following incubation, 3 drops (3*mg* each) of Kovacs reagent were added to TSI reactions. An alkaline slant-acid butt (red or yellow) indicates fermentation of dextrose only. An acid slant-acid butt (yellow or yellow) indicates fermentation of dextrose, lactose or sucrose. An alkaline slant-alkaline butt (red or red) indicates lactose were not fermented (non-fermenter). A black precipitate in butt indicates hydrogen sulfide production.

6. Voges-proskaeur test: A 4 *ml* Voges-Proskauer (VP) broth was prepared and inoculated with a bacterial colony from a pure culture. After incubation, 3 drops (3*mg* each) of Voges-Proskauer (VP) 1 and Voges-Proskauer (VP) 2 solutions were added to the 4 *ml* Voges-Proskauer (VP) reactions. A strong red colouration formed within 30 min indicates positive reaction.

7. Methyl red test: A 2 *ml* Methyl red (MR) broth was prepared and inoculated with a bacterial colony from a pure culture and incubated for 24 hours at 37°C. Following incubation, 4 drops (3*mg* each) of MR solution were added to the 2 *ml* Methyl Red/Voges-Proskauer (MR-VP) reactions. A red colour indicates a positive reaction.

8. Urease test: A 4 *ml* Urea broth was prepared and inoculated with a bacterial colony from a pure culture and incubated for 24 hours at 37°C. A colour change from a reddish-orange to a bright pink indicates a positive reaction. A negative reaction is indicated by no colour change.

9. Citrate Utilization Test: A 4 *ml* Citrate broth was prepared and inoculated with a bacterial colony from a pure culture and incubated for 24 hours at 37°C. A change from green to blue indicates utilization of the citrate.

Figure 3: Flow chart representing the Biochemical characterization performed to characterize the isolated bacteria

3.7 DNA extraction and PCR amplification

The amplification of the 16S rRNA gene was performed to detect the bacterial 16S rRNA gene to further identify species of the isolated bacterium. DNA was extracted using a bacterial DNA extraction kit (ZYMO Research Extraction Kit, Inqaba, South Africa) according to the manufacturer's instructions.

Two universal primer pairs (27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R 5'-TACGGYTACCTTGTTACGACTT-3') (Altimira et al., 2012; Fredriksson, Hermansson and Wilén, 2013) were used to amplify the 16S rRNA gene in a 50 µl reaction, 25 µl of Master Mix was used, 16 µl nuclease free water, 4 µl DNA template and 2.5 µl each of the forward and reverse primers with the following conditions: Initial denaturation for 5mins at 95°C, 35 cycles of Denaturation at 95°C for 1min, Annealing at 52°C for 1 minute, Extension at 72°C for 1 minute 30 seconds and final extension at 72°C for 5minutess. The obtained amplicons were analysed using 1% agarose gel in a 1X buffer solution. PCR reactions were performed in the Eppendorf Mastercycler machine (Eppendorf: Hamburg, Germany).

PCR amplicons were then sent to Inqaba Biotechnical Industries (Pty) Ltd - Pretoria, South Africa for sequencing. When sequences were obtained, they were edited using the BIOEDIT sequence alignment editor (Hall, 1999) and then a consensus DNA sequence was generated for comparison with reference sequences in NCBI database. A putative bacterial taxonomic affiliation of each library was assigned based on the closest match to sequence in NCBI database using the consensus sequence generated. The resulting sequences were matched against those available in the GenBank using the BLAST

algorithm method (Altschul *et al.*, 1997) to determine their approximate taxonomic affiliation and 16S rRNA gene sequence similarities.

3.8 Determination of the prevalence of potential zoonotic bacterial species

Prevalence was evaluated in terms of percent (%) occurrence, the denominator was the total number of bacterial species recovered from the animal or tissue. (Sum of the bacteria species recovered in each animal species or in each tissue divided by the total number of bacteria species x 100).

3.9 Phylogenetic analysis

The partial 16S rRNA gene sequences used in this study were aligned using the multiple sequence alignment program CLUSTAL X 2.0 with default parameters (Larkin *et al.*, 2007). The phylogenetic analyses were conducted in MEGA7 (Kumar, Stecher and Tamura, 2016). Firstly, the analysis involved 8 DNA aligned sequences of the 16S rRNA fragment gene obtained in this study. Secondly, the 16S rRNA gene sequences obtained in this study were aligned with (24) 16S rRNA gene sequences of several bacterial genera obtained from the GenBank and were analysed. The comparative analysis was conducted in order to determine the phylogenetic relationship between the study bacterial species and to reconstruct phylogenetic relationships of the study bacteria strains with those already known from the NCBI database.

The phylogenetic analysis was conducted applying the Maximum Parsimony (MP) method of gene sequence evolution as described by Nei and Kumar, (2000). The bootstrap consensus tree inferred from 1000 replicates was used to represent the evolutionary history of the taxa analysed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The

percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates). All positions containing gaps and missing data were removed.

CHAPTER 4: RESULTS

4.1 Isolation and identification of isolated bacteria

Bacteria from the different 44 tissues samples kidney, liver, heart, spleen, lung and lymph node of buffalo, Kudu, Elephant, Roan, Hippo and Sable were successfully identified and characterized using biochemical tests (Table 4) following Markey's identification key (Markey *et al.*, 2013) and the 16S rRNA gene sequences (Table 5). The 16S rRNA sequence genes were compared to the NCBI database to establish the closest genus neighbour type strain (Table 5). The analysis of the sequence amplification led to the identification of 16 different bacterial genera (Table 6), namely: *Escherichia coli, Acinetobacter haemolyticus, Klebsiella pneumonia, Proteus mirabilis,* Bacillus spp, Pseudomonas spp, Streptococcus spp, *Micrococcus caseolyticus, Lactococcus spp, Enterobacter aerogenes, Moellerella wisconsensis,* Carnobacterium sp. *Serratia marcescens, Pantoea agglomerans, Morganella morganii* and *Aeromonas punctate.* According to (Guentzel, 1996; Cleven *et al.*, 2006; Cardentey-Reyes *et al.*, 2009; Hoenigl *et al.*, 2010; Van Hoek *et al.*, 2015; Karaaslan *et al.*, 2016; Maseke, 2017) all isolates are potential pathogens.

The isolated bacteria in this study belonged to two bacterial phyla namely: *Proteobacteria* 68.75% (11 of 16) and *Firmicutes* 31.25% (5 of 16) (Table 6). The phyla *Proteobacteria* was dominated by one Class *Gammaproteobacteria. Firmicutes* were also represented by one Class namely *Bacilli.* Furthermore, *Klebsiella pneumonia* was prominent in kidney and heart tissues. *Streptococcus spp.* were well represented across the tissue organs but was absent in the spleen (Table 6).

Table 4: Biochemical characterization of isolated bacteria incubated for 24 hours at 37°c in this study

	Biochemical test									
Species	Gram reaction	Indole	Methyl red	Voges-Proskauer	Citrate	TSI	Oxidase	Catalyse	(OF)	Shape
1. Acinetobacter haemolyticus	-	-	-	-	+	-	-	+	O	B/R
2. Bacillus spp.	+	+	+	-	+	K/A	-	+	F	B/R
3. Enterobacter aerogenes	-	-	-	+	+	K/A	-	+	F	B/R
4. Escherichia coli	-	+	+	-	-	A/A	-	+	F	B/R
5. Klebsiella pneumonia	-	-	-	-	+	A/A	-	+	F	B/R
6. Lactococcus spp.	+	-	-	+	-	-	-	-	F	C/S
7. Micrococcus caseolyticus	+	-	-	-	+	K/NC	-	+	F	C/S
8. Proteus mirabilis	-	-	+	-	+	K/A	-	+	F	B/R
9. Pseudomonas spp.	-	-	-	-	+	K/K	+	+	F	B/R
10. Streptococcus spp.	+	-	-	-	-	K/NC	-	-	F	C/S

Key reactions: O= oxidative; F = Fermentative; + = positive reaction; - = negative reactions; A/A= acidic slant/acidic butt; K/A=Alkaline slant/acidic butt; K/K= alkaline slant/Alkaline butt; K/NC= Alkaline slant/no change in butt; BR=bacili/ rod; C=cocci/ Spherical

Table 5: Identification, sequence similarity and the closest type strain from the NCBI Database on the basis of the 16S rRNA gene sequences

Bacterial species **Top GenBank match**	**GenBank accession number**	**Similarity**
1. Aeromonas punctate	AM184292.1	99%
2. Carnobacterium sp.	AF076637.1	87%
3. Escherichia coli	JN644544.1	97%
4. Micrococcus caseolyticus	MG543828.1	97%
5. Moellerella wisconsensis	NR_104939.1	99%
6. Morganella morganii	AB089245.1	99%
7. Pantoea agglomerans	EU879089.1	97%
8. Serratia marcescens	MF399280.1	92%

The BLAST homology search tool revealed that the generated sequences had closest homology to their closet match in the Gene database. Partial 16S rRNA gene sequences of the bacterial species have been achieved in GenBank under accession numbers AM184292.1 through MF399280.1. A total of (8) 16S rRNA bacterial gene sequences were obtained in this study. They were compared to the NCBI database to establish their closest bacterial sequences match (Table 5). The closest similarity match were *Aeromonas punctate* (99%), *Moellerella wisconsensis* (99%) *and Morganella morganii* (99%) and Carnobacterium sp. (87%) had the lowest similarity to its top match in GenBank. Therefore, the need for more sensitive gene and techniques to determine whether they belong to same species.

Table 6: Identification and distribution of bacteria isolated per tissue organ. n= sample size of tissue organ

Bacteria species	Tissue organ									
	Kidney (n=12)	Liver (n=7)	Heart (n=4)	Lung (n=6)	Spleen (n=6)	Lymph node (n=3)	Phyla		Class	
							Proteobacteria	*Firmicutes*	*Gammaproteobacteria*	*Bacilli*
1. *Acinetobacter haemolyticus*	+	+	+	-	-	-	+	-	+	-
2. *Aeromonas punctate*	+	-	-	-	-	-	+	-	+	-
3. Bacillus spp	-	-	+	-	+	+	-	+	-	+
4. Carnobacterium sp.	+	+	-	-	-	-	-	+	-	+
5. *Enterobacter aerogenes*	+	+	-	-	+	-	+	-	+	-
6. *Escherichia coli*	+	+	-	-	+	-	+	-	+	-
7. *Klebsiella pneumonia*	+	+	+	+	-	-	+	-	+	-
8. Lactococcus spp.	+	-	-	-	-	-	-	+	-	+
9. *Micrococcus caseolyticus*	+	+	+	+	+	-	-	+	-	+
10. *Moellerella wisconsensis*	-	+	-	-	-	-	+	-	+	-
11. *Morganella morganii*	+	-	-	-	-	-	+	-	+	-
12. *Pantoea agglomerans*	-	+	+	-	-	-	+	-	+	-
13. *Proteus mirabilis*	-	+	-	+	+	+	+	-	+	-
14. Pseudomonas spp.	+	-	-	-	-	-	+	-	+	-
15. *Serratia marcescens*	-	-	-	+	-	-	+	-	+	-
16. Streptococcus spp.	+	+	+	+	-	+	-	+	-	+
Total bacteria	11/16	10/16	6/16	5/16	5/16	3/16	11/16	5/16	11/16	5/16
Prevalence	68.8%	62.5%	37.5%	31.3%	31.3%	18.7%	68.75%	31.25%	68.75%	31.25%

Table 7: Identification and distribution of isolated bacteria per animal and tissue organ. n= sample size of individual animals

	Animal					
Tissue organ	**Kudu (n=3)**	**Sable (n=1)**	**Hippo (n=1)**	**Buffalo (n=2)**	**Roan (n=1)**	**Elephant (n=1)**
1. Kidney	-*Acinetobacter haemolyticus* -*Enterobacter aerogenes* -*Micrococcus caseolyticus* -*Escherichia coli* -Lactococcus spp.	-Pseudomonas spp. -Carnobacterium sp. -*Aeromonas punctate*	-*Klebsiella pneumonia*	-*Acinetobacter haemolyticus* -*Escherichia coli* -*Klebsiella pneumonia*	-*Enterobacter aerogenes* -*Morganella morganii*	- *Klebsiella pneumonia* -Streptococcus spp.
2. Liver	-*Acinetobacter haemolyticus* -*Enterobacter aerogenes* -*Pantoea agglomerans* -*Moellerella wisconsensis* -*Escherichia coli* -Streptococcus spp.	-*Enterobacter aerogenes* -Carnobacterium sp.		-*Klebsiella pneumonia* -*Proteus mirabilis*	-*Micrococcus caseolyticus*	
3. Heart	-*Micrococcus caseolyticus* -*Pantoea agglomerans*	-Streptococcus spp.	-Bacillus spp. -*Klebsiella pneumonia*	-*Acinetobacter haemolyticus* -*Klebsiella pneumonia*		-*Klebsiella pneumonia*
4. Spleen	-*Enterobacter aerogenes* -*Escherichia coli* -*Micrococcus caseolyticus*		- Bacillus spp. -*Micrococcus caseolyticus*	-*Proteus mirabilis*		
5. Lung		-*Micrococcus caseolyticus*	-*Klebsiella pneumonia* -*Serratia marcescens*	-*Proteus mirabilis*	-*Micrococcus caseolyticus*	-Streptococcus spp.
6. Lymph node			-*Bacillus spp.* -Streptococcus spp.	-*Proteus mirabilis*		
Frequency distribution	**8**	**6**	**5**	**4**	**3**	**2**

4.2 Prevalence of isolated bacteria from the six trophy hunted species (elephant, buffalo, kudu, sable, roan and hippo) in BNP

A total of 16 bacterial species were obtained from 44 tissue samples (11 buffalo samples, 6 elephant samples, 8 kudu samples, 5 roan samples, 4 sable samples and 5 hippo samples) in this study. The prevalence of the isolated bacteria recovered from the combined tissue samples and per animal is presented in Table 8. The Kudu had the highest prevalence 50% (8 of 16) of the isolates followed by Sable 37.5% (6 of 16) and Hippo 31.25 % (5 of 16). The prevalence of the isolates in Buffalo was 25% (4 of 16). The lowest prevalence 18.75% (3 of 16) and 12.5% (2 of 16) was recorded in Roan and Elephant respectively. By contrast, the highest prevalence 68.8% (11 of 16) of the isolates was recorded in kidney tissues, followed by 62.5% (10 of 16) in liver, 37.5% (6 of 16) in heart, 37.5% (5 of 16) in lung, 31.25% (5 of 16) in spleen tissues, whereas the lymph nodes recorded the lowest prevalence 18.75% (3 of 16). *Klebsiella pneumonia* dominated the tissues organs 18.9% (recovered 8 times from 44 tissues), while *Micrococcus caseolyticus* dominated the animal species 66.7% (4 times from 6 animals).

Table 8: Prevalence of isolated bacteria recovered from six trophy hunted animals in BNP

	Animal					
	Kudu	**Sable**	**Hippo**	**Buffalo**	**Roan**	**Elephant**
Total	8/16	6/16	5/16	4/16	3/16	2/16
Prevalence	50%	37.5%	31.25 %	25%	18.75 %	12.5%

4.3 Molecular phylogenetic analysis of 16S rRNA gene sequences

From the resulting sequences obtained in this study, a consensus DNA sequences was edited and generated using the BIOEDIT sequence alignment editor (Hall, 1999) and subjected to phylogenetic analyses using MEGA7 (Kumar, Stecher and Tamura, 2016) to gain an understanding of their relationship to each other and their phylogenetic positions relative to known sequences in the GenBank database. The evolutionary history was inferred using the Maximum Parsimony (MP) method in order to predict the evolutionary tree and generate the observed variation in the sequences from common ancestral sequences (Kannan and Wheeler, 2012).

The present study used 16S rRNA bacterial gene sequences of 8 isolated bacteria, and (24) 16S rRNA bacterial gene sequences obtained from the GenBank. The phylogenetic tree of (8) 16S rRNA bacterial gene sequences obtained in this study as presented in figure 4, showed that the (8) 16S rRNA species were divided into four distinctive moderate to high supported clusters (58-100% nodal support), henceforth referred to as Cluster 1, Cluster 2, Cluster 3 and Cluster 4.

Cluster 1 appeared as the most branching group of bacterial species and was composed of three species, namely: Carnobacterium sp., *Micrococcus caseolyticus* and *Aeromonas punctate* (Figure 4). Within this cluster, *Aeromonas punctate* isolated from sable (kidney) formed a distant cluster basal to Carnobacterium sp. isolated from sable (kidney and liver) and its closest relative *Micrococcus caseolyticus* from in kudu (kidney), which appeared as sister taxa to each other, forming a moderate to strong supported cluster (70%-100%). On the other hand, Carnobacterium sp. and *Micrococcus caseolyticus* both identified from kidney tissues although not from the same animal were grouped together

forming a strong supported cluster (100%). Cluster 2 contained 2 species comprised of *Moellerella wisconsensis* isolated from kudu (liver) and *Morganella morganii* isolated from roan (kidney) which appeared as sister taxa to each other and revealed a moderate phylogenetic relationship supported by 89% bootstrap value. Cluster 4 contained two sequenced species comprised of *Pantoea agglomerans* isolated from kudu (heart and liver) and *Escherichia coli* isolated from kudu (kidney) forming the weakest supported cluster, bootstrap value 58%. Additionally, *Serritia marcescens* isolated from hippo (lung) formed a separate branch from all the (8) 16S rRNA bacterial gene sequences obtained in this study, indicating a greater evolutionary divergence of this species. The branching order and phylogenetic placement of all the cluster groups was the same in all phylogenies

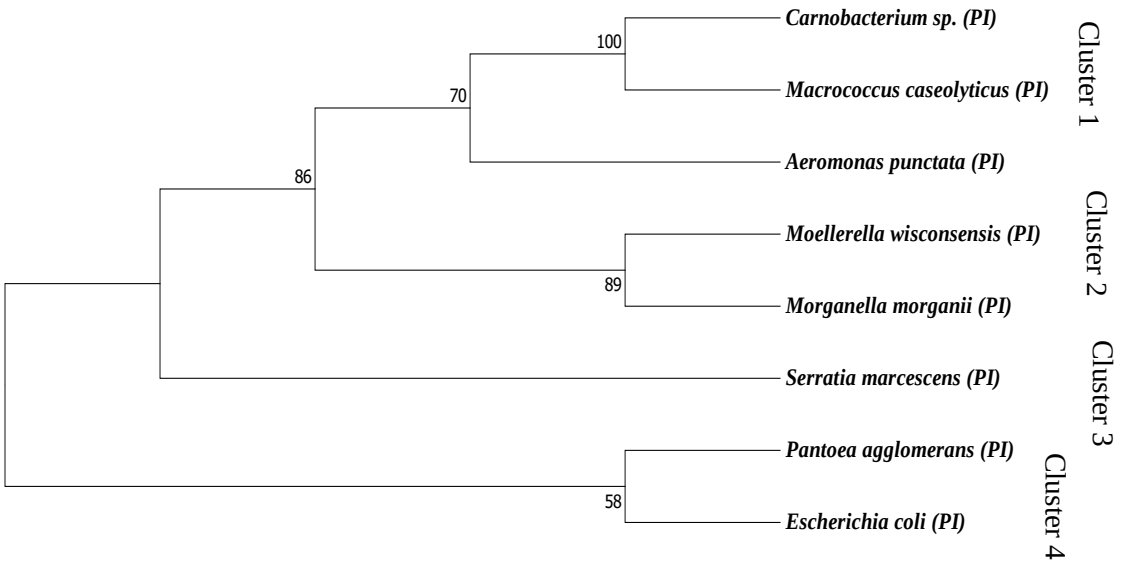

Figure 4: Maximum parsimony tree of eight 16S rRNA bacteria species generated from this study, showing the phylogenetic relationship of bacterial species obtained from tissue organs of six trophy hunted animals. Numbers at the nodes show the level of bootstrap support based on 1000 replicates, only values >50% were represented

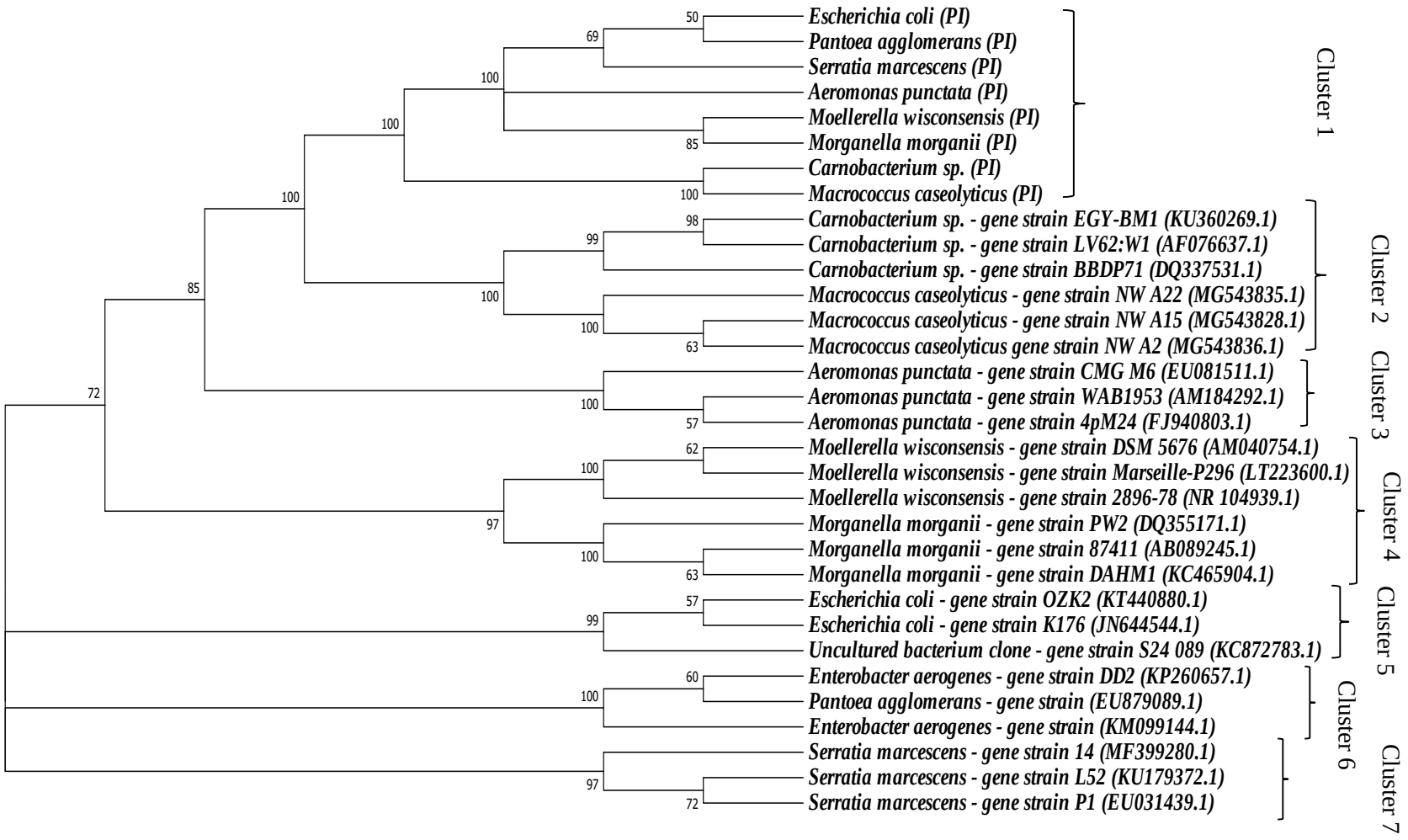

Figure 5: Maximum parsimony tree of 16S rRNA bacterial partial sequence similarity, showing the position of bacterial species obtained from tissue organs of six trophy hunted animals and their closest match from the NCBI database. Numbers at the nodes show the level of bootstrap support based on 1000 replicates, only values >50% were represented. Bacteria species represented by PI were generated from this study.

The phylogenetic tree of (32) 16S rRNA gene sequences comprised of 8 sequenced bacteria species obtained in this study and 28 reference species found in the data base showed that the 32 species were divided into seven distinctive clusters (Figure 5).

Cluster I predominantly consisted of all the 8 species from this study which formed the largest and diverse cluster. Cluster 1 was further subdivided into three groups. Within these groups, *Serritia marcescens* isolated from hippo (lung) formed a distant cluster to *Escherichia coli* isolated from Kudu (kidney) and *Pantoea agglomerans* isolated from Kudu (heart and liver) which appeared as sister taxa to each other, presenting a weak to moderate relationship ranging between 50% to 69 % bootstrap value. The other group belonged to *Moellerella wisconsensis* recovered from Kudu **(**liver) and *Morganella morganii* recovered from Roan (kidney) appeared as sister taxa to each other and revealed a moderate phylogenetic relationship supported by 85% bootstrap value. In the same cluster, *Aeromonas puntata* isolated from Sable (kidney) formed a separate branch from all other bacteria species recovered in this study indicating a greater evolutionary divergence. Furthermore, the third group in cluster 1 showed Carnobacterium sp. recovered from Sable (kidney and liver) and its closest relative *Micrococcus caseolyticus* recovered from Kudu (kidney), forming a strongly supported cluster (100%) bootstrap value (Figure 5). The smallest clusters (6 and 7) contained 3 reference species from the GenBank (*Enterobacter aerogenes*, *Pantoea agglomerans* and *Serritia marcescens*) forming a strongly supported cluster with bootstrap value ranging from 60% to 100%. All reference bacteria species from the GenBank branched together as sister taxa forming moderate to strong supported clusters (57% to 100%).

CHAPTER 5: DISCUSSIONS

Understanding the occurrence of potential bacterial zoonoses, their putative effects on human health and how they are transferred from animals to humans, is an ultimate concern beyond the scale of an individual case which requires intensive surveillance and assessment especially in a trophy hunting based destination. To date, very few microbiological records on bacterial zoonoses in trophy hunted species are available (Alexander *et al.*, 2012; Cantlay, Ingram and Meredith, 2017).

5.1 Identification of isolated bacteria, their prevalence and potential zoonoses

A total of 44 tissue samples comprising of - (13) from Kudu, (11) Buffalo, (6) Elephant, (5) Roan, (5) Hippo and (4) Sable samples, were processed for isolation and identification of potential bacterial zoonoses using growth on culture media, biochemical tests and the 16S rRNA gene sequences.

The present study isolated 16 bacteria species from 6 trophy hunted animals in BNP. The isolated bacteria were identified as: *Acinetobacter haemolyticus, Escherichia coli, Enterobacter aerogenes,* Lactococcus spp., Streptococcus spp., *Pantoea agglomerans, Morganella morganii, Micrococcus caseolyticus, Moellerella wisconsensis, Aeromonas punctate,* Carnobacterium sp., *Serratia marcescens, Proteus mirabilis*, Pseudomonas spp., *Klebsiella pneumonia* and Bacillus spp.

According to Forsythe, Abbott and Pitout, (2015), members of the Enterobacteriaceae spp. including the genera *Klebsiella, Enterobacter, Escherichia coli, Proteus mirabilis, Morganella morganii and Serratia* as recovered in the present study are reported to cause a broad range of infections in humans. Several enteric genera contain important

human pathogens, responsible for a variety of diseases such as: *Escherichia coli* - nteritis, *Klebsiella*-pneumonia, *Serratia*-abscesses, *Enterobacter* - enteritis, *Morganella morganii* - urinary tract infections, *Proteus* - urinary tract infections (Guentzel, 1996). The infections caused by these bacteria warrant serious consideration in Namibia, following their isolation from trophy hunted animals in BNP. Furthermore, the infections caused by these bacteria are in most cases underreported.

Some of the bacteria species in our study have been isolated from various wild animals. In Iran, *Escherichia coli,* Streptococcus spp., Proteus spp., Klebsiella spp. were isolated from 353 slaughtered buffalos (*Bubalus bubalis*) (Nikvand *et al.*, 2014). Subramanian *et al.*, (2012) isolated *Pseudomonas aeruginosa* from Asian elephants of Tamil Nadu in India. Dubay *et al.,* (2000) reported Aeromonas spp, and Serratia sp. in a hunted mule deer in Wyoming and Utah (United States of America).

The bacteria isolated from the 44 tissue samples of the carcasses in the present study is in agreement with the study of Kumar *et al.*, (2015) who isolated *Escherichia coli* from various tissues organs including intestine, lymph nodes, liver, spleen, heart, lungs, and kidneys in wild and domesticated animals in Kruger National Park. He stressed that this bacterium is pathogenic since it had caused pathomorphological effect on organs it was isolated from (Kumar *et al.*, 2015).

In the present study, the prevalence of bacteria isolated from Kudu was 50% (8 of 16). The isolated bacteria were identified as: *Acinetobacter haemolyticus, Enterobacter aerogenes, Micrococcus caseolyticus, Escherichia coli,* Lactococcus spp., *Pantoea agglomerans, Moellerella wisconsensis, and* Streptococcus spp. A recent study by Van

Hoek *et al.*, (2015) investigated *Enterobacteriaceae* family of bacteria in humans living in areas with high and low broiler (chicken) density in Germany. Their study isolated bacteria similar to those in the present study, *Escherichia coli 37%, Enterobacter aerogenes 2.3%* and *Pantoea agglomerans 0.6%*. Following 7.9% human admission to a hospital with 6.4% urinary tract infection caused by these bacteria, their study concluded that contact with broilers pose as a risk factor of poultry in the transmission of bacteria to humans through the environment or the food chain (i.e. eating broiler meat).

Likewise, a previous study by Goldberg *et al.*, (2007) isolated *Escherichia coli* 4.80 ± 0.34 % from humans and 5.65 ± 0.19% from chimpanzees and livestock, around Kibale National Park in Uganda while studying patterns of gastrointestinal bacterial exchange between chimpanzees and humans. Their study indicated that humans and apes interacting in the wild, share genetically and phenotypically similar bacteria. A second study by Goldberg *et al.*, (2008) of the gut bacterium *Escherichia coli* in humans, livestock, and wildlife around Kibale National Park in Uganda isolated 75% *Escherichia coli* in humans living near forest fragments genetically similar to those bacteria from nonhuman primates in the forest fragments than to bacteria carried by non-human primates living in nearby undisturbed forest. The degree of similarity increased with the level of human induced disturbance in the forest fragment (Goldberg *et al.*, 2008). An increase in potential bacterial zoonotic transmission from wildlife-human and vice versa, is therefore likely to arise from greater ecological interfaces.

A study by David J. Wilson and Kurz, (2017) isolated *Moellerella wisconsensis* (23%) and *Pantoea agglomerans* (28%) from hunted wild elk in Utah, United States of America. These bacteria were similar to those isolated in the current which contributed

to 50% (8 of 16) of the recovered bacteria from Kudu in this study. In the same vein, Casalinuovo and Musarella, (2009) isolated *Moellerella wisconsensis* from lungs of a goat in Italy. Equally, Anurag *et al.*, (2018) isolated *Moellerella wisconsensis* from humans with diarrhea in India. This bacterium was also previously isolated from humans in Belgium by Cardentey-Reyes *et al.*, (2009). The two studies indicated that *Moellerella wisconsensis* is an opportunistic pathogen which may seriously cause sickness when transmitted (Cardentey-Reyes *et al.*, 2009; Anurag *et al.*, 2018).

The current study further isolated Lactococcus spp. from Kudu, which also contributed to the 50% (8 of 16) of the total bacteria recovered from Kudu. Lactococcus spp. has been isolated from cows and buffalos with mastitis in a study by Vela *et al.*, (2000) and from clinical specimens of human with chronic diarrhoea from blood, urine and skin in previous studies by (Karaaslan *et al.*, 2016). The zoonotic potential of *Lactococcus garvieae* has further been reported in a recent study by (Reguera-Brito *et al.*, 2016) on genetic analysis of human clinical isolates of *Lactococcus garvieae* and the relatedness with isolates from foods, including fish, meat, milk and dairy products. Although the prevalence was not indicated in their study, the human isolates grouped with *Lactococcus garvieae* strains isolated from meat, dairy and fish, indicating a genetic overlap between isolates from human and those from foods which might represent important sources of human *Lactococcus garvieae* infections. Therefore, the isolation of Lactococcus spp. from Kudu in BNP possibly indicates a potential health risk for human in the BNP and surrounding areas.

Little is known about the prevalence of potential bacteria zoonoses in Sable. However, 37% (6/16) of bacteria were recovered from tissue samples of the Sable in the present study. The species were identified as: Streptococcus spp, Pseudomonas spp, *Enterobacter aerogenes, Micrococcus caseolyticus, Aeromonas punctate* and Carnobacterium sp. Similar bacteria were reported in 44 live and 226 hunter-harvested mule deer (*Odocoileus hemionus*) from Wyoming and Utah (USA) in a previous study by Dubay *et al.*, (2000) on the bacteria and nematodes in the conjunctiva of mule deer. In their study, the prevalence was not determined. Micrococcus spp. were however the most common Gram positive bacteria isolated, and Enterobacter spp., *Escherichia coli,* and Pseudomonas spp. were the common Gram negative bacteria. According to Chantida *et al.*, (2014) Micrococcus spp. can be an opportunistic pathogen, especially in hosts with broken and compromised immune system.

The isolation of Streptococcus spp. from the Sable in our study has also been recovered from humans in previous studies. In 2011, 3 unrelated human severe cases of one of the Streptococcus spp., *Streptococcus equi,* subspecies. *Streptococcus zooepidemicus* infections occurred in men working with horses in eastern Finland (Pelkonen *et al.*, 2013). To this end, *Streptococcus spp.*, including *Streptococcus zooepidemicus* are known to be zoonotic pathogen for persons in contact with domestic and wild animals including horses (Pelkonen *et al.*, 2013). In Korea, previous studies isolated *Streptococcus suis* from humans associated with domestic and wild pigs Choi *et al.*, (2012) and in Vietnam (Thi Hoang Mai *et al.*, 2008).

The isolation of *Aeromonas punctate* and Carnobacterium sp. from Sable in the present study has also been reported in a recent study by Wilson and Kurz (2017) on genetic

sequencing using 16S rRNA to identify bacteria pathogens in wild elk. In contrast, a study by Rieder *et al.*, (2012) which aimed at determining bacterial load in ten portions of fresh pork meat fillet or loin from different supermarkets or butcheries, isolated 23 bacterial species. Among these bacteria, *Carnobacterium divergens* was the most frequently isolated species in their study, 50% (5/10) prevalence in 10 samples. In several of the analysed pork meat samples, their study further isolated bacteria similar to those of the present study namely: *Serratia proteamaculans* 30% (3/10) and *Pseudomonas fluorescens 40*% (4/10). Their study revealed a high bacterial load from fresh pork meat supporting the potential health risk of meat for those handling pigs, and for end users consumers even when pork is under refrigerated conditions. This could be a plausible explanation why our study could still isolate these species from our samples even after the samples were transported under cooled conditions and stored and persevered in the fridge for laboratory analysis.

Furthermore, reports of isolation of Carnobacterium sp. from humans have been published. The first report described the isolation of Carnobacterium sp. from blood cultures from a man who had prepared fish before onset of fever (Hoenigl *et al.*, 2010). The second report described isolation of *Carnobacterium divergens* from a woman with a history of diabetes mellitus, severe under nutrition, and chronic alcoholism in France (Smati *et al.*, 2015). Of these two studies, the prevalence was not determined. However, the second study highlighted that because *Carnobacterium divergens* seems to be able to cause life-threatening infection in immunocompromised patients, its safe use in such patients and in the food industry should be monitored (Smati *et al.*, 2015).

Our study further isolated *Aeromonas punctate* from Sable, which contributed to the 37% (6/16) of bacteria recovered from tissue samples of the Sable. *Aeromonas punctate* has been previously reported as a potential zoonotic pathogen by various studies. Lehane and Rawlin, (2000) investigated zoonoses acquired from fish and reported that Aeromonas spp. (aeromonads) caused cellulitis, myositis, and septicemia infections in human as a result of injuries from handling fish, working in aquaculture, or keeping fish as pets. The prevalence was not indicated in their study. Another study by Castro-Escarpulli *et al.,* (2003) isolated Aeromonas spp. from 53 samples of raw chicken meat in Mexico. Their study found 47.17% of the sample to be positive for motile Aeromonas spp. Of the 47.17%, 28.30% represented *Aeromonas hydrophila* and 9.43% of *Aeromonas sobria*. Their study indicated that the presence of these pathogenic organisms in the raw chicken samples possessed a serious potential risk for public health (Castro-Escarpulli *et al.*, 2003). In addition to this, there have been published reports of proved cases of human infections with Aeromonas spp. In Limpopo Province of South Africa, Obi and Bessong, (2002) isolated Aeromonas spp. from 13.3% HIV patients with chronic diarrhoea in rural communities. Equally, Chan *et al.*, (2003) also documented the incidence of aeromonads in 6.9% of adult patients with acute diarrhoea in Hong Kong.

Despite the sable antelope being recognized as a trophy hunted animal, little research has been conducted to assess the prevalence of bacterial zoonoses in sable antelope. Nevertheless, between 2009 and 2010 sable antelope in Zambia were reported with a variety of bacterial zoonotic diseases associated with importation risk into South Africa. The diseases were reported as Brucellosis and bovine Tuberculosis (Sergeant, 2014).

The present study isolated *Klebsiella pneumonia,* Bacillus spp, *Streptococcus spp.* and *Micrococcus caseolyticus, Serratia marcescens* from the Hippo. The prevalence of isolated bacteria in our study was poorly represented in Hippo 31.25 % (5 of 16). From the five bacteria, Bacillus spp, was isolated from gastrointestinal tract and composting of a hippopotamus in a study by da Cruz Ramos *et al.,* (2016) who investigated the cellulolytic and proteolytic ability of bacteria. In their study, the prevalence was not indicated. Their analysis revealed that all the bacteria isolated were affiliated to the genus Bacillus. Additionally, Rensburg *et al.*, (2016) reported brucella spp. infection in Sable antelope (*hippotragus niger*) in South Africa. Brucella spp. was also reported in Springbok (*Antidorcas marsupialis*) in Namibia (Magwedere, 2013; Magwedere *et al.*, 2013). Similarly, a recent outbreak of anthrax caused by *Bacillus anthraces* caused high mortalities of over 100 hippopotamus and several water buffaloes in the Kavango River, Mahango game reserve of BNP (Maseke, 2017). Here, the prevalence was not determined. Anthrax caused by *Bacillus anthraces* was however not reported in any of the individual species in the present study.

Anthrax (*Bacillus anthraces*) has been isolated from humans in recent years. It has been isolated from humans where livestock and wildlife anthrax outbreaks had occurred, with several cases in humans who had eaten affected livestock carcasses (50% case-fatality rate, 4 deaths and 8 cases in 7,538 individuals) in the Serengeti Ecosystem, Tanzania between 1996-2009 with a prevalence of 46% in Buffalo and 19% Wildebeest by Lembo *et al.,* (2011). Henceforth, this outbreak possesses high risk of zoonotic penitential to communities and livestock living in proximity to Parks. Surprisingly,

although the nearby communities and livestock in BNP utilize the same water from Kavango River (MET, 2013), no human or livestock cases were reported. Equally, a recent study by Barandongo (2015) demonstrated the seasonality of dust bathing as a possible contributor to inhalational anthrax in zebra, blue wildebeest and African elephants, in relation to the seasonality of anthrax mortalities in Etosha National Park, Namibia. The study concluded that it was unlikely for dust bathing behaviour to serve as an important risk for inhalational of anthrax for herbivores in Etosha National Park. However, the risk for inhalation of anthrax by humans particularly tourist in the Etosha National Park might be high.

More recently, Flacke, (2017) conducted a study on pygmy hippos (*Choeropsis liberiensis*) which aimed to identify primary causes of diseases and mortality as well as potential implications of polycystic kidney disease for viability of the captive hippo population. His study isolated the following bacteria; *Escherichia coli*, Proteus spp., Streptococcus spp. similar to the result of the current study. The prevalence was not determined in this study.

In all cases, *Serratia marcescens* was only recovered from Hippo (lung). These findings are different from those obtained in a recent study by Flacke (2017) were *Serratia marcescens* was not detected in this tissue. The isolation of *Serratia marcescens* from the lung of an herbivore such as a Hippo suggests that hippos may have been infected with this bacterium through grazing and water sources. This follows a recent study by Khan *et al.*, (2017) who isolated *Serratia marcescens* from plants.

The current study recovered *Acinetobacter haemolyticus, Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis* from Buffalo, the prevalence here was 2.5% (4 of 16). A corresponding study by Nikvand *et al.*, (2014) isolated the same bacteria *Escherichia coli (21%), Streptococcus spp. (15.8%),* Proteus spp. (15.8%), Klebsiella spp. from 353 slaughtered buffalos (*Bubalus bubalis*) in South West of Iran. Similarly, a recent study by Eze *et al.*, (2015) isolated *Escherichia coli* and Proteus spp. from humans which were similar to those isolated from wild animals including *Tragelephus scriptus* (Cape Bushbuck) at the human-wildlife interface in Nigeria. Equally, King, (2017) isolated 47% of *Escherichia coli* from zebra in a study which investigated wild and domestic animals as reservoirs of antibiotic resistant *Escherichia coli* in South Africa. The two studies mentioned above, suggested the possibility of bacteria gene transmission at the human-wildlife interface, indicating similarity between *Escherichia coli* and Proteus spp. isolated from humans and wild animals (Eze et al., 2015; King, 2017).

Our study further isolated *Acinetobacter haemolyticus* and *Klebsiella pneumonia* from Buffalo. Similar bacteria were isolated from imported cattle hide and rabbit wool in a study by (Yu et al., 2012) and from beef, pork and chicken by (Min *et al.*, 2004). Correspondingly, Gupta *et al.*, (2015) isolated (12%) *Acinetobacter haemolyticus* from 111 human clinical samples in a hospital in India. Their study demonstrated that Acinetobacter is a clinically important pathogen and human infections caused by Acinetobacter spp include pneumonia (Gupta *et al.*, 2015). Regardless of the isolation of *Acinetobacter haemolyticus* in our study, it is worth noting that *Acinetobacter haemolyticus* can survive in vital tissue organs as isolated from kidney, liver and heart in our study, where these organs may act as a potential reservoir for infection. It should

also be stressed that *Acinetobacter haemolyticus* is a saprophyte and the environment, soil, water and animals is its natural habitats (Almasaudi, 2018), hence Buffalo in BNP might have been exposed to *Acinetobacter haemolyticus* through feeding, water sources or soils. However, this finding requires focused studies to determine factors underlying the occurrence of this bacterium in BNP.

The presence of *Escherichia coli* and *Klebsiella pneumonia* which contributed to 2.5% (4 of 16) of bacteria recovered from the Buffalo in the present study has been reported with high prevalence, 24.1% in Buffalo populations in Egypt (Hakim *et al.*, 2017) and 85.04% in Southern India (Srivani *et al.*, 2017). Similarly, a study by Najjuka *et al.*, (2016) isolated *Escherichia coli* and *Klebsiella pneumoniae* from human in Uganda. Their study recovered (97 %) *Escherichia coli* and (3 %) *Klebsiella pneumonia* from human stool samples, whereas human urine samples yielded (89 %) *Escherichia coli* and 39 (11 %) *Klebsiella pneumonia*. A second study by Stanley *et al.*, (2018) isolated (89%) *Escherichia coli* and 23 (11%) *Klebsiella pneumoniae* from gut of out-patients from pastoralist communities of Kasese district, Uganda. The two studies demonstrated high antimicrobial resistance, including multidrug resistance, among *Escherichia coli* and *Klebsiella pneumonia* isolates from pastoralist out-patients which could complicate treatment options for community-acquired infections caused by Enterobacteriaceae (Najjuka *et al.*, 2016; Stanley *et al.*, 2018). The current study confirms that Buffalo populations are carriers of some potential zoonotic pathogens which could be highly transmitted to other wildlife, humans and livestock populations. Therefore, communities, trophy hunters, veterinarians and game ranchers dealing with buffalo and their derivatives should be cautioned.

Based on the findings of our study, out of the total of 6 animals analysed, the isolation of *Proteus mirabilis* contributed to the prevalence of 2.5% (4 of 16) bacteria from Buffalo. In the current study, *Proteus mirabilis* was isolated 4 times from the different Buffalo tissues. Similarly, *Proteus mirabilis* has previously been reported in various animals. A recent study by (Yu, He and Huang, 2015) investigated *Proteus mirabilis* infection in newly weaned infant rhesus monkeys (*Macaca mulatta*) and ferrets (*Mustela putorius furo*) with diarrhoea during the period 2011-2012 in China. Their study detected (7/74, 9.5%) and (4/12, 30%) *Proteus mirabilis* strains in the stool samples collected from the monkeys and ferrets, respectively. Equally, sequence analyses in their study showed that the isolated *Proteus mirabilis* was closely related to *Proteus mirabilis* strain HI4320, which was isolated from the urine of a patient with a long-term indwelling urinary catheter. These findings highlight the potential zoonotic nature of *Proteus mirabilis* from wildlife to human.

Additionally, the isolation of *Proteus mirabilis* from several Buffalo tissues in our study could be possibly due to the skinning process. After skinning, it is assumed that *Proteus mirabilis* diffused from other body parts of the carcass through contaminated blood at the time the animals were shot in the present study.

Furthermore, our study isolated (18.75%) 3/16 of bacteria from the Roan which were identified as: *Enterobacter aerogenes, Morganella morganii* and *Micrococcus caseolyticus.* These bacteria were among those previously isolated from different mammals, reptiles, birds and water sources in a study on zoo animals at Asa Zoological Park, Hiroshima, Japan as a potential reservoir of gram-negative bacteria harbouring integrons and antimicrobial resistance genes in a study by (Ahmed *et al.,* 2007). Their

study recovered more species than the current study. Their study showed that the most prevalent species was *Escherichia coli* (52.6%), followed by *Klebsiella pneumoniae* (7.3%), *Proteus mirabilis* (6.9%), *Enterobacter aerogenes* (6.0%), *Pseudomonas aeruginosa* (5.2%), *Morganella morganii* (1.7%), *Serratia marcescens* (0.9%), and a single isolate (0.43%) of Acinetobacter spp, and Aeromonas spp. Among these bacteria some are known to cause fatal infections in humans leading to deaths. Singla *et al.*, (2010) isolated *Klebsiella pneumoniae* along with *Morganella morganii* 15 times in patients admitted in Intensive Care Units (ICU) in India.

With this mentioned, since humans and wildlife in BNP share water from the Okavango River (MET, 2013), the use of water by both humans and animals from this River might expose communities to some of the above mentioned zoonotic bacteria which are capable of causing diseases. Accordingly, a study by Harris (2013) on antimicrobial resistance profiles of selected commensal bacteria isolated from impala (*Aepyceros melampus*) and their water sources in the Kruger National Park detected 404 bacterial species belonging to different genera and species. Of the 404 species, 10 were similar to those identified in this study namely: Acinetobacter spp. *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia,* Pantoea spp., Proteus spp., *Pseudomonas aeruginosa, Serratia marcescens,* Bacillus spp., and Streptococcus spp.

The high number of bacterial species isolated from the impala and its associated water sources suggest that water sources can be contaminated with microbes which could be of potential zoonotic transmission to humans, livestock and wildlife sharing the same water bodies. The same bacteria were reported in a previous study by Saleem *et al.*, (2011) on the isolation, identification and seasonal distribution of bacteria in a water source which

aimed at determining the bacteriological characteristics of the waters of Dal Lake, Kashmir. Their study revealed a total of 894 bacterial species, of which most of them are similar to the ones revealed in the current study. Their study reported that out of the bacteria species isolated, *E. coli* was most prevalent (15.77%) followed by *Enterobacter aerogenes* (12.19%), Bacillus spp (11.96%), *Micrococcus caseolyticus*, (10.17%), *Pseudomonas aeruginosa* (8.27%), *Klebsiella pneumoniae* (6.71%) and *Serratia marcescens* (5.92%).

Despite the fact that communities in the BNP share water from the Kavango River with wild and domestic animals, this suggests that there is a potential zoonotic for bacterial transmission between wildlife, water sources and human in BNP and the surrounding. Therefore, the presence of these organisms in water sources should receive particular attention, because their presence indicate public health hazard and give warning alarm for the possible occurrence of water born infections. Previous studies in Uganda have reported similar findings of transmission of potential zoonotic bacteria from water including potential bacteria isolated from the animals in our study namely: *Escherichia coli, Klebsiella pneumoniae* and *Morganella morganii* in human associating with wildlife (Goldberg *et al.*, 2007; Najjuka *et al.*, 2016).

In addition to this, *Morganella morganii* isolated from the Roan Antelope in our study has been recognized as an increasingly important pathogen in recent years (Liu *et al.*, 2016). There have been various reports of *Morganella morganii* causing urinary tract infections, skin and soft tissue infections, meningitis and bacteraemia often with fatal consequences (Liu *et al.*, 2016). In India, sporadic cases due to infection with *Morganella morganii* have been reported in human several times (Thomas *et al.*, 2007).

Chen *et al.,* (2012) collected 82,861 samples from patients who presented symptoms of Gram-negative bacterial infections at Changhua Christian Hospital, Taiwan. Of these samples, 1,219 (1.47%) were positive for *Morganella morganii*.

Given the prevalence (18.75%) 3/16 (*Enterobacter aerogenes, Morganella morganii* and *Micrococcus caseolyticus*) bacteria from the Roan antelope in our study. The distribution of these bacteria in BNP may increase, considering the mechanisms for which they cause diseases in both humans and animals. Similarly, (Fischer *et al.,* 2016) assessed the carriage of *Enterobacteriaceae* in the anterior nares of humans exposed to pigs. Their study demonstrated that 66.7% (76/114) of the participants were positive for *Enterobacteriaceae* bacteria, with the major species being *Proteus mirabilis* 14.9% (17/114), followed by *Pantoea agglomerans* 11.4% (13/114), *Morganella morganii* 7.9% (9/114), *Citrobacter koseri* 7.9% (9/114), *Klebsiella pneumoniae*, *Escherichia coli*, and *P. vulgaris* (each 7.0%, 8/114). Their study suggested a possible transmission pathway between humans and a close contiguous animal may exist.

Further, the present study isolated *Micrococcus caseolyticus* from Kudu, Sable, Hippo and Roan Antelope. Although none of the species of the genus Macrococcus are thought to be human pathogens (Mašlaňová *et al.*, 2018), there are reports of *Micrococcus caseolyticus* associated with animal infections. However, in these reports the prevalence was not indicated. Recently, Li *et al.,* (2018) isolated *Micrococcus caseolyticus* from commercial broiler chickens in China, causing inflammatory infiltration, haemorrhages and multifocal necrosis in various organs. It was also isolated in dog (Cotting *et al.*, 2017) and from the nares of a dog with rhinitis (canine infection) by (Gómez-Sanz *et al.*,

2015). These findings suggest the possible zoonotic potential and pathogenicity of *Micrococcus caseolyticus*.

Enterobacter aerogenes isolated from Kudu, Sable and Roan, and *Klebsiella pneumonia* isolated from the Hippo and Elephant in our study were also among the bacteria isolated from 15 marketed meat samples (chicken, turkey-hen, beef, sheep, pig, dromedary, ostrich, and fish) in a study by Messaoudi *et al.*, (2009). Over a period of 3 years, 2010 to 2012 in the city of Sanandaj, Kurdistan province, Iran, a study by (Ramazanzadeh *et al.*, 2016) isolated 31.36% *Enterobacter aerogenes* out of 118 *Enterobacter spp.* isolates from two thousand patient specimens (urine, wound, respiratory tube, blood, cerebrospinal fluid and stool) in a government hospital of Toohid and Besat. This indicates that *Enterobacter aerogenes* is a potential zoonotic bacterium able to colonize human and a range of wildlife life species. Therefore, the isolation of this bacterium from trophy hunted species in BNP cautions trophy hunters, communities and those involved in handling these animals and their derivatives to be very cautious as they pose potential bacterial zoonoses of Public Health concern.

Even though there are few publications about the prevalence of bacteria zoonoses in Roan antelope, in 2004, an outbreak of member of the Bacillus spp. similar to the bacterial taxa recovered in our study killed 42% of the Roan antelope (*Hippotragus equinus*) in the Malilangwe Wildlife Reserve in Zimbabwe as well as almost all of the approximately 500 kudu in the reserve, 68% of the nyala (*Tragelaphus angasi*), 48% of the bushbuck (*Tragelaphus scriptus*), 44% of the waterbuck (*Kobus ellipsiprymnus*) and about 6% of the buffalo (*Syncerus caffer*) were reported dead (Clegg *et al.*, 2007). Based on the results of our study, it is suggested that Roan populations may carry few

potential bacteria zooneses. However, this should not undermine the fact that such bacterial species could be potential zoonotic pathogens.

The prevalence of potential bacterial zoonoses species recovered from Elephants in our study was very low, 12.5% (2 of 16). This could presumably be due to the individual differences in the immunological status or susceptibility of the host specimens. Here, the species were identified as: *Klebsiella pneumonia* and *Streptococcus spp.* Our results are in agreement with those obtained by Magwedere *et al.*, (2012) who reported similar findings with few cases, although different bacteria namely: *Salmonella spp.* and *Bacillus anthracis* in elephants in their report. In other reports, the causes of *Streptococcus suis* human infection outbreaks in Jiangsu and Sichuan province in 2005 have been reported by several researchers (Yang *et al.*, 2006; Yu *et al.*, 2006). The analysis of the two studies concluded that the outbreaks were closely related to a large outbreak of *Streptococcus suis* infection in pigs, since all the 233 human cases occurred in the endemic regions of pig infection, and approximately 80 000 pigs were estimated to be infected by *Streptococcus suis* in this province at that time (Tambyah and Lee, 2001; Tang *et al.*, 2001). Almost all the human patients had a history of direct contact with infected pigs or pork. Of the 205 patients investigated, 199 (97%) had contact with sick pigs, and among these patients, 134 (67%) had slaughtered sick pigs and 100 (50%) had skin cuts (Tambyah and Lee, 2001; Tang *et al.*, 2001).

In previous studies, Streptococcus spp. was detected in the gut of other farm animals such as bovine, horses, pigs and dogs (Sasaki *et al.*, 2004). The fact that elephants in our study carry 12.5% (2 of 16) bacteria including *Klebsiella pneumonia* and Streptococcus spp., this should not undermine that they carry few potential zoonotic bacteria, as some

potential zoonotic bacterial microbes are uncultured organisms in nature and might have not been detected by the methods used in this study. By contrast, Subramanian *et al.*, (2012) isolated more bacteria than our study, from 49 elephants at Mudumalai and Anaimalai tiger reserve: They were identified as *Bacillus cereus, Streptococcus agalactiae, Salmonella entritidis, Proteus mirabilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The bacteria species recovered from our study were also among those reported in a previous study by Kayode and Kolawole, (2008) on the β-lactamase production of bacterial isolates from smoked bush meats including meat samples from antelopes. Their study recovered *Klebsiella pneumoniae* (75.0%), *Escherichia coli* (69.7%), Proteus sp. (33.3%), *Pseudomonas aureginosa* (25.9%), and Streptococcus spp. (12.5%). Of these bacteria, a study by Bhuyan *et al.*, (2017) isolated *Klebsiella pneumoniae* (9%) and Streptococcus spp. 5.5% from children with acute respiratory infections in hospital settings of Dhaka, Bangladesh. Accordingly, (Dumke *et al.*, 2015) revealed the potential transmission of Streptococcus spp. from an infective endocarditis patient and his laying hen flock in Bad Oeynhausen, Germany. Here, the prevalence was not determined. Their study however showed the same Streptococcus spp. isolates of an infected person who had close contact with his colonized laying hens based on a clonal identity with a similar DNA fingerprinting pattern, which highlighted the question about the zoonotic potential of isolates from poultry and should be considered in future studies. Overall, based on the above findings, it is suggested that the frequency of occurrence of the potential zoonotic bacteria derived from elephants and bush meat indicates a potential zoonotic transmission of these bacterial species to humans.

There are only a few recent reports on Streptococcus spp. infections in elephants even though this bacterium has been isolated often in this endangered animal population. Few studies have isolated Streptococcus spp. in elephants (Subramanian *et al.,* 2012; Eisenberg *et al.,* 2017). Streptococcus spp. have also been isolated from elephant skin lesions (Vodička, 2008) and from elephant oral cavities (Saito *et al.*, 2014; Shinozaki-kuwahara *et al.*, 2016). Moreover, Mahanti *et al.,* (2015) isolated *Escherichia coli* from faecal samples of healthy buffaloes in West Bengal, India. While *Klebsiella pneumoniae* was isolated from buffaloes by Ahmed *et al.*, (2016) and Osman *et al.*, (2014) while studying the phenotypic, antimicrobial susceptibility profile and virulence factors of *Klebsiella pneumoniae* in buffalo and cow milk. In comparison *Escherichia coli* was only isolated from the Kudu and Buffalo in the present study. In previous studies in African wildlife, similar findings were observed (Jobbins and Alexander, 2015).

Although a few cases, 12.5% (2 of 16) were recorded in Elephants in the current study, the role in the transmission of potential zoonotic bacteria from elephants to rural communities in BNP and the surrounding areas should not be neglected because there could be a high possibility of zoonotic transmission from elephants to humans through the wildlife livestock human interactions in the BNP.

Correspondingly, previous studies on elephants in Tamilnadu at Mudumalai tiger reserve, Anamalai tiger reserve and Arignar Anna Zoological park, India, and (crocodile, *Crocodylus niloticus*; spotted hyena, *Crocuta crocuta*; leopard, *Panthera pardus*; otter, *Aonyx capensis*), herbivorous (Cape buffalo, *Syncerus caffer*; bushbuck, *Tragelaphus scriptus*; domestic cattle, *Bos primigenius*; elephant, *Loxodonta africana*; giraffe, *Giraffa camelopardali*s; hippopotamus, *Hippopotamus amphibious*; impala,

Aepyceros melampus; greater kudu, *Tragelaphus strepsciseros*; sable, *Hippotragus niger*; waterbuck, *Kobus ellipsiprymnus*), or omnivorous (Chacma baboon, *Papio ursinus*; guineafowl, *Numida meleagris*; banded mongoose, *Mungos mungo*; velvet monkey, *Chlorocebus pygerythrus*; warthog, *Phacochoerus africanus*) in Chobe National Park, Botswana to explore key attributes and behaviors that may increase exposure and allow resistance to move between humans, animals, and ecosystems revealed bacteria similar to bacteria isolated in the current study. These bacteria were identified as: *Escherichia coli* (41.3%), *Klebsiella pneumonia, Proteus mirabilis,* Pseudomonas spp, Acinetobacter sp. and Streptococcus spp (Subramanian *et al.*, 2012; Jobbins and Alexander, 2015). Of these bacteria, Jobbins and Alexander, (2015) isolated *Escherichia coli* from animals and demonstrated similar patterns of resistance to human *Escherichia coli*, from environmental and clinical sources in their study area. Jobbins and Alexander, (2015) highlighted that the occurring pathogens in these animals will find new windows of opportunity to invade, with terrible consequences for public health in Botswana.

Considering a fair bacteria composition, other bacterial species were only isolated few times from the animals tissues in our study. For example, Pseudomonas spp. was isolated only once from Sable liver. The low detection numbers of bacteria from the tissues might be partly due to the media used, sample size, sampling period or transportation of tissue sample from the field to the laboratory.

Some of the genera and bacterial species identified in the current study such as Enterobacteriaciae spp, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis,* Pseudomonas spp. *Klebsiella pneumonia and* Acinetobacter spp. are similar to those

identified by Bender and Shulman (2004) while studying zoonotic disease outbreaks associated with animal exhibits and availability of recommendations for preventing zoonotic disease transmission from animals to people in settings including petting zoos, farms, and a zoological parks. These results highlight concerns for potential bacterial zoonoses transmission to the public in such settings.

In Namibia, the majority of wildlife animals in BNP still retain their natural environment, thus wildlife particularly large mammals can roam around freely in large areas which are not fenced off, therefore dispersing bacteria with zoonotic potential as highlighted in the current study. Therefore, to assist with identification and prevention of potential bacteria zoonoses in Namibia, Mashood, Minga and Machangu (2006) recommended that the public must be educated on the risks of consuming meat from wild animals including meat from trophy hunted animals and such products must be inspected by veterinarians.

The results of the current study revealed that all individual animals showed an indication of potential bacterial zoonoses. Regardless of their tissues and location, they carried a variety of bacterial species which could be of zoonotic potential. To the best of my knowledge, this is the first study in Namibia on the prevalence of potential bacterial zoonoses in trophy hunted species.

The results of our study strongly support the study hypothesis: trophy hunted species in BNP carry species specific diversity of pathogenic bacterial zoonoses. The study predicts that potential zoonotic bacteria may be frequent in BNP. The present study strongly demonstrates that consuming wild meat from trophy hunted species in BNP

may present a potential zoonotic risk, since the findings identified diverse bacterial pathogens which may be potentially zoonotic.

5.2 Phylogenetic relationship of potential bacterial zoonoses isolated from trophy hunted animal from BNP

Based on phylogenetic analysis of 16S rRNA gene sequences, the current study revealed a significant bacteria diversity represented by a strong clear phylogenetic relationship which can be considered orthologous, suggesting that the potential zoonotic bacteria represent typical relatedness. Furthermore, the phylogenetic relationship of potential zoonotic bacteria isolated from trophy hunted animal in BNP was determined and members of Proteobacteria were dominant 37.5% (6 of 16).

The present study showed that Carnobacterium sp. isolated from sable (kidney and liver) and *Micrococcus caseolyticus* from kudu (kidney) are highly closely related, 100 % bootstrap value. Carnobacterium sp. have been previously isolated from the gastrointestinal tract of animals as well as from foods of animal origin such as meat (Iskandar *et al.*, 2017). Other species, including *Carnobacterium maltaromaticum* and *Carnobacterium divergens*, have been found in animal-associated habitats (Iskandar *et al.*, 2017). In addition to this, *Micrococcus caseolyticus* previously classified as *Staphylococcus caseolyticus* (Kloos *et al.*, 1998) has been isolated from sucuk samples produced from a mixture of meat derived from cattle, sheep and water buffalo in the Aegean region of Turkey (Geniş and Tuncer, 2018). The presence of *Micrococcus caseolyticus* can also be isolated from milk and meat products of sheep, goats and cattle (Musa *et al.*, 2012).

Aeromonas punctate isolated from sable (kidney) formed a distant cluster basal to Carnobacterium sp. isolated from sable (kidney and liver) and its closest relative *Micrococcus caseolyticus* from kudu (kidney). Aeromonas spp. are opportunistic pathogens causing a wide range of human illnesses such as gastroenteritis, chronic diarrhoea and wound infections (Stratev, Vashin and Rusev, 2012). Stratev, Vashin and Rusev, (2012) further reported that about 85% of gastrointestinal disorders in humans are attributed to Aeromonas spp. especially *Aeromonas hydrophila, Aeromonas caviae* and *Aeromonas veronii biovar sobria.* Similarly, a recent study by Alhazmi, (2015) on the isolation of Aeromonas spp. from food products and their significance in public health in Riyadh, Saudi Arabia isolated 47% Aeromonas spp. from raw meat samples. Alhazmi, (2015) further suggested that the ability of Aeromonas spp. to grow in foods stored in a refrigerator poses a substantial threat for human health.

The current study confirmed a close relationship between *Moellerella wisconsensis* from kudu (liver) and *Morganella morganii* from roan antelope (kidney). The close relationship between *Moellerella wisconsensis* and *Morganella morganii* is supported by the 89 % bootstrapping tree. The presence of *Moellerella wisconsensis* has been commonly isolated from these tissues, in Portugal it was isolated from liver and kidney tissues of a cow (Anastácio and Leão H., 2016) and from a lung of a goat in Italy (Casalinuovo and Musarella, 2009).

In this study, *E. coli* isolated from roan antelope (liver) and *Pantoea agglomerans* from kudu (heart and liver) presented a weak phylogenetic relationship supported by weak bootstrap value (58%). These findings are also comparable with those of a recent study (Wang, Wang and Jing, 2017).

It will be necessary to have a more comprehensive knowledge of *Moellerella wisconsensis, Pantoea agglomerans* and *Micrococcus caseolyticus* genetic variation in kudus to be able to interpret the significance of the relative distribution of bacteria species found in these animals. Furthermore, the phylogenetic relationship of the bacteria species recovered in this study revealed that *Serratia marcescens* recovered from hippo (lung) indicated a greater evolutionary divergence. This could be attributed to the geographic origin of these species. This finding is in agreement with Stackebbrandt and Ebers (2006) and Burbano *et al.*, (2015) who consider this as a constant boundary in species distribution. Besides, Gaby and Buckley (2012) suggested that this could be a result of high differences in 16S rRNA sequences dissimilarities. Secondly, it appeared that Kudu carried potential zoonotic bacteria representing a broad phylogenetic continuum compared to the rest of the trophy hunted species in this study. Potential zoonotic bacteria detected with 92% to 99% homology to known bacteria in the NCBI data base using the 16s rRNA gene sequences are shown in (Table 8). Ecologically, it is worth noting that none of the potential zoonotic bacteria in this study where grouped together with those known in the NCBI data base. This suggests that it is possible that the process of speciation itself may have resulted in the observed phylogenetic relationship results of the potential bacterial zoonoses in the present study.

CHAPTER 6: CONCLUSIONS

The objective of this study was to determine the prevalence of potential bacterial zoonoses in trophy hunted species (African Elephant, African Buffalo, Kudu, Hippopotamus, Sable antelope, and Roan antelope) with respect to human exposure and risk in BNP.

This study showed that African Elephant, African Buffalo, Kudu, Hippopotamus, Sable antelope, and Roan antelope carry a wide spectrum of bacteria which could be of zoonotic potential. The Kudu and Sable potentially host the highest prevalence of potential bacterial zoonoses with the elephant hosting the lowest prevalence. Considering the role of wildlife as a carrier of potential bacterial zoonotic pathogens in BNP, products derived from trophy hunted animals may be an important public health risk in Namibia.

At the genus level, 16 different genera were recovered as potential bacterial zoonoses. In addition to this, there were no members of a specific genera that were common across the trophy hunted animal tissues or organs. Based on phylogenetic analysis of the 16S rRNA gene sequences, the results showed that the potential bacteria zoonotic species are strongly related, supported by moderate bootstrap values ranging between 58% and 100%. This is in consistent with the possibility that the bacterial species recovered in this study coevolved with their host, following an ancestral beginning of the relatives.

CHAPTER 7: RECOMMENDATIONS

The next step in this work is to carry out the potential pathogenicity activity to determine which of these bacteria are zoonotic in order to start a targeted disease management programme. Similarly, a metagenomic study should be carried out to determine the zoonotic bacterial community associated with different tissues and animals in the BNP. In contrast, when analysing microbiological communities, the prevalence of potential zoonotic bacterial variation between different animal species must be interpreted carefully because the number of detected bacteria taxa may be influenced by the number of different Operational Taxonomic Unit (OTU) types present in a given community, habitat or sample size and the species being investigated (Hughes and Bohannan, 2004; Fakruddin and Shahnewaj, 2013). In addition, long term study of bacteria zoonoses in trophy hunted species in different localities including other national parks, conservancies and game ranches should be conducted in Namibia. It will further help in effectively determining points of infections.

Finally, trophy hunting activities should be performed with utmost care to ensure minimum cross contamination from wildlife to human. In order to achieve this, it is important to have veterinary personnel or microbial clinicians present during trophy hunting activities to inspect hunted carcasses. The present study thus strongly advices the community to take safe precautions when handling and preparing trophy hunted derivatives including meat. Therefore, the public in the BNP should be very cautious in considering the consuming meat from trophy hunted animals in BNP as it may harbour potential zoonotic bacteria which could result in human health implications.

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APPENDICES

Appendix 1: Biosafety plan

BIOSAFETY PLAN

Research project

Master of Science in Biodiversity Management

Department of Biological Science- University of Namibia

Title: Prevalence of bacterial zoonoses in selected trophy hunted species and the potential of human health risk in Bwabwata National Park, Namibia

Approved: by……Date……

A. Principle investigator Information

Principal Investigator (PI): Ameya Matheus-Auwa Department: Biological Sciences Address: Phone number: Alternative phone: Email: shoeameya@gmail.com

B. Emergency Contact Information

Primary Contact:	
Name: Ameya Matheus-Auwa	**:** Principle Investigator
Cell Phone Number:	
Secondary Contact:	
Name: Dr. Jean Damascène Uzabakiriho	**Affiliation:** Main Supervisor (UNAM)
Office Phone Number:	
Email:	
Cell Phone Number:	
Tertiary Contact:	
Name: Dr. Seth J. Eiseb	**Affiliation**: Core Supervisor (UNAM)
Office Phone Number:	

Cell Phone Number:
Email:

It is the responsibility of all parties whom operate under this plan to follow all safe work practices and procedures to the best of their abilities.

C. Sampling (Collection and submission of diagnostic specimens)

1. Samples will be collected from trophy hunted species and any other accidental mortalities

1.1. Study animals for the intended project include:

a. Elephant
b. Buffalo
c. Kudu

1.2. Type of samples for the intended Research Project:

a. Liver
b. Heart
c. Lung
d. Kidney
e. Spleen
f. Lymph node

1.3. Location where the research project will be conducted (Study area)?

a. Bwabwata National Park

4. Purpose for which the samples are to be transported

Specimens for Laboratory diagnostic analysis for MSc final year project

5. Analysis\Experiment Locations

Table 1 will be used to document the locations where the tissue samples will be diagnosed and bio-hazardous materials will be used and experimental work will be carried out.

Location		Type of Research	Purpose of Work Area	Containment Level
Building	**Section**			
Central Veterinary Laboratory	Clinical Microbiology	Bacteriology	Diagnosis and Analysis	CVL standard measures

Laboratory Telephone Number: …….

Primary Contact Name: Dr. Medical veterinary, Umberto Molini (Sub-Division Head-Biotechnology)

Cell:

Telephone:

Email:

1. **Procedures of sample collection**

- Specimens were collected aseptically as possible

- Samples were collected and submitted in forms such as tissue blocks, approximately 500 cm^3.

- Each stage during skinning made use of different sterile equipment's; this was to avoid the contamination of vital organs (specimens).

- Cross-contamination between samples was avoided.

- Sterile instruments were used to cut off the tissue samples.

- The samples were placed in separate sterile plastics or sterile screw cap containers containing a liquid that would preserve the cell during their transportation (Bacteria transporting medium).

- Precautions will be taken to avoid human infection throughout the experiment.

2. **Sample submission**

Samples were submitted to the Central Veterinary Laboratory as early as possible accompanied by a Test Request/ Sample submission Form.

The laboratory or transporting entity was notified that the samples/diagnosis includes agents that are potentially infectious for people.

3. **Transportation, preservation and packaging**

The tissue samples that were transported were potential Infectious Biological Substances

3.1. Entities involved in packaging and transportation

a. Packaging:

1. Principle Investigator
2. Veterinary officials

b. Transportation:

1. Principle Investigator
2. NamPost Courier Services (was notified of the potential infectious tissue samples accompanied by a copy of this Biosafety plan).

- Samples were packed in 3 (layers) sterile plastics, primary plastics with a seal surrounded by enough absorbent material to completely contain a spill. In general, samples were packed in triple packaging as required by CVL.
- Samples were stored and transported in contact with liquid nitrogen units or RNA*later* in contact with a cooling gel/agent or cold packs inside a cool box.
- If transportation to laboratory was delayed, samples were kept or preserved in a refrigerator between 2 - 8 °C.
- Samples were clearly labelled (sample identity, animal identification and the date of collection) and submitted inn separate leak proof containers to avoid leakage.
- If samples were sent via the post (NamCourier), they were packed and labelled in accordance with the regulations of the postal authorities. The outer packaging

labels included a list of contents, the transporting entity's label, including name, address and phone numbers as well as the PI contact details.
  - Samples were always sealed with a sufficient absorbent material to soak up the entire sample in the case of breakage of leakage.
- The package was clearly labelled with the words 'Biological Substances'

4. Facility and equipment care and maintenance

4.1 Personal Protective Equipment (PPE) Needed for Procedure:

Examples of Personal protective equipment (PPE) during the experiment included safety glasses or goggles, laboratory coats, gloves-latex, face shields.

4.2 Laboratory equipment

A. Biological safety cabinets

B. Refrigerators

D. Autoclaves

C. Disinfectants

The PI followed the CVL guidelines for proper procedures

5. Field Waste management or disposals

For waste disposal, the principle investigator followed the Directorate of Veterinary Biological Waste Management Plan, such that all utilized instruments were sterilized /disinfected.

a. Decontamination methods

The following levels of decontamination methods were used during this study.

1. Sterilization, a method which destroys all microbes, including bacterial spores. Autoclaving (dry or steam) is one method of sterilization which was used in this study.

2. Disinfection, a method that reduces all forms of disease causing organisms on the working surfaces or equipment.

3. Note; used samples were incinerated

5.2. Equipment Disposal

The principle investigator ensured that the equipment is cleaned of all hazardous elements and it was well disposed. Equipment to be disposed-off were incinerate or autoclaved.

Appendix 2: Equipment management form

EQUIPMENT MANAGEMENT FORM

Objective: The Equipment Management Form is intended to protect the health and safety of the environment, all personnel involved during the field and laboratory experiments and the public from being exposed unnecessarily to equipment's and associated areas or contaminants that may contain potential infectious hazards.

It is the responsibility of the Principle Investigator to ensure that any equipment and areas associated with the tissue sample(s) and the equipment being used or disposed-off, are appropriately cleaned and decontaminated after any experimental work.

Equipment information	
Equipment name:	Associated Area:
Associated animal\sample used on:	User(s) name:
Nature of work	
☐ Disposal ☐ Decontaminated ☐ Removal\cutting of organ(s) ☐ Other	
Brief description of nature of work performed by equipment	
Health and safety hazards	
Hazardous materials used with the equipment ☐ Biological ☐ Infectious ☐ Chemical	
Has the equipment and the associated area been disinfected?	☐ Yes ☐ No
If yes, describe the disinfection process\method employed. If no, explain why.	
Required PPE when handling the tissue samples and equipment's ☐ Leather gloves ☐ Latex gloves ☐ Eye protection ☐ Foot protection ☐ Disposal coveralls Other: …… ☐ None	

Environmental health and safety precautions that must be adhered to.	
I certify that the information provided in this form is correct to the best of my knowledge.	
Principle investigators name: …….. Date: ……..… Signature: ……...…	Supervisors name: ..... Date: ……. Signature…….

Equipment's used during the field experimental associated with any potential biological organs/tissues will be decontaminated with an appropriate disinfectant following the Veterinary Services sampling and laboratory standard measures. If the Principle Investigator is unsure about which method to use he\she will contact the nearest State Veterinary Offices across Namibia.

Appendix 3: Ethical clearance certificate for the study

UNAM
UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: FOS /190/2017 **Date: 24 April, 2017**

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: Prevalence Of Bacterial Zoonosis In Selected Trophy Hunted Species And The Potential Human Health Risk In North East Namibia

Nature/Level of Project: Masters

Researcher: Ameya Matheus-Auwa

Student Number: 201030292

Faculty: Faculty of Science

Supervisor: Dr Jean Damascène Uzabakiriho (Main) Dr. Seth J. Eiseb (Co)

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. P. Odonkor: UREC Chairperson

Ms. P. Claassen: UREC Secretary

NAMIBIAN POLICE WINDHOEK 18 -05- 2017

Appendix 4: Research or collecting permit 1

REPUBLIC OF NAMIBIA

MINISTRY OF AGRICULTURE, WATER AND FORESTRY

Tel.: (061)208 7512\3
Fax: (061) 2087779

Chief Veterinary Officer
Government Office Park
Private Bag 12022
WINDHOEK

27 March 2017

Deputy Director Academic Centres
University of Namibia
P/bag 13301
Windhoek

Dear Mr Eiseb

MSC STUDENT: MATHEUS AMEYA

Your email on the above refers.

I am pleased to inform you that we have approved the bio safety plan as presented by Mr Ameya to the Central Veterinary Laboratory in Windhoek.

You may therefore commence with research activities in line with the approved bio safety plan. Kindly note we reserve the right to withdraw this approval at any time to safe guard the interest of the Directorate.

Yours sincerely,

Dr A Maseke
CVO

MINISTRY OF AGRICULTURE WATER AND FORESTRY
CHIEF VETERINARY OFFICER
27 MAR 2017
P/BAG 12022
REPUBLIC OF NAMIBIA

Appendix 4: Research or collecting permit 2

MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 2290/2017
Valid from 7 April 2017 to 31 March 2018

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Mr. M.A. Ameya**
Address: **P.O. Box 60196**
Katutura
Windhoek
Namibia
Coworkers: **Dr. J.D. Uzabakiriho, Dr. S.J. Eiseb and Mr M. Christopher**

Prevalence of bacterial zoonosis in selected trophy hunted species and the potential human health risk in North East Namibia at Bwabwata National Park, subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
12 APR 2017
WINDHOEK
Private Bag 13306, Windhoek
Tel: 2842111 • Fax: 258861

.....
Authorising Officer

IMPORTANT
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Conservation Scientist, email iita.matheus@met.gov.na
Private Bag 13306, Windhoek, Namibia