A survey of Tuberculosis and Brucellosis in wildlife and cattle in the South-East Lowveld of Zimbabwe

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Declaration

1. BY CANDINDATE

This thesis is my own original work and has not been presented for a degree in any other University.

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2. BY SUPERVISORS

This thesis has been submitted for examination with our approval as University supervisors.

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List of Acronyms and Abbreviations

Acronym	Expanded form
AFB	Acid Fast Bacilli
AHEAD	Animal Health for the Environment and Development
ARC-OV1 Onderstepoort	Agriculture Research Council-Veterinary Institute
bTB	Bovine Tuberculosis
CA	Contagious Abortion
c-ELISA	Competitive Enzyme Linked Immunoabsobert Assay
CIRAD	French Agricultural Research Centre for International Development
DTH	Delayed Type Hypersensitivity
GLTFCA	Greater Limpopo Trans-frontier Conservation Areas
GNP	Gonarezhou National Park
HNP	Hwange National Park
KNP	Kruger National Park

IFN-γ	Interferon Gamma
OIE	Office International Des Epizooties
PPD	Purified Protein Derivative
SCITT	Single Comparative intradermal Tuberculin skin Test
SA	South Africa
TFCAs	Trans-frontier Conservation Areas
TST	Tuberculin Skin Test

Abstract

A cross-sectional study was conducted to determine the seroprevalence of bovine brucellosis and the prevalence of bovine tuberculosis (bTB) in cattle and wildlife at a wildlife-livestock interface in the south-east lowveld of Zimbabwe. Study areas were selected to include those with close proximity to wildlife from GNP and KNP and those without a wildlife-livestock interface area. For both cattle and wildlife, sera were screened for anti-Brucella antibodies using the Rose Bengal test as a presumptive test and the competitive-ELISA as a confirmatory test. The Single Comparative Intradermal Tuberculin Skin Test was used to identify reactor cattle for bTB and positive animals were confirmed using the gamma interferon test, culture and histopathology. For wildlife, bTB was tested in African buffaloes by using the gamma interferon test, culture and histopathology. Age, sex, location, abortion and grazing history were considered as risk factors for Brucella seropositivity while age, sex, location and grazing history were considered as risk factors for bTB in cattle. A total of 1158 cattle were tested and the overall seroprevalence of brucellosis was 9.9%. A total of 97 wild animals (47 buffaloes, 33 impala, 16 kudu, and 1 giraffe) were tested and only one animal (giraffe) (1%) was seropositive for brucellosis. In the interface area, cattle with a history of grazing in the park recorded a significantly ($P<0.05$) higher Brucella seroprevalence (13.5%) compared to those with no history of grazing in the park (4.9%). A total of 477 cattle were tested for bTB and only five (1%) reactors were recorded. The five cattle reactors were all found to be negative on the confirmatory test, culture and histopathology. Of the 38 buffaloes tested for bTB and 4 (10.5%) were positive and bacterial culture of two gamma interferon-positive buffaloes yielded *Mycobacterium bovis*. The results of the present study established the presence of

brucellosis in communal cattle in the studied areas and of bTB in GNP African buffaloes for the first time.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

In 1880, Hutcheon reported the first case of bovine tuberculosis (bTB) caused by *M. bovis* in cattle. In 1990 the disease was first confirmed in the African buffalo in South Africa's Kruger National Park (KNP) (Bengis *et al*., 1996). However, a number of cases of bTB caused by *M. bovis* had previously been reported in free-ranging African wildlife during the 20th century, illustrating the susceptibility of a wide range of free-ranging mammals to the disease which has been primarily recognized as a disease of livestock (Guilbride *et al*., 1963; Gallagher *et al*., 1972). The African buffalo (*Syncerus caffer)* and the Lechwe (*Kobus leche*) in Uganda Queen Elizabeth and Zambia's Kafue National Parks respectively, proved to act as maintenance hosts for *M. bovis* (Krauss *et al*., 1984). Evidence suggests that 10 other small and large mammalian species, including large predators, are spillover hosts (Michel *et al*., 2006). Over the past 15 years, the disease has been reported to spread northwards in the KNP leaving only the most northern buffalo herds unaffected (Michel *et al*., 2006).

Brucellosis has also been described in several free-ranging ecosystems infecting predominantly buffalo, hippopotamus and waterbuck (De Vos and van Niekerk, 1969; Condy and Vickers, 1972; Gradwell *et al*., 1977). In South Africa, several species of wildlife: African buffalo (*Syncerus caffer*), hippopotamus (*Hippopotamus amphibius*),

zebra (*Equus burchellii*), eland *(Taurotragus oryx)*, waterbuck (*Kobus elipsiprymnus*) and impala *(Aepyceros melampus)* have tested serologically positive for brucellosis (De Vos and van Niekerk, 1969) and serological surveys revealed up to 23% positive reactors in Africa buffalo from the KNP (Herr and Marshall, 1981). In Zimbabwe, 48% of African buffalo serum samples were sero-positive (Madsen and Anderson, 1995). These samples were collected from game areas where contact with domestic cattle, sheep and goats could be excluded. It was concluded that brucellosis might be a sustainable infection in African buffalo populations, which consequently should be considered a possible source of re-infection for domestic stock (Madsen and Anderson, 1995). From an economic point of view, wildlife tuberculosis and brucellosis has resulted in national and international trade restrictions for affected species (Michel *et al*., 2006).

In Zimbabwe, the current status of bTB and bovine brucellosis in cattle and wild animals in wildlife-livestock-human interface areas is not known. As of 2007, the national cattle herd of Zimbabwe has been declared to be free of bTB however, the prevalence of brucellosis in non-interface areas has been reported to be increasing and this has been attributed to the uncontrolled movements of cattle, disruptions of the Brucellosis Accreditation Scheme and lack of foreign currency to procure the strain 19 vaccine to vaccinate the animals (Director of Veterinary Services Report, 2007).

Expansion of ecotourism-based industries, changes in land-use practices and an escalating competition for resources have been reported to increase the contact between free-ranging wildlife, domestic animals and humans (Bengis *et al.*, 2002). In addition,

the joint development of the Greater Limpopo Trans-frontier Conservation Area with South Africa and Mozambique would increase such contacts. Although human and domestic animal presence in wildlife areas may provide an important economic benefit through ecotourism, exposure to human and domestic animal pathogens may represent a health risk for wildlife and vice-versa. As a result of changes in population dynamics, an increased interaction of livestock with wild animals at a livestock-wildlife interface and changes in disease trends associated with a livestock-wildlife interface as indicated by studies done in neighboring countries such as South Africa; bovine brucellosis and bTB epidemics would have a number of implications, which might only be seen in the long-term (Michel *et al*., 2006). In this context, the development of the wildlife farming industry could contribute to the re-emergence of bTB and bovine brucellosis. The risk of spillover infection to neighboring communal cattle raises concerns about human health at the wildlife–livestock–human interface areas of the Gonarezhou, Hwange and other National Parks.

These concerns would be exacerbated by the declining human population immunity due to the Human Immunodeficiency Virus (HIV) infection leading to an upsurge of new human Mycobacterium infections. Increased poverty levels force people to rely on informal food markets for commodities such as fresh un-pasteurized milk and un-inspected meat and thereby aggravating the situation. In addition, the unavailability of convenient diagnostic tools for most species, the absence of an effective bTB vaccine making it impossible to contain and control the disease within an infected free-ranging environment, the impractical treatment of infected animals and the costly treatment of infected human beings would also have a negative impact on prevention and control of

bTB. Furthermore, eradication of bTB and bovine brucellosis from wildlife reservoirs is difficult and currently, the test and slaughter control policy is not feasible under the existing socio-economic conditions of Zimbabwe. Hence, veterinary researchers and policy-makers in sub-Saharan Africa have recognized the need to intensify research on these diseases and the need to develop tools for their control, initially targeting the African buffalo and the lion *(Panthera leo)* (Michel *et al*., 2006). It is of public health concern for people living closely with livestock population with high incidence of brucellosis (Magona *et al*., 2009). The aim of this study was to determine the prevalence of bTB and bovine brucellosis in cattle and wildlife at a wildlife-livestock-human interface in the south-east lowveld of Zimbabwe and also to determine whether the prevalence of these diseases in cattle is influenced by close proximity to wildlife and other factors such as sex and age of cattle.

1.2 Justification of the study

The study sites, Malipati and Pesvi communal areas in the lowveld of Chiredzi South district in southeastern Zimbabwe, are located adjacent to Gonerezhou National Park (GNP) and Kruger National Park (KNP), respectively (Figure 3.1 and 3.2). A treaty was signed in December 2002 with the prospect of uniting the GNP, Mozambique's Limpopo National Park and the KNP, creating the largest trans-frontier game reserve in Southern Africa. This will result in marked increase interaction between wildlife and livestock since all the parks are adjacent to communal areas. Hence, there is great need for information on diseases which occur at a livestock-wildlife interface. Considering the spread of bTB in KNP, bTB and bovine brucellosis are some of the diseases of

zoonotic and economic importance which need to be investigated at the livestock-wildlife interface. Preventing the transmission of the diseases is more economical compared to the costly control of the diseases when they have already spread in both livestock and wildlife. The risk of spillover infection from wildlife between neighboring countries and ultimately to communal cattle and vice-versa raises concerns about human health at the wildlife-livestock-human interface, not only along GNP, but also other national parks like Hwange and also with regards to the joint development of the Great Limpopo Transfrontier Conservation Area (GLTCA) with South Africa and Mozambique.

1.3 Hypotheses

1. Bovine brucellosis and bTB are present in communal cattle and wildlife.
2. The prevalence of bTB and bovine brucellosis in cattle is influenced by close proximity to wildlife and other factors such as sex and age of the cattle.

1.4 Objectives

1.4.1 General objective

To investigate/explore the presence of bTB and bovine brucellosis in communal cattle and wildlife at a wildlife-livestock interface in the south-east lowveld of Zimbabwe.

1.4.2. Specific objectives

1.4.2.1. To determine the prevalence of bTB and bovine brucellosis in communal cattle and wildlife.

1.4.2.2. To determine whether the prevalence of bTB and bovine brucellosis in cattle is influenced by close proximity to wildlife.

1.4.2.3. To investigate the role of individual animal risk factors on the prevalence of bTB and brucellosis.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Definition of a livestock-wildlife interface

A livestock-wildlife interface may be linear as along a fence line or focal as at a shared water point or diffuse as where range and resources are shared (Bengis *et al.*, 2002). This livestock-wildlife interface can be a direct physical interaction such as sharing the same space at the same time or it can be an indirect contact through soil, forage and water with which another animal has recently been in contact with and has left bodily secretions (Bengis *et al.,* 2002). The interface is associated with a potential two-way avenue for the transmission of pathogens from wild to domestic animals and vice-versa. Diseases with a major epizootic potential are generally the highly contagious viral diseases such as foot-and-mouth disease, rinderpest, Newcastle disease and African swine fever and these may have a significant impact on domestic livestock populations, agricultural-based economies and wildlife (Bengis *et al.,* 2002). Vector borne diseases, especially tick borne diseases such as babesiosis, erhlichiosis and theileriosis affect the development of communal and commercial agriculture. Disease transmission at a livestock-wildlife interface depends on a number of factors such as indirect/direct presence or absence of maintenance hosts, mode of transmission, presence or seasonal abundance of vectors and presence of susceptible populations. Direct or indirect contact at the interface is of paramount importance for an outbreak to occur (Bengis *et al.,* 2002).

According to Bengis *et al*. (2002), diseases transmitted at the livestock-wildlife interface can be divided into three main categories: the wildlife-maintained (indigenous), multi-species and alien/exotic diseases. Wildlife-maintained diseases include foot-and-mouth disease, African swine fever, Malignant catarrhal fever, trypanosomosis, theileriosis or Corridor disease, African horse sickness, classical swine fever or hog cholera, heartwater (erhlichiosis), bluetongue, Rift valley fever, lumpy skin disease and Newcastle Disease (Bengis *et al.,* 2002). Multi-species diseases occur on most continents and these diseases (e.g. anthrax, brucellosis and rabies) occur in both wildlife and domestic livestock. Alien/exotic diseases are certain diseases that were probably introduced into the African continent with importation of domestic livestock from Europe and Asia during the colonial era. Examples include rinderpest, canine distemper, bovine tuberculosis, African swine fever and African Horse Sickness and most recently avian influenza (Bengis *et al.,* 2002).

2.2 Bovine tuberculosis

2.2.1 Aetiology

The disease is caused by *M. bovis* (Hope and Villarreal-Ramos, 2007). These tubercle bacilli are rod-shaped and variable in length; Gram-positive, acid-fast, non-motile and non-spore forming slow growing bacteria. They are also reported to be relatively resistant to chemical disinfectants and are strict aerobes that grow on Lowenstein-Jensen medium (Hirsh and Zee, 1999). *M. bovis* together with *M. tuberculosis, M. africanum, M. microti* and *M. leprae* are all obligate parasites, usually being transmitted only by mammalian hosts and make up the *M. tuberculosis* (MTB) complex also known as tubercle bacilli. Attempts have been made to group *Mycobacterium* spp. based on

pigment production, growth rate, biochemistry, pathogenicity and currently genetic studies. However, with the exception of *M. leprae, Mycobacterium* species can be distinguished into two groups; the *M. tuberculosis* (MTB) complex and *Mycobacterium* outside tuberculosis Complex (Sooligen, 2001).

M. bovis is the usual cause of tuberculosis in cattle, although other species such as *M. tuberculosis* and *M. avium* occasionally have been able to establish and produce localized lesions in cattle. However, *M. bovis,* the etiological agent of bovine tuberculosis (bTB) has one of the broadest host ranges of all pathogens including carnivorous mammals (De Lisle *et al*., 2001). In 1880, Hutcheon made the first reference of bovine tuberculosis, which is caused by infection with *M. bovis*, in cattle in South Africa (Hutcheon, 1880). Cattle, African buffalo and bison (*Bison bison)*, are considered the maintenance hosts of *M. bovis*, but nearly all warm–blooded animals are susceptible to the infection (Tschopp *et al*., 2010). Compared to other bacteria of the MTB complex, *M. bovis* has a broad range of animal hosts and this complicates the control of bTB, particularly when the infection becomes self-sustaining in wildlife species, which in turn can become reservoirs of *M. bovis* for domestic animals. A potential link between tuberculosis in livestock and wildlife was first suggested by Paine and Martinaglia (1929) when they reported bovine tuberculosis in greater kudu *(Tragelaphus strepsiceros)* and small ungulates in the Eastern Cape Province of South Africa. Hence, *M. bovis* is associated with tuberculosis in wild animals especially the African buffaloes and the greater kudus *(*Michel *et al*., 2006).

2.2.2 Epidemiology

2.2.2.1 Distribution

Bovine tuberculosis occurs in almost all developed and developing nations of the world. Of the 55 African countries, 25 reported sporadic/low occurrence of bovine TB; six reported enzootic disease and two; Malawi and Mali, were described as having a high occurrence (Cosivi *et al*., 1998). Because it is a chronic disease, bTB is mainly found in old animals. It affects both young and old animals but old animals are more susceptible. Distribution of bTB is not affected by sex; both females and males appear to be infected at the same rate. However, in Hluhluwe-iMfolozi park (HiP) in South Africa, African buffalo bulls spent only a limited period, generally not exceeding 3–4 months, with breeding herds, but their *M. bovis* infection rates were found to be higher than those of cows (Jolles, 2004).

2.2.2.2 Transmission

Mycobacterium bovis is excreted in exhaled air, sputum, faeces, urine and milk of infected animals (Blood *et al*., 2000). In the early stages of the disease, before any lesions are visible, cattle may also excrete viable mycobacteria in nasal and tracheal excretions (McIlroy *et al*., 1986; Cadmus *et al*., 2004). Inhalation is the almost invariable mode of infection in housed cattle, and even in those at pasture and, it is considered the principal mode of transmission (Cadmus *et al*., 2004). The most common method of disease transmission between cattle and wildlife is inhalation of the bacteria. Transmission can also occur through ingestion of contaminated feed (Michel *et al*., 2007). However, transmission of *M. bovis* between herd members occurs most

frequently by aerosol, whereas spillover to other species requires different modes of transmission (Michel *et al*., 2008). Predators and scavengers contract the infection commonly by ingestion of infected tissues (Michel *et al*., 2006). The organism can remain viable in the environment for 6-8 weeks depending on temperature and humidity. Only 1–5% of infected cattle shed the organism in their milk and transmission from infected dam to calf can occur through the consumption of the dam's milk (Michel *et al*., 2004). Farm employees in contact with infected cattle may serve as mechanical vectors of the bacterial agent on their clothing or shoes (Michel *et al*., 2004). In rare cases, humans infected with *M. bovis* can transmit the disease to cattle through sputum and urine (Gabashane, 2008). There is evidence that bTB was introduced into KNP by cattle to buffalo transmission (Grobler *et al.,* 2002). Spill over of infection by direct and indirect transmission occurred in wildlife species (Grobler *et al.,* 2002).

Bovine tuberculosis is a zoonosis and the transmission to humans constitutes an important public health problem (Cadmus *et al*., 2004). The bacteria can be transmitted from cattle to humans through consumption of contaminated unpasteurised milk and meat products (Berg *et al.,* 2009).

2.2.2.3 Prevalence

The incidence of the bTB is not only higher in developing nations but in the absence of any national control and eradication programs, it is also increasing worldwide particularly in the Asian, African and Latin American countries (Hope and Villarreal-Ramos *et al.,* 2003). Most of the developed countries have managed to reduce the prevalence of bTB. In Great Britain for instance, about 5.6% of the herds were reported

to be affected by tuberculosis restrictions in 2004 (Reynolds, 2006). Developed countries have used the test and slaughter policy where all positive reactors were slaughtered to control the disease (Thoen *et al.,* 2006; Durez et al., 2009). In Africa, the prevalence is still high because of difficulties encountered in implementation of the test and slaughter policy. Bovine tuberculosis is endemic in African buffalo and a number of other wildlife species in the Kruger National Park (KNP) and Hluhluwe-iMfolozi Park (HiP) in South Africa (Michael *et al*., 2009). The prevalence is even increasing as evidenced by outbreaks in wildlife, especially in African buffaloes in KNP (Rodwell *et al.,* 2000). The disease has spilled over to other animals such as lions, cheetah, kudu and leopards (Keet *et al*., 1996). In the southern and central region of KNP the incidence increased from 4% to 16% while the initially bTB free area to the north showed an overall prevalence of 1.5% in 1998 (Michel *et al*., 2009). Since African buffaloes are considered to be one of the four preferential prey species of lions (Mills, 1995), the frequent exposure of lions to large amounts of infectious buffalo tissue led to a spatial spread of bTB within lion prides in areas where the prevalence of the disease is high in African buffaloes (Michel *et al*., 2006). Surveillance of bTB at abattoirs in Zimbabwe indicated that there were no cases of the disease since 1980 (Director of Veterinary Services Report, 2007). In addition, studies on the disease in 2003 in the south-east lowveld areas of Zimbabwe (Pesvi communal lands) using SCITT did not confirm bTB cases in cattle (Dutlow, unpublished report, 2003). In Zimbabwe, the current status of bTB and brucellosis is not well documented. High bTB prevalence has been recorded in Zambian Kafue Lechwe (*Kobus Leche Kafuensis*) (Munyeme *et al*., 2009). In Ethiopia, bTB is considered an endemic disease, and has been reported in many regions (Ameni, 2007; Damelash *et al*., 2009). In Ethiopia a nine-year meat inspection record was

analyzed to elucidate the trend of bTB in the local cattle population and of the carcasses inspected, 10.2% had lesions suggestive of tuberculosis (Damelash *et al*., 2009). In another study in Ethiopia the prevalence of bTB infection as determined by SCITT was 9.7% whereas the non-specific infection prevalence was 10.8% (Fetene and Kebede, 2008). In a study in Tanzania the overall prevalence of the bTB ranged from 13, 2% to 51% (Kazwala *et al*., 2001). In the same study older animals were found to be infected with bTB more than yearlings and calves (Kazwala *et al.,* 2001). In Mali a high prevalence of bTB has been reported but surveillance and control schemes are restricted to abattoir inspections only (Muller *et al*., 2008). In a recent prevalence study in dairy cattle herds from the peri-urban region of Bamako, 19% of the animals reacted positively to the comparative tuberculin skin test (Muller *et al.*, 2008). In Algeria bTB was found to be prevalent despite governmental attempts to control the disease (Sahraoui *et al*., 2009). In Uganda, bTB was reported to be common among the long-horned Ankole cattle of the western part of the country (Oloya *et al*., 2006). Earlier studies revealed a prevalence of 19.7% in pastoral cattle in that region. Surveillance through the abattoir slaughter reviews showed that 1.8% of slaughter animals originating from the eastern region showed generalized tuberculosis based on gross pathological lesions (Oloya *et al*., 2006). In Zambia a study was conducted focusing on tuberculosis in indigenous cattle breeds in the Zambian livestock/wildlife interface areas across different cattle grazing strategies (Munyeme *et al*., 2009). Animal bTB prevalence in Lochinvar was recorded at 5.2% and Blue Lagoon 9.6%, both found in the wildlife/livestock interface areas whilst Kazungula which is outside the interface area had a prevalence of 0.8% (Munyeme *et al*., 2009). In a prevalence study carried out in

Malawi from selected dip tanks and dairy cattle the prevalence of bTB reactions was found to be 3.85% (Bedard *et al*., 1993).

2.2.2.4 Factors associated with transmission at a wildlife-livestock interface

In single host systems the density of a host population needs to exceed a threshold for the disease to invade and persist in the population (Renwick *et al.,* 2006). The rate of inter-species transmission is dependent on the interaction rate between the host species (Renwick *et al*., 2006). Indirect and/or direct contact is important for the transmission of the disease (Bengis *et al.,* 2002). A wildlife deterrent fence is usually meant to separate domestic from wild animals, but despite great efforts and costs for its maintenance, this man-made barrier cannot guarantee the absolute separation of livestock from wildlife populations. Elephant (*Loxodonta africana*) activities or natural disasters such as the water floods experienced early in the year 2000 can cause damage to the fence, allowing African buffaloes to mingle with domestic cattle (Michel *et al.,* 2006). On the other hand, fences cannot prevent the movement of wild animals in all cases, e.g. greater kudu and warthogs. Once contact between infected wild animals with livestock is established, the potential of *M. bovis* transmission to cattle exists, as demonstrated in New Zealand, Great Britain and North America (Cheeseman *et al*., 1989; Morris and Pfeifer, 1994). Once infected, many wild animals have shown the potential to act as reservoirs of infection for both domestic cattle and other valuable wildlife species (Renwick *et al*., 2006). The brush-tail possum (*Trichosurus vulpecula*), European badger (*Meles meles*), bison, African buffalo, Kafue lechwe and white-tailed deer (*Odocoileus virginianus*) can all act as maintenance hosts for bTB, allowing the persistence of the infection in wildlife and enabling the horizontal transmission of the

pathogen between species (Renwick *et al*., 2006). With the increasing *M. bovis* infection rate in the buffalo population in KNP, the infection spilled-over into other wildlife species (Keet *et al*., 1996).

To date no evidence of outbreaks of bovine tuberculosis in communal cattle herds in South Africa has been demonstrated, despite intensified monitoring of cattle health at the interface (Michel *et al.,* 2006). However, unlike in commercial productions, communal livestock and their products are largely excluded from veterinary public health control measures and this could probably account for no evidence of the disease in communal cattle (Michel *et al*., 2004). Infection of communal cattle with bovine tuberculosis could be detrimental to the livelihood of small scale farmers. The objectives of livestock keeping in rural areas of sub-Saharan Africa, over and above that of food production, also include the generation of traditional wealth, social status and marriage dowries (Michel *et al.,* 2006). As a result of this value system, life expectancy of livestock is generally higher than on commercial farms, livestock are moved in exchange of goods or services, and owners often live in close proximity with their animals. Bovine tuberculosis, as a chronic and progressive disease manifests itself more often in older animals under nutritional or productive stress. Taking this into account, people who are frequently exposed to either livestock infected with bovine tuberculosis or infected products such as un-pasteurized milk should be considered at risk. Spillover or dead-end hosts have only a limited possibility of maintaining the disease in the population in the absence of a persistent alternate source of infection (De Lisle *et al*., 2002). Lions, leopards (*Panthera pardus*), cheetahs (*Acinonyx jubatus*) and other carnivore species do not appear to be able to maintain infection in the absence of an

infected maintenance host in the system (Renwick *et al*., 2006). The African buffalo is considered the main reservoir of bTB (Michel, 2002) and is thought to be responsible for infection of other sympatric wildlife and the possible re-infection of cattle (Renwick *et al*., 2006).

2.2.3 Clinical signs

Bovine tuberculosis is a chronic, primarily respiratory disease which can affect all mammals including humans (Berg *et al.,* 2009) characterized by the formation of granulomatous lesions (tubercles) seen in lungs and draining lymph nodes (Berg *et al.,* 2009). It is a slowly progressive disease often taking months or years to develop. Coughing with a pronounced difficulty in breathing, reduced milk production, severe chronic weight and production loss, rough hair coat, a variable appetite and fluctuating fevers are some of the common clinical signs in animals (Blood *et al.,* 2000; De Lisle *et al*., 2002). Other clinical signs are swollen lymph nodes especially of the head, discharging abscesses of lymph nodes and skeletal and synovial lesions associated with lameness especially in lions (De Lisle *et al*., 2002). Cattle and wildlife with bovine tuberculosis infections are without clinical signs 90% of the time, but may eventually exhibit weight loss and a gradual decline in general health (De Lisle *et al*., 2002). Udder infection is rare (<2% of cases) but have serious public health implications (Hirsh and Zee, 1999). In humans, because of the route of infection, disease often manifests itself as extra-pulmonary TB (Berg *et al.,* 2009).

2.2.4 Diagnosis

Early laboratory diagnosis of tuberculosis still relies on microscopic examination of stained smears (Gabashane, 2008). Acid-fast staining and microscopic examination is used to detect the pathogen in the lung lesions, lymph nodes of infected animals and sputum of adults (in humans). However, sensitivity of direct microscopy has been shown to be 25-65% leading to a possibility of under-diagnosis in under-resourced countries which cannot afford to send samples for culture (Gabashane, 2008). Bacterial culture is the gold standard for diagnosis of tuberculosis, while histopathology is limited by difficulties to distinguish lesions caused by *M. bovis* and other mycobacterial species (De Lisle *et al*., 2002). Culturing allows species identification and determination of susceptibility to antimicrobial agents to be accomplished. The culture of *Mycobacterium* spp. can take up to 8 weeks and 10-20% cases of the bacillus are not cultured (Andersen *et al.,* 2000). Probability of isolating *M. bovis* from test reactors with visible lesions (VL) is well over 90% (Rua-Domenech, 2006).

Delayed-type hypersensitivity (DTH) skin testing has been the most common test used in cattle (Monaghan *et al*., 1994) but alternative *in-vitro* assays of cellular immunity including lymphocyte proliferation, the release of interferon-gamma (IFN-γ) and the production of soluble interleukin-2R (IL-2R) have also been developed (Outteridge and Lepper, 1973; Wood *et al*., 1991). Skin-testing with purified mycobacterial protein derived antigens is still the standard test for diagnosis of tuberculosis in man and domestic animals (OIE, 2008). The tuberculin skin test (TST) assesses the degree of cellular immune response to purified protein derivative of *M. bovis* (PPD-B). In

sensitized animals, intradermally inoculated PPD elicits indurations at the injection site within 48–72 hours post-injection. The size of the indurations depends on the number of infiltrating and accumulating cells during this period of time and a positive test implies past or present infection (Hirsh and Zee, 1999). The SCITT is done by shaving two sites in the cervical region of each animal at a distance of about 12 cm apart and record the skin fold thicknesses. Equal volumes (0.1 ml) of avian PPD tuberculin and bovine PPD tuberculin are injected intradermally into the shaved upper and lower sites of the neck, respectively and measurement of the skin fold thicknesses at injected sites is done after 72 hours using calipers (OIE, 2008). Reviews of SCITT done estimates the sensitivity values of the tuberculin test to be 90% (Morris and Pfeiffer,1994; Aranaz *et al*.,2006).The major drawback of PPD is that most protein components in this substance are shared between mycobacterial species or with unrelated species of the bacteria thereby decreasing the specificity of TST (Andersen *et al*., 2000). Errors in placement and reading of the TST can yield false positive results (Mazurek *et al.,* 2001). However, the use of comparative tuberculin skin tests has resulted in improvements in specificity of skin tests (Pollock *et al*., 2000). Intradermal tuberculin skin test has been used in wildlife with varying degrees of sensitivity and specificity (Cousins and Florisson, 2005). In farmed red deer, studies carried out in New Zealand established 82–86% sensitivity and 46–76% specificity of the comparative skin test (Ferna´Ndez-De-Mera *et al*., 2009).

The gamma interferon test has been recently introduced to test for bTB in animals and humans (Michel *et al.,* 2004). Recently available interferon gamma (IFN-γ) release assays have been shown to have better specificity and sensitivity in the diagnosis of

both latent and active bTB (Wood and Jones, 2001). The sensitivity of the (IFN-γ) assay has been found to vary from 81.8% to 100% for culture-confirmed bovine TB and specificity between 94% and 100% (Wood and Jones, 2001). The test measures IFN-γ in the supernatant of antigen-stimulated cells by enzyme-linked immunosorbent assay (ELISA). Laboratory diagnosis of suspected cases of bovine tuberculosis in wildlife is essential for confirmation of infection and, in combination with molecular characterization of *M. bovis*, provides a powerful tool to assist in studying spatial, temporal and inter-species transmission of *M. bovis* (Michel, 2002). Restriction fragment length polymorphism (RFLP) has been used to track transmission from cattle to KNP African buffalo, from African buffalo to lion (and other spillover species (Michel, 2002). Ante-mortem diagnosis of bovine TB in free-living wildlife is difficult as animals need to be located and immobilized to collect blood for in vitro diagnostic assays (de-Garine-Wichatitsy *et al.,* 2010)

2.2.5 Treatment and control

The treatment of bTB in cattle or wildlife is not recommended because treated animals can continue to shed the bacteria and act as sources of infection to others (Hirsh and Zee, 1999). In addition, because of public health hazards and drug resistance, chemotherapy of animals is not recommended (Hirsh and Zee, 1999). Control and eradication programmes for bTB have been focused mainly in domestic cattle because they are the traditional hosts and have economic importance (Renwick *et al*., 2006). The control of bTB in South Africa is based on intradermal tuberculin testing and slaughter as well as on abattoir surveillance (Michel *et al*., 2008). A number of strategies have been employed against bTB, but the approach has generally been based on government-

organized programmes by which animals deemed positive to defined screening tests are slaughtered (Pollock *et al.,* 2000). Eradication of bTB from cattle populations during the 20th century using test and slaughter measures were based on SCITT (Grobler *et al.,* 2002; Collins, 2006). Of all nations in Africa, only seven apply disease control measures as part of a test-and-slaughter policy and consider bTB a notifiable disease; the remaining 48 controls the disease inadequately or not at all (Cosivi *et al*., 1998).

M. bovis can spread to humans through the consumption of raw milk or un-pasteurized or improperly pasteurized dairy products from infected animals (Berg *et al.,* 2009). Hence, regulations for milk pasteurization temperatures are designed to protect consumers from contracting bTB. All raw milk dairies must be tested annually to ensure safe products for the consumers. The control program must rely on two strategies for the detection of bTB; live animal and slaughter surveillance (Renwick *et al.,* 2006). For live animal surveillance, field veterinarians conduct the tuberculin skin test on cattle for movement, herd accreditation and disease investigations. Animals with a response to the initial skin test are subjected to additional confirmatory testing using IFN-γ assay by veterinarians. For routine slaughter surveillance, cattle slaughtered at abattoirs are inspected for granuloma lesions. Suspected lesions undergo laboratory diagnostics to confirm presence of *M. bovis.* Any carcass with bTB confirmed lesions is not used for human consumption. Additionally, the herd of origin for the condemned carcass is bTB tested (Blood *et al*., 2000).

Eradication of bovine, human and avian tuberculosis is reported to reduce infection hazards for other species (Hirsh and Zee, 1999). Despite great efforts the Officially

Tuberculosis Free status has not yet been achieved in some countries. This lack of success has been attributed, among other causes, to the insufficient sensitivity of the diagnostic tests under field conditions (Alvarez *et al*., 2009). Wildlife reservoirs and dissemination due to movement of infected animals (Johnston *et al*., 2005; Collins, 2006) have been pointed out as possible causes of failure to eradicate bTB (Alvarez *et al*., 2009). Diagnostic accuracy is a key issue in the test-and-slaughter programs, especially where the prevalence is low and detection of all infected animals is crucial (Pollock *et al*., 2001). It has been proposed that in the presence of a wildlife reservoir, the test and slaughter policy will not be sufficient to control the incidence of bTB and hence there is an urgent need to develop improved control measures (Hope and Villarreal-Ramos, 2007). The typing of *M. bovis* and identification of *M. bovis* wildlife reservoirs in countries where a wildlife–livestock interface exist is crucial to the effective management of bTB control schemes (Skuce and Neill, 2001; Haddad *et al*., 2004).

2.3 Bovine brucellosis

2.3.1 Aetiology

The disease is caused by a group of bacteria belonging to the genus Brucella, which are Gram-negative cocccobacili that posses surface antigens located on the lipopolysaccharide (Hirsh and Zee, 1999). *Brucella abortus* (7 biovars) principally affects cattle and African buffaloes; *B. suis* (5 biovars) affects swine and reindeer but also cattle, *B. melitensis* (3 biovars) affects goats but can also infect sheep and cattle, *B. canis* affects dogs and *B. ovis* affects sheep (Gee *et al*., 2004; Huber *et al*, 2009).

Brucellosis in cattle is usually caused by biovars of *B. abortus* with biovar 1 being the most frequently isolated type in Zimbabwe and worldwide (Matope, 2009)

2.3.2 Epidemiology

2.3.2.1 Distribution

Bovine brucellosis caused by *Brucella abortus* biovars is a disease of both economic and public health importance in many geographical regions of the world (Matope *et al*., 2010). Brucellosis has been reported worldwide (Muma *et al*., 2006). Some developed countries have managed to eradicate the disease. Animal brucellosis is still endemic in Mediterranean countries, Africa, the Middle East, South Asia and Central and South America (Theegarten *et al*., 2008). Bovine brucellosis is known to occur in 40 of the 55 African countries for which investigative reports are available, and the prevalence ranged from less than 1% in East Africa to 30% in West Africa (Bedard *et al.,* 1993). The disease is common in sub Sahara and is mainly found in dairy animals (Godfroid *et al.,* 2005; Pappas *et al.,* 2006). Brucellosis is more important in female animals where it causes abortions (Hirsh and Zee, 1999). Although many countries have eradicated *Brucella abortus* from cattle, in some areas *Brucella melitensis* has emerged as a cause of infection in this species as well as in sheep and goats (Corbel, 1997).

2.3.2.2 Transmission

Cows are infected through licking of infected materials or the genital area of other infected cows or through ingestion of the disease-causing organism from contaminated water (Hirsh and Zee, 1999). The general rule is that brucellosis is carried from one

herd to another by an infected animal and this mode of transmission occurs when an owner buys replacement cattle that are infected (Crawford *et al*., 1990). Aborted foetuses, placental membranes or fluids and other vaginal discharges present after an infected animal has aborted or calved are reported to be highly contaminated with infectious *Brucella* organisms (Godfroid *et al.,* 2002). Both wild and domestic animals are susceptible to infection with Brucella and may serve as carriers for other animals (Ahmad and Majali, 2005). The disease may also spread when wild animals from an infected herd mingle with brucellosis-free herds (Godfroid *et al.,* 2002). Insects (face flies) play a minor role in transmission and maintenance of the infection in herds (Hirsh and Zee, 1999). In non-endemic countries with a successful eradication of animal brucellosis the disease is imported by travelling (Theegarten *et al.*, 2008). Brucellosis is commonly transmitted to susceptible animals by direct contact with infected animals or with an environment that has been contaminated with discharges from infected animals (Blood *et al*., 2000). This disease is transmitted by direct or indirect contact with infected excretors (Verger, 1985; Blood *et al*., 2000).

Brucellosis is considered to be an occupational disease for workers in contact with farm animals and for laboratory personnel (Seleem *et al.,* 2010). Examples of human-to-human transmission by tissue transplantation or sexual contact are occasionally reported but are insignificant (Corbel, 1997). This organism has also been implicated as a possible agent of bioterrorism (Valdezate *et al*., 2007).

2.3.2.3 Prevalence

Most developed countries, appear to have eradicated brucellosis in dairy cattle (Mohan *et al.,* 1996). The disease is endemic in Sub-Saharan African countries, including Zimbabwe and the prevalence rates vary according to agro-ecological regions and livestock husbandry system (Matope *et al*., 2010). Brucellosis in dairy herds in Zimbabwe was reported as early as 1913, when serologically positive animals were identified following abortion storms around Harare (Bevan, 1931). Zimbabwe initiated control measures such as compulsory calf vaccination on commercial farms aiming at eradicating brucellosis (Mohan *et al.,* 1996). Bovine brucellosis is endemic among domestic cattle in Zimbabwe (Madsen and Anderson, 1995). Control of brucellosis in cattle in Zimbabwe did not target small ruminants kept together with cattle as found on a number of farms in Zimbabwe (Director of Veterinary services Zimbabwe, 2007). The introduction of the land reform programme in Zimbabwe in the year 2000 brought about increased movement of cattle between the commercial and smallholder farming sectors resulting in increased prevalence of bovine brucellosis (Matope *et al*., 2010). Although *Brucella* spp. tend to discern host predilection in causing overt disease, cross infection between cattle and small ruminants is not uncommon (Verger *et al.,* 1985). Brucellosis in game animals in Zimbabwe was documented by serological studies done in 1991 (Madsen and Anderson, 1995). Muma *et al*. (2006) conducted a study in Zambia at wildlife-livestock interface, Kafue flats and he recorded high prevalence in area close to wildlife areas. Previous surveys conducted in Uganda revealed seroprevalence levels ranging from 3% to 16, 7% depending on the type of production system; pastoral dairy system and semi-intensive dairy system (Magona *et al*., 2009).

2.3.2.4 Factors associated with transmission at a wildlife-livestock interface

The introduction of an infected individual is not a sufficient indicator of transmission of *Brucella* spp. to other animals of the recipient species (Godfroid, 2002). The probability of brucellosis becoming established and being sustainable in a species will be equal or less than the probability of infection and in some cases will be close to zero because a combination of factors must be taken into account (MacDiarmid *et al.,* 1987). The factors associated with transmission at a wildlife-livestock interface are similar to those of other infectious diseases such as bTB (Bengis *et al*., 2002). Some of the factors include stock density, production cycle, cattle movements, and abortion occurrence, horizontal and vertical infections. Considering the contagious nature of *Brucella* spp., sharing grazing land and drinking water between cattle and wildlife is likely to facilitate transmission of the disease (Jiwa *et al.,* 1996; Reviriego *et al*., 2000). Herd size and animal density are related to prevalence and difficulty in controlling the infection in a population (Hirsh and Zee. 1999). Geographical area (grazing site), contact with wildlife, herd size and breed type are major factors associated with Brucella herd seropositivity and counts of seropositive (Muma *et al*., 2007).

2.3.3 Clinical signs

It is a contagious costly disease of ruminant animals that also affects humans. *Brucella abortus* main threat is to cattle and buffaloes (Carter *et al*., 1995). Animals do not exhibit overt systemic illness (Hirsh and Zee, 1999). The course of brucellosis in cattle is governed primarily by the age of the animal when exposed to infection and to a lesser extent by the severity of the challenge in terms of numbers of organisms and their virulence (Hirsh and Zee, 1999). Following entry into the body via mucous membranes,

organisms multiply in the local lymph node. A bacteriaemia ensues and the organisms colonize their predilection sites such as the mammary glands, gravid uterus, testes, seminal vesicles, and associated lymph nodes. Other sites are joints, bursae, liver and spleen (Blood *et al.,* 2000).

Clinical signs of the disease include orchitis in males, abortion in females, and bursitis in both sexes (Forbes, 1991). It is only during pregnancy when a placenta exists (i.e. second half of pregnancy) that the uterus is invaded and the classical signs of brucellosis are seen (McGiven *et al.,* 2003). Abortions depends upon the immune status of the herd, in naïve herds which are highly susceptible abortion after fifth month is the cardinal feature of the disease in cows (Blood *et al*., 2000). In endemic areas pregnancy is usually carried to full term although second or third abortions can occur in the same cow (Blood *et al*., 2000). Prepubescent calves lose the infection once removed from the source of contamination (Blood et al., 2000). After puberty the chances of cattle becoming permanently infected increases (Hirsh and Zee, 1999). Abortions decrease with parity as adult cows develop some resistance. A necrotic placentitis which may be acute or widespread is characteristic (Blanco, 1990). The cells of the villi and the walls or crypts become swollen and there is considerable leucocyte infiltration leading to necrosis (Moreno and Moriyo, 2006). Infected hygroma may occur in males including steers and females, and are regarded as highly indicative of infection (Blood et al., 2000). Infected males show no clinical signs though frequently there is testicular enlargement (Hirsh and Zee, 1999). In bulls, there may be seminal vesiculitis, epididymitis and orchitis which may lead to abscess formation resulting in infertility

(Hirsh and Zee, 1999; Moreno and Moriyo, 2006). There may be degenerative changes in semen with pus (Hirsh and Zee, 1999).

In humans, undulant fever and malaise are the major clinical signs seen in most patients (Theegarten *et al*., 2008). Focal manifestations are found in joints and bones (spondylitis, sacroilitis, arthritis), the respiratory tract (pneumonia, pleuritis), in the cardiovascular (endocarditis, pericarditis, vasculitis), and nervous system (radiculitis, meningoencephalomyelitis), in the uro-genital system (nephritis, epididymitis, orchitis) as well as in the liver, spleen and the skin (Theegarten *et al*., 2008).

2.3.4 Diagnosis

2.3.4.1 Culture and isolation

The isolation and identification of *Brucella* spp. offers a definite diagnosis of brucellosis (Abdoel *et al*., 2008). When culturing Brucella spp. samples are inoculated onto Farrell's medium, Blood agar and MacConkey and placed in a jar with 6% O_2, 10% CO_2 and 84% N_2 (Farrell's and Blood agar) and in air (MacConkey plate); and incubated at 37 ^{0}C for 3 days (Alton *et al*., 1988; Quinn *et al*., 1994). On blood agar, Brucella colonies are small (1 mm diameter), round, grey and non-haemolytic. On Farrell's medium, the colonies are small, round, convex, translucent, have smooth margins, and are of a pale honey colour (Alton *et al*., 1988; Quinn *et al*., 1994). Gram stain reveals Gram negative coccobacilli, usually arranged singly, but may occur in pairs or small groups, the bacteria are partially acid fast and stain red against the blue background with the modified Ziehl Neelsen (Stamp's) stain (Alton *et al*., 1988; Quinn

et al., 1994). Brucella species are catalase and oxidase positive (Alton *et al*., 1988; Whatmore, 2009). Testing of livestock for brucellosis is done by culture and serology or by testing milk samples (Nielsen *et al*., 2002). Confirmation of diagnosis of brucellosis as the cause of abortion is done by demonstrating organisms in smears or culture (Nielsen, 2002). The gold standard in brucellosis diagnosis remains the isolation of *Brucella* spp. (Godfroid *et al.,* 2002). Culture provides definitive proof of brucellosis, but culturing the microorganism takes time because it is a relatively slow-growing bacterium, and needs experienced laboratory personnel and properly collected samples (Zeytinoglu *et al*., 2006). Although culture is the standard test for brucellosis, the culture positivity rate is high in acute cases, but the isolation level decreases significantly in chronic cases (Zeytinoglu *et al*., 2006). In dairy cattle, milk samples and selective media are used most often. However, when testing large numbers of cattle, this direct diagnostic test is often impractical (Romero *et al*., 1995). Smears of placenta, foetal stomach or vaginal discharge are stained by Modified Ziehl- Nielsen or Koster methods (Carter *et al.,* 1995). However, *Brucella* spp. may be confused with Q- Fever organisms *(Rickettisia. burnetti)* (Blood *et al.,* 2000). Tissues for culture include foetal stomach contents or lung, placenta, vaginal discharge, milk and semen (Blood *et al.,* 2000). At post mortem the best samples are mammary tissue and supramammary or iliac lymph nodes (Blood *et al*., 2000). Polymerase chain reaction (PCR) can provide both a complementary and molecular epidemiological typing method based on specific genomic sequences (Whatmore, 2009). PCR diagnosis remains promising for the rapid diagnosis of acute but not chronic brucellosis since bacteriaemia is present only in the acute stages of infection (Sharma *et al*., 2008). Hence, serological investigation remains the mainstay for diagnosis (Sharma *et al*., 2008). In humans, due to its variable clinical

features and lack of truly diagnostic tests, brucellosis remains a difficult disease to diagnose particularly in non-endemic countries with a low prevalence (Seleem *et al.,* 2010).

2.3.4.2 Serological tests

No single serological test is appropriate for epidemiological studies (OIE, 2008). Antibody detection is not wholly satisfactory because not all infected animals produce significant levels of antibodies and several bacteria can produce cross reacting antibodies (Romero *et al*., 1995). Indirect tests for detecting antibodies in serum or milk are used routinely to screen for cattle suspected of being infected with *Brucella spp*. (Romero *et al*., 1995). None of these tests is 100% sensitive or specific and a combination of screening and confirmatory tests does not result in 100% sensitivity or 100% specificity (Mari'n *et al.,* 1999). Interpretation of the tests is based on experience and knowledge of the epidemiology of the disease in an area in particular herds (Godfroid *et al*., 2002). For *Brucella spp*. antibody testing, individual samples are often tested by two or more serologic test methods (Zarnke *et al*., 2006). Non-specific reactions are caused by vaccination with S19 and occasionally by infection with other Gram-negative bacteria such as *Yersinia* and *Salmonella.* A false-positive serological reaction can occur either in cattle, sheep, goats and or in pigs and this is due to a cross-reactivity between the smooth-lipopolysacchrides of *Brucella* species and those of other bacteria (i.e., *Yersinia enterocolitica* O: 9, *Salmonella urbana*, *Vibrio cholerae*, and *Escherichia coli* O: 157) (Weynants *et al.,* 1996; Lucero *et al.,* 1999). Potentially, *Y. enterocolitica* O: 9 presents the most serious source of confusion in the diagnosis of

brucellosis and this is because the O chains of the smooth-lipopolysacchrides of *Y. enterocolitica* O: 9 and *Brucella* species are identical (Weynants *et al.,* 1996).

Screening tests are cheap, fast and highly sensitive and not necessarily highly specific (Gail and Nielsen, 2004). These tests are used to detect animals which are most likely to be infected but are not definitive and other confirmatory tests have to be carried to confirm the diagnosis (Gail and Nielsen, 2004). Screening tests should have high sensitivity i.e. the number of false negatives should be low (Nielsen, 2002). The Rose Bengal Test (RBT) because of its relatively high sensitivity, ease and speed of use, as well as its low cost, have made it the most commonly used screening test (Ruiz-Mesa *et al*., 2005). The RBT is the main screening test for non-lactating animals and is also useful where non-specific reactions are encountered with the Milk Ring Test (MRT) (Nielsen, 2002). The RBT is carried out on serum and its sensitivity approaches 98% but it is not appropriate for latent (usually in heifers) and chronic infections (Weynants *et al.,* 1996). In an area where no brucellosis exists, approximately 3% of non-specific reactions can be expected (Gall and Nielsen, 2004).

Confirmatory tests are required to be both sensitive and specific (Stemshorn *et al*., 1985). Conventional serological tests cannot distinguish vaccinal antibodies (Strain 19) from active infections. The competitive enzyme-linked immunosorbent assay (c-ELISA) and the Fluorescence polarisation assays used for serum have the capability to distinguish between animals vaccinated with the widely used *Brucella abortus* strain 19 vaccine and animals naturally infected with *B. abortus* (Gall and Nielson, 2004). The c-ELISA competing antibodies inhibit binding of vaccinal but not field strain-induced

antibodies (Nielsen, 2002). Hence, the c-ELISA is a prescribed test by Office International Des Epizooties (OIE) for international cattle trade and an alternative test for swine brucellosis (Nielsen, 2002; OIE, 2008).

2.3.5 Treatment and control

Testing of livestock is cumbersome when dealing with farms located in remote areas or with animals from nomadic populations and migratory farmers (Abdoel *et al*., 2008). Treatment of brucellosis is not recommended in animals because the success rate is very low and expensive. Treatment in wildlife is almost impossible because it is expensive, time consuming and stressing to the animals (Godfroid, 2002). Tetracycline, rifampicin and the aminoglycosides such as streptomycin and gentamicin are effective against human brucellosis (Carter *et al.,* 1995). However, because the bacteria are intra-cellular, the use of more than one antibiotic for several weeks is recommended. In humans the gold standard treatment for adults is daily intramuscular injections of streptomycin but intramuscular injection of gentamicin is also an acceptable substitute (Wilkinson and Lise, 1993). The best way to deal with brucellosis in a herd is to vaccinate all heifers between 3 months and 10 months of age with strain 19 vaccines and to remove those which react positive to convectional serological tests (OIE, 2008). The test and slaughter policy is another method that is used to control brucellosis and move towards eradication. Where animals (both cattle and wildlife) are to be translocated, the animals should be screened for the disease using screening tests such as the RBT, with positive animals being re-tested using confirmatory tests such as the cELISA or CFT (Blood *et al.,* 2000).

The disease still persists in United Kingdom and New Zealand even though for over 30 years these countries operated classical test and slaughter programmes that have been used successfully elsewhere to eradicate *M. bovis* from domestic livestock (De Lisle, 2001).

CHAPTER 3

MATERIALS AND METHODS

3.1 Location and selection of study areas and sites

The study was conducted in Gonarezhou National Park (GNP) and surrounding areas in the southeast lowveld of Zimbabwe with an annual rainfall of below 500 mm. Study areas were purposively selected to include those sites with close proximity to wildlife from the Gonarezhou National Park (GNP) and Kruger National Park (KNP) and those without a wildlife-livestock interface area. GNP located in the southeast lowveld, is Zimbabwe's second largest game reserve covering an area of 5000 km^2 of open grasslands and dense woodland. The Park forms a natural migratory triangle with wildlife populations from the Mozambique's Limpopo National Park (LNP) where animals move freely between the two sanctuaries and adjoining South African KNP separated from GNP by Sengwe communal lands (Figure 3.1). Collectively these areas constitute the Greater Limpopo Transfrontier Conservation Areas (GLTCA), where management is the responsibility of the three neighbouring countries.

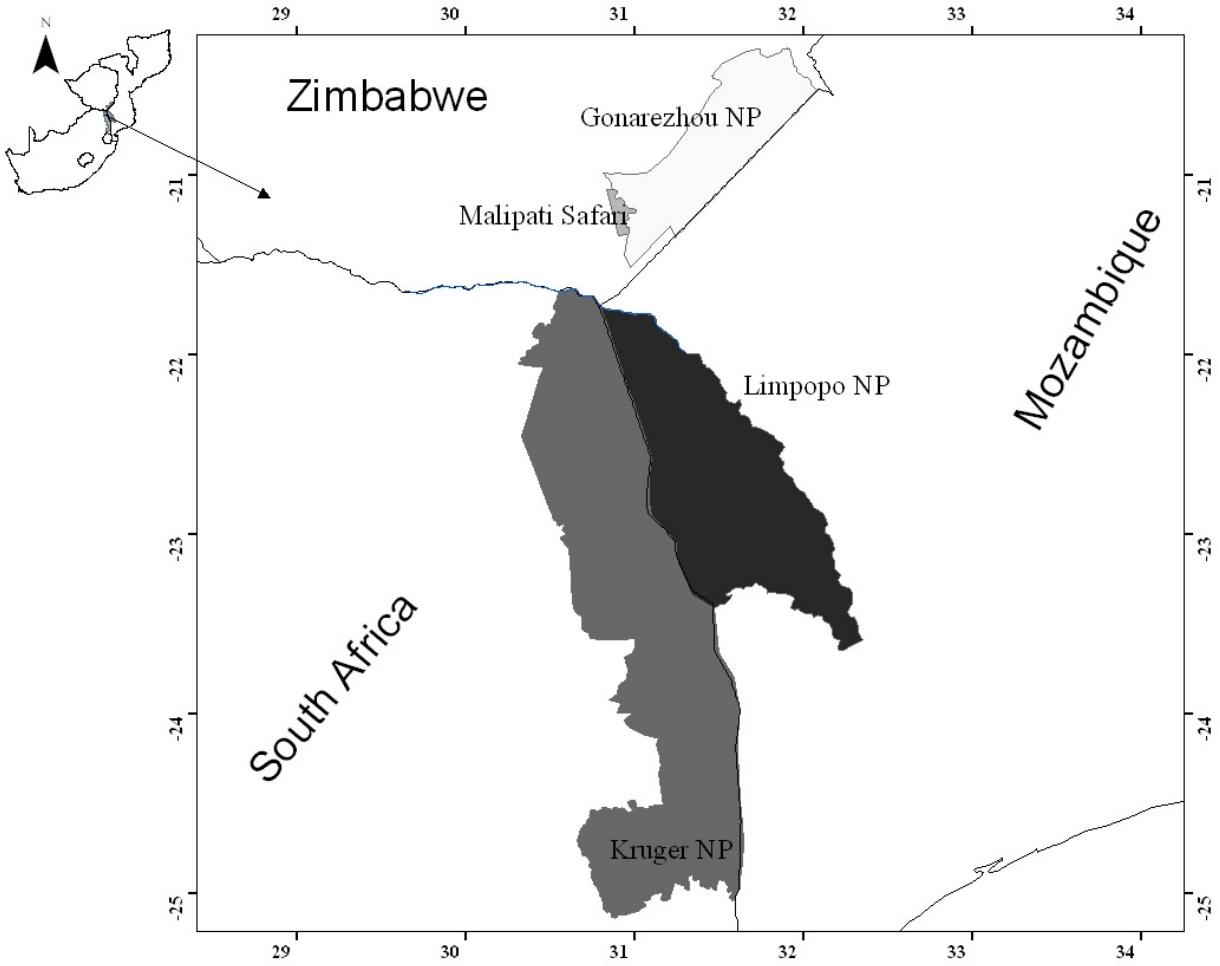

Figure 3.1 Map showing location of three parks included in GTFCA: GNP in Zimbabwe, KNP in RSA and Limpopo NP in Mozambique.

The selected study areas with a wildlife-livestock interface were Malipati and Pesvi communal (subsistence farming) areas. Malipati lies adjacent to GNP and the selected dip tank lies less than 5km from the unfenced areas of the Park, allowing direct and indirect contact between domestic and wild animals. Cattle from Malipati share common grazing and watering sources with wild animals (e.g. African buffaloes, bushbucks, elands and impalas) particularly during the dry season (August to October) when there is limited pasture and water sources for communal livestock farmers. Pesvi

lies adjacent to KNP across the Limpopo River and the selected dip tank lies less than 3km from the unfenced northern boundary of the park (Figure 3.2). During the dry season, when the Limpopo River is dry, wild animals (e.g. African buffaloes) from KNP cross into Pesvi communal areas and cattle from Pesvi communal areas cross into KNP in search of grazing. The comparative study areas without a wildlife-livestock interface were Chomupani and Pfumare communal areas. These areas are situated more than 40km from GNP boundary and more than 100km from KNP and wildlife is either absent or occurs at very low densities (PARSEL unpublished data, 2009). Cattle reared in these areas have no apparent direct contact with wild animals.

For cattle, owing to the availability of animal handling facilities and access to large populations of cattle, dip tanks were chosen as the study sites. One dip tank was selected from each study area, giving a total of 4 dip tanks – 2 from an interface area (Malipati and Pesvi dip tanks) and 2 from a non-interface area (Chomupani and Pfumare dip tanks) (Figure 3.2). In these areas cattle were dipped weekly with acaricides (Amitraz) during the rainy season and monthly during the dry season for the control of ticks. For wildlife, the study sites were GNP and Malilangwe Conservancy which is adjacent to the north of GNP.

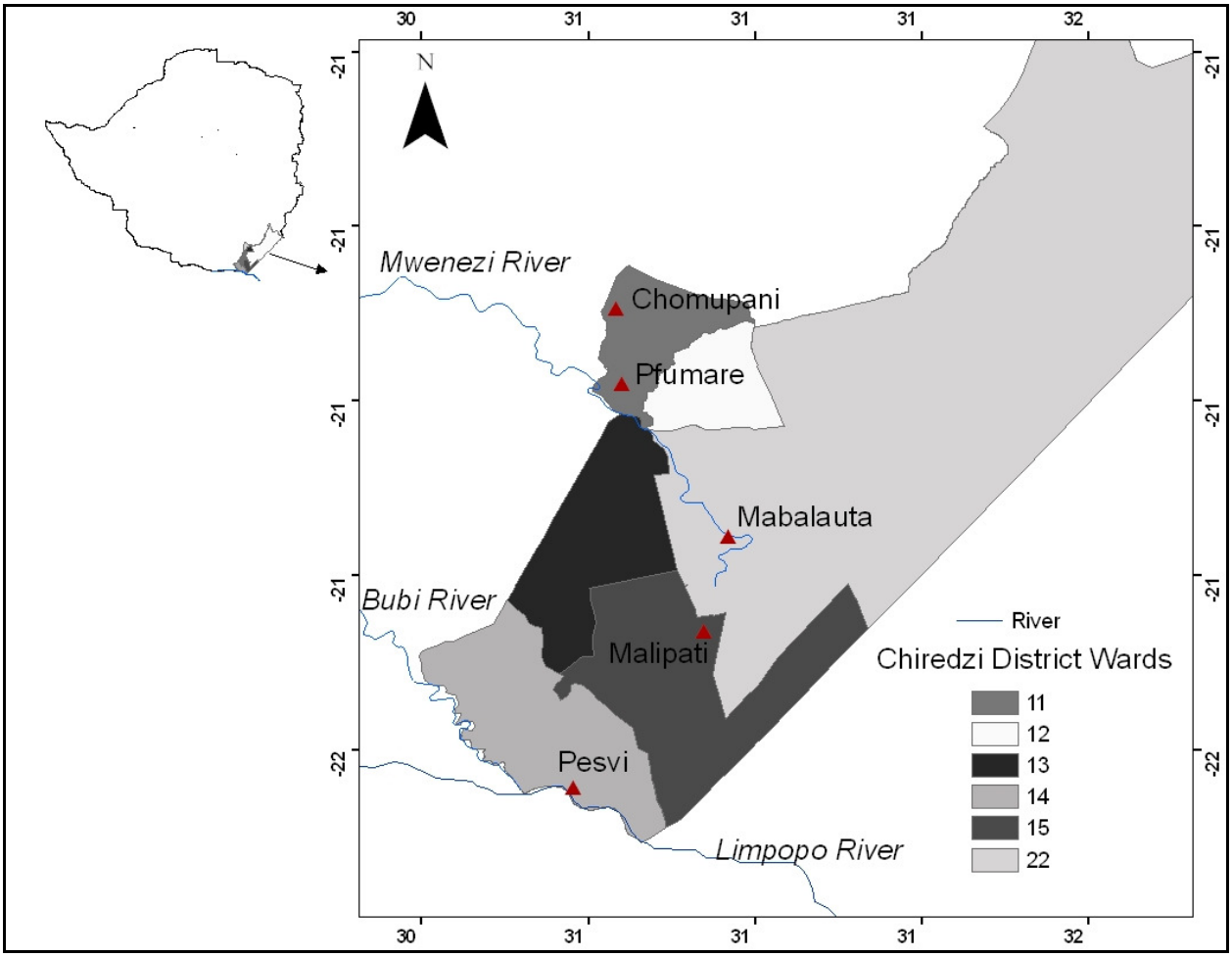

Figure 3.2 Location of the interface area (Malipati and Pesvi), non-interface area (Chomupani and Pfumari) and GNP.

3.2 Sampling of animals and sample collection

The survey covered the period from July 2007 to October 2009. For cattle, all herds which were present on the day of sample collection were included for sampling. Systematic random sampling (i.e. 1/10 animals interval) was used to select individual animals. For the detection of antibodies against *Brucella* spp., only those animals > 7 months old were selected while for bovine tuberculosis only those animals > 12 months old were sampled. Local indigenous cattle used in the study were Sanga type (a

stabilized *Bos taurus* x *Bos indicus* cross), commonly known as "Mashona". Blood samples for the detection of antibodies against *Brucella* spp. and *M. bovis* were collected from all sampled animals. Lymph node samples from the head, thorax and suspected lesions from the lungs of cattle which tested tuberculin-positive were taken for histopathological examination and bacterial culture. Blood samples were collected in plain tubes and serum from plain tubes was transferred to separate sterile 5ml cryovials and these were stored in cooler boxes in the field. At the laboratory (CVL) 2ml of serum from 5ml cryovials was aloquoted into 2ml cryovials for use and remaining 3ml s stored in -20 degrees Celsius fridge for future use. The lymph nodes were put into; one piece into 10% formalin and another one into sterile 10ml container and frozen at Camp site freezer and at CVL the samples were transferred into -20 degrees Celsius until further use. Samples from free-ranging wildlife were collected from various sources. Wildlife samples were collected from those slaughtered for the GNP staff rations, hunter kills, animals captured for translocation and those captured for the purpose of this study. An organized capture of wildlife for the purpose of this study was done during the month of October 2008 where 38 buffaloes from four different herds were captured in a boma in GNP. All captured buffaloes were marked and ear-tagged with three adult females from each group (total of 12) being fitted with radio-collars before being released. For each sampled wild animal, blood samples were collected for the detection of antibodies against *Brucella* spp. using the Rose Bengal Test (RBT) and the c-ELISA and for the detection of bovine tuberculosis (bTB) using the gamma interferon test. RBT and c-ELISA were carried out at CVL, Harare Zimbabwe. Blood collected in heparin tubes for IFN-γ assay were sensitised with PPDs before they were sent to ARC-OVI Tuberculosis Laboratory in South Africa. In February 2009, two collared buffaloes which were

positive to bTB on the gamma interferon test were traced by helicopter, darted, euthanized and necropsies were performed in the field where lymph node samples from the head and thorax as well as from suspected lesions were taken for histopathology examination and bacterial culture.

3.3 Epidemiological data

Information about each animal (cattle) such as sex, age, abortion history and history of grazing in the Park according to the owner (GNP or KNP) were collected and entered into a data sheet.

3.4 Testing for bovine brucellosis and bovine tuberculosis

3.4.1 Testing for bovine brucellosis

Brucellosis serological test were carried out at Central Veterinary laboratory, Harare Virology/serology section by Calvin Gomo. For both cattle and wildlife, antibodies to *Brucella* spp. were detected by using the RBT and the c-ELISA tests. Testing of serum samples using the RBT was done as described earlier (Alton et al., 1988; Matope, 2009). Briefly, as outlined earlier (Matope, 2009), *Brucella abortus* antigen (VLA, UK) was used to screen sera for the presence of antibodies to *Brucella* spp. The test was performed on round-bottomed welled Pyrex plates where 25µl of the serum were mixed with equal amounts of stained Rose Bengal antigen (pH 3.65). The samples were mixed on a rocker for five minutes. The degree of agglutination was graded on an ordinal scale from 0 (no agglutination) to 3 (coarse clumping), with RBT scores of 2 and 3 being considered positive. All RBT seropositive sera were further tested using the Svanovir^{TM} Brucella-Ab c-ELISA test kits (Svanova Biotech, Uppsala, Sweden). The c-ELISA was

conducted according to the manufacturer's instructions. Briefly, as outlined earlier (Matope, 2009), the test was carried out in 96 well polystyrene plates (Nalge Nunc, Denmark) that were pre-coated with *Brucella* spp. lipopolysaccharides (LPS) antigen. Serum diluted 1:10 was added to each well, and immediately followed by equal volumes of pre-diluted mouse monoclonal antibodies specific for a common epitope of the O-polysaccharide (OPS) of the smooth LPS molecule. The reactivity of the mouse monoclonal antibody was detected using goat antibody to mouse IgG that was conjugated to horseradish peroxidase. Hydrogen peroxidase substrate and ABTS chromogen were developed for 10 minutes. The reaction was stopped using 1M H_2SO_4. Optical densities (OD) were read at 450nm using a Titertek Multiscan® PLUS reader (Flow Laboratories, UK). The threshold for determining seropositivity was based upon the manufacturer's recommendations (≥ 30%), with antibody titers recorded as percentage inhibition (PI) as defined by the ELISA kit supplier. In this study a serial testing protocol (Matope, 2008) was used and hence, a serum was considered positive for antibodies to *Brucella spp* if it was positive for both the RBT and c-ELISA.

3.4.2 Testing for bovine tuberculosis

3.4.2.1 The Tuberculin test

The Single Comparative Intradermal Tuberculin skin Test (SCITT) using purified protein derivatives tuberculin (PPD, Bovituber, Synbiotics Corporation, France) was performed only in sampled cattle as described by Lesslie and Herbert (1975). Briefly, two sites were shaved in the cervical region of each animal at a distance of about 12 cm apart and the skin fold thicknesses were recorded. Equal volumes (0.1 ml) of avian PPD

tuberculin and bovine PPD tuberculin were injected intradermally into the shaved upper and lower sites of the neck, respectively and the animals were released. Seventy-two hours post-injection the skin folds thicknesses were re-measured using calipers. The tuberculin results were interpreted based on standard interpretation (OIE, 2008). Bovine and avian-positive reactors were obtained using the following formulae: $[(Bov_{72}–Bov_0)–(Av_{72}–Av_0)]$ and $[(Av_{72}–Av_0)–(Bov_{72}–Bov_0)]$, respectively (Shirima *et al*., 2003). Bov_0 and Av_0 were the skin fold thicknesses pre-injecting bovine and avian PPDs tuberculin, respectively, and Bov_{72} and Av_{72} being the skin fold thicknesses after 72 h of inoculation of bovine and avian PPDs tuberculin, respectively. A result of <2 mm was regarded as a negative reaction to tuberculin, a result of 2-4 mm as a doubtful reaction and a result of >4 mm as a positive reaction to tuberculin (OIE, 2008).

3.4.2.2 The Gamma interferon test

This test was conducted on blood samples collected from African buffaloes (n=38) and five cattle which were positive to the tuberculin test. Blood samples collected were transferred into heparin tubes and incubated with bovine and avian tuberculin PPD as well as crude protein extract of *M. fortuitum* following the protocol described by Grobler *et al*. (2002). After incubation at 37°C for 20-24 hours, the samples were frozen until submission to Onderstepoort Veterinary Institute (Pretoria, South Africa), where the gamma-interferon assays was performed. Briefly, stimulation with avian and bovine PPDs was carried out as previously described (Wood *et al.,* 1991; Liebana *et al.,* 1998), and plasma samples were analyzed in duplicate using a sandwich EIA for the detection of bovine IFN-γ (Bovigam™ Bovine Gamma Interferon Test, Prionics, Schlieren, Switzerland). Results were interpreted as previously described (Aranaz *et al.,* 2006).

Animals were considered positive if the mean optical density (OD) of a sample stimulated with bovine PPD minus the mean OD of avian PPD was ≥0.20 and if mean OD of fortuitum PPD minus the mean OD of control was ≤0.15 and if mean OD of control was ≤0.25 (if $OD_{bov} - OD_{av} \geq 0.20$ and if $OD_{fort} - OD_{control} \leq 0.15$ and if $OD_{control} \leq 0.25$).

3.4.2.3 Culture and Histopathology

Culture for the isolation and identification of *M. bovis* was done at the Tuberculosis Laboratory of ARC-OVI in South Africa. For bacterial isolation, tissue samples were processed and cultured according to the protocol described by Bengis *et al*. (1996). Briefly, the samples were cultured using the Lowenstein-Jensen (L-J) medium after the decontamination/digestion procedure was carried out using the Becton Dickinson method (Anonymous, 1999). The samples were cultured at 37^{o}C for 10 weeks on paired L-J media, enriched with pyruvate (L-J-P medium) and others enriched with glycerol (L-J-G medium). All cultures were evaluated for colony growth on a weekly basis up to 10 weeks.

Histopathology examination was done at the Wildlife Veterinary Unit in Harare and at the National Zoological Gardens of South Africa. For histopathology, selected tuberculous lesions were examined microscopically by Ziehl-Nielsen (Z-N) staining technique for the detection of acid-fast bacteria.

3.5 Data analysis

3.5.1 Brucellosis

The recording and editing of data was performed using Microsoft Excel®. Statistical analyses were performed using Stata, STATA® version SE/9.0 for Windows (StataCorp, Taxes, USA). The overall number of sero-positive animals was calculated from the total number of samples tested over the study period and expressed as a percentage. For cattle, sero-positive animals were examined in relation to age, sex, location, abortion history and grazing history. The variable categories for age, sex, location, abortion and grazing history were generated as follows: four for age (≤ 2.5, >2.5-4, > 4-6 and > 6 years), two for sex (male and female), two for location (interface and non-interface), two for abortion history (aborted and not aborted) and two for grazing history (grazing in Park and not grazing in Park). Descriptive statistics for grazing history was restricted to animals originating from the interface only. The proportion test for independent groups was used to measure differences in proportions between categories. Values of ($P < 0.05$) were considered as significant

The logistic regression analyses were conducted in Stata SE/9.0 for Windows to investigate the individual animal risk factors for infection with *Brucella* spp. Cattle in communal farming areas share common grazing and watering sources, and hence, herd risk factors were not investigated. Logistic regression analysis was used to test the *Brucella*-seropositive status of cattle (negative = 0, seropositive =1) according to age, sex and location as the predictor variables. The predictor variables were assessed for collinearity by cross tabulations using the two sided Fisher's exact test hence; abortion

history and grazing history were not included in the first model as they had high collinearity with sex and location, respectively. Hence, another model was restricted to females, where the history of abortion and grazing were included as additional predictors of sero-positivity. The models were constructed by forward selection applying the maximum likelihood estimation procedure and statistical significance contribution of individual predictors to the models was tested using the likelihood ratio test as described (Dahoo *et al.,* 2003). The presence of interaction between variables was checked by constructing a two-product term and forcing it into the model and examining the changes in the coefficients and p-values of main effects. Evidence of confounding was checked by dropping one of the variables and assessing changes of the coefficients. Goodness-of-fit of the logistic regression models were assessed using the Hosmer-lemeshow test while predictive ability was determined using the receiver operating characteristic (ROC) curve (Dahoo *et al*., 2003)

3.5.2 Bovine tuberculosis

The recording and editing of data was performed using Microsoft Excel®. The overall number of animals positive to SCITT was calculated from the total number of samples tested over the study period and expressed as a percentage. For cattle, positive animals were examined in relation to age, sex, location, and grazing history. Three age categories (≤ 3, >3-5 and >5 years) were generated and categories for the other variables are similar to those generated for brucellosis. Since the number of positive animals was very small only descriptive statistics were performed without detailed analytical statistics.

CHAPTER 4

RESULTS

4.1 Brucellosis

4.1.1 Cattle

The distribution of individual sampled cattle and their seroprevalence according to different categories are shown in (Table 4.1). A total of 1158 cattle were sampled and the overall sero-prevalence was 9.9%. The seroprevalence showed an increasing trend with increasing age, with adult cattle (> 6 years) recording the highest seroprevalence but the differences were not statistically significant (P>0.05). Females recorded a relatively higher seroprevalence compared to males but the difference was not statistically significant (P>0.05). In females, the seroprevalence was significantly higher (P<0.001) in those which had a history of abortion. The seroprevalence varied from 9.6% to 11.2% for cattle originating from the interface areas while it varied from 5% to 8.3% for those originating from the non-interface areas. Overall, the seroprevalence was relatively higher for cattle sampled from the interface areas compared to those sampled from the non-interface areas but the difference was not significant (P>0.05). From the interface areas, cattle with a history of grazing in the park recorded a significantly (P<0.01) higher seroprevalence compared to those with no history of grazing in the park.

The overall logistic regression results showed an independent effect of age, sex and location (Table 4.2). The model showed a non-significant increase in prevalence with

age, sex and for cattle sampled from the interface areas. Logistic regression results restricted to female animals showed an independent effect of age, location, and history of abortion and that of grazing (Table 4.2). The results showed a non-significant association between antibodies against *Brucella* spp. and an increasing age of female animals and, also for those sampled from the interface areas. However, the results demonstrated a strong association (P<0.001) between antibodies against *Brucella* spp. and a history of abortion (OR = 35.8, 95% CI: 19.0, 67.6) and that of grazing in the park (OR = 3.3, 95% CI: 1.8, 6.1).

4.1.2 Wildlife

A total of 97 wild animals which included 47 buffaloes, 33 impala, 16 kudu, and 1 giraffe were tested for antibodies against *Brucella* spp. Only one animal (giraffe) was positive for brucellosis (1.03%) and the rest were negative.

4.2 Bovine tuberculosis

4.2.1 Cattle

The distribution of individual cattle sampled for the detection of bTB using the tuberculin skin test according to age, sex and location are shown in (Table 4.3). A total of 478 cattle were sampled and tested for bTB. The prevalence of *M. bovis*-infections and atypical mycobacterioses in cattle based on SCITT is shown in (Table 4.3). The overall prevalence of *M. bovis*-infections and atypical mycobacterioses in cattle was 1% and 2.3%, respectively. Animals originating from the interface areas had an overall prevalence of 1.3% of *M. bovis*-infections while none of those originating from the non-

interface areas were positive for *M. bovis*-infections. However, the prevalence of atypical mycobacterioses was relatively higher (3.8%) in animals coming from the non-interface areas compared to those coming from the interface areas (1.9%). Adult cattle (> 5 years) recorded the highest prevalence of both *M. bovis*-infections and atypical mycobacterioses compared to other age groups. Males also recorded a relatively higher prevalence of both *M. bovis*-infections and atypical mycobacterioses compared to females. At the interface, all the animals which tested positive for *M. bovis*-infections based on the SCITT had a history of grazing in the park (2 in GNP and 3 in KNP).

The five cattle which tested positive for *M. bovis*-infections based on the tuberculin test were all found to be negative on the gamma-interferon test. In addition, two of the tuberculin positive animals were also negative on culture and histopathology.

4.2.2 Wildlife

A total of 38 buffaloes were tested for *M. bovis*-infections using the gamma interferon test and 4 (10.5%) were positive. Of the positive animals, three were adult females and one sub-adult male; three were from the same herd and one was from another herd. The histological appearance of tissues sampled from the euthanized two gamma interferon-positive buffaloes was strongly suggestive of paucibacillary tuberculosis and bacterial culture of these samples yielded *M. bovis.*

Table 4.1: The distribution of *Brucella* seroprevalence according to different categories in traditional cattle (n=1158) of Zimbabwe – July 2007-October 2009.

Category	Level	Number tested	Positive	% seroprevalence and 95% confidence interval (CI)
	All animals	1158	115	9.9 (8.2-11.7)
Age group	≤ 2.5yrs	244	19	7.8 (4.4-11.2)
	>2.5-4yrs	362	35	9.7 (6.6-12.2)
	> 4 – 6yrs	265	29	10.9 (7.2-14.7)
	> 6yrs	287	32	11.1 (7.5-14.8)
* Sex	Females	836	90	10.8 (8.7-12.9)
	Males	318	25	7.9 (4.9-10.8)
Location	Pesvi	526	56	10
	Malipati	512	51	12.4
	Overall Interface area	1038	107	10.3 (8.5-12.2)
	Chomupani	60	5	8.3
	Pfumare	60	3	5
	Overall Non-interface area	120	8	6.7 (2.2-11.2)
Abortion history	Aborted	58	41	[a]70.7 (58.9-82.5)
	Not aborted	777	49	[c]6.3 (4.6-8.0)
Grazing history in the interface area	Grazing in Park	653	88	[a]13.5 (7.8-15.6)
	Not Grazing in Park	385	19	[c]4.9 (2.3-6.1)

* Four animals had their sex not recorded

*Figures with different superscripts for each category are significantly different at P<0.05

Table 4.2: Results of the logistic regression analysis for identification of individual animal risk factors for Brucella seroposotivity in traditional cattle (n=1158) of Zimbabwe during the period July 2007 to October 2009

Predictor variable	*OR	95% CI	P value
Overall analysis			
>2.5-4yrs vs. ≤ 2.5yrs	1.2	0.7-2.2	0.41
> 4 – 6yrs vs. ≤ 2.5yrs	1.3	0.7-2.5	0.32
> 6yrs vs. ≤ 2.5yrs	1.4	0.8-2.6	0.22
Males vs. females	0.8	0.5-1.2	0.36
Interface vs. non-interface	1.6	0.8-3.0	0.29
Analysis restricted to female animals			
>2.5-4yrs vs. ≤ 2.5yrs	1.3	0.6-2.7	0.38
> 4 – 6yrs vs. ≤ 2.5yrs	1.5	0.7-3.2	0.32
> 6yrs vs. ≤ 2.5yrs	1.9	0.9-3.9	0.16
Interface vs. non-interface	1.8	0.9-4.1	0.23
History of abortion vs. no history of abortion	35.8	19.0-67.6	0.000
History of grazing in Park vs. no history of grazing in Park	3.3	1.8-6.1	0.000

*OR = odds ratio

Table 4.3: The prevalence of *M. bovis*-infections and atypical mycobacterioses in cattle according to age, sex and location in the southeast lowveld of Zimbabwe, based on SCITT

			Tuberculin Result			
			Positive		Suspect	
			Bovine	Avian	Bovine	Avian
Category	Level	Total sampled	No (%)	No (%)	No (%)	No (%)
	All animals	478	5 (1.0)	11 (2.3)	36 (7.5)	48 (10.0)
*Age group	≤ 3yrs	156	1 (0.6)	3 (1.9)	9 (5.8)	19 (12.2)
	>3-5yrs	169	2 (1.2)	4 (2.4)	20 (11.8)	16 (9.5)
	> 5yrs	152	2 (1.3)	4 (2.6)	7 (4.6)	13 (8.6)
Sex	Female	334	2 (0.7)	7 (2.1)	19 (5.7)	30 (9.0)
	Male	144	3 (2.3)	4 (2.8)	17 (11.8)	18 (12.5)
Location	Interface area	374	5 (1.3)	7 (1.9)	31 (8.3)	32 (8.6)
	Non-interface area	104	0 (0.0)	4 (3.8)	5 (4.7)	16 (15.1)

* One of the animals had no age recorded

CHAPTER 5

DISCUSSION

5.1 Brucellosis

Calves from seropositive dams have been reported to be usually seropositive for up to 4-6 months due to colostral antibodies and later test negative (Blood *et al*., 2000). Hence, in order to minimise false positive reactions due to maternal antibodies in younger animals, only animals at least 7 months old were included in the present study. The antibodies detected were more likely to be due to natural infection with *Brucella* spp. rather than by *B. abortus* S19 vaccine because according to the local veterinarian and livestock technicians, none of the cattle from the studied areas had been vaccinated against brucellosis. In addition, the c-ELISA test used to confirm seropositive animals has been reported to differentiate antibodies due to *B. abortus* S19 vaccine from those of natural infection (Nielsen *et al.,* 2002). Serological cross-reactions due to *Yersinia enterocolitica* were unlikely to influence the results since this pathogen is assumed to be rare or absent in the tropics (Godfroid, 2002; Nielsen *et al.,* 2004) and the use of specific tests such as c-ELISA results in a substantial decrease in the number of such false positive reactors (Nielsen *et al.,* 2004). The RBT was used to screen individual animals because the test has a high sensitivity (>90%), thus reducing the possibility of false negative reactions (OIE, 2008). Although the *B. abortus* antigens used in these two tests extensively cross-react with antibodies produced against the lipopolysaccharides of *B. melitensis* and *B. suis* (Nielsen *et al*., 1999) both species have not been isolated in cattle in Zimbabwe (Madsen, 1989). Therefore, it is unlikely that the classification of

tested animals in the present study was biased towards false negative and false positive results due to cross-reactions with other *Brucella* spp. It is important to note that the test regime (RBT and c-ELISA) were likely to miss positive animals which were false negative with RBT test since the sensitivity of the test is 90%.

In Zimbabwe, brucellosis was reported as early as 1913 in dairy herds when seropositive animals were identified following abortion storms around Harare (Madsen, 1989). The disease is endemic in Sub-Saharan African countries, including Zimbabwe with the prevalence rate varying according to agro-ecological regions (McDermott and Arimi, 2002; Mohan *et al*., 1996; Muma *et al*., 2007; Matope *et al*., 2010). The source or origin of brucellosis in the present study area could not be accurately ascertained as there have been no previous studies on the disease in the area. However, earlier studies have demonstrated the presence of brucellosis in the commercial and smallholder sectors and, other communal areas other than the present study area (Manley, 1969; Swanepoel *et al*., 1975; 1976; Madzima, 1987; Madsen, 1989; Mohan *et al*., 1996; Matope *et al*., 2010). The spread of *Brucella* spp. from one herd and one area to another is often due to the movement of an infected animal into a non-infected susceptible herd (Crawford *et al*., 1990). The purchase of unknown *Brucella*-status cattle from the commercial to the communal sector for the purpose of restocking herds and genetic improvement and an increased uncontrolled movement of cattle due to agrarian reforms in the country have been attributed as the source of spread of brucellosis into the communal sector (Matope, 2009). Unknown *Brucella*-status cattle purchased from commercial farms and translocated to the communal sector were mixed and cross-bred with the indigenous *Bos indicus* breeds. As pointed out earlier (Matope, 2009), these

farming practices brought about mixing of naïve cattle between the commercial, communal and smallholder sectors in the country and could be the source of brucellosis in the latter sectors.

The prevalence rate reported in this study was lower than previous reports for commercial dairy farms in Zimbabwe (Manley, 1969; Swanepoel *et al*., 1975; 1976) but higher than that reported from small-holder farms (Madzima, 1987; Madsen, 1989; Mohan *et al*., 1996; Matope *et al*., 2010). The most important spread of brucellosis takes place from cow to cow, with infected cows contaminating the pasture and uninfected animals becoming infected by ingestion when grazing (Madsen, 1989). However, the final prevalence rate is determined by the intensity of cattle contacts within and between herds and with infected pasture and water (Madsen, 1989). In sub-Saharan Africa, the highest incidences of brucellosis have been reported in pastoral production systems (Schelling *et al*., 2003). Omer *et al*. (2000) reported that the prevalence of brucellosis varied with production systems, with pastoral systems recorded the highest (46.1%), followed by dairy systems (35.9%) and the lowest was recorded in the mixed crop-livestock systems. Mixed crop-livestock systems are practiced in the south east lowveld of Zimbabwe which also recorded a low prevalence in present study. Hence, brucellosis risk increases with change from a purely extensive, nomadic to a more-intensive form of cattle management (Thimm and Wundt, 1976). This probably explains the higher prevalence reported in commercial dairy farmers compared to the lower prevalence reported in traditional purely extensive form of cattle management in the study area.

However the low prevalence reported in study could also be attributed to a long sampling interval and the inclusion of sexually immature animals. Chimana *et al*. (2010) conducted a study in Zambia and the seroprevalence was 18.7% higher than observed in this study.

During the present study a significant association between Brucella seropositivity and abortion history was observed and this is consistent with earlier reports (England *et al*., 2004; Muma *et al*., 2006; Matope *et al*., 2010) as abortion is one of the main signs of brucellosis in cattle. Cows are particularly susceptible to infection during early pregnancy and this has been attributed to the presence of erythritol which is a growth stimulant for *B. abortus* (Quinn *et al*., 1994; Matope, 2009). Such infections can result in late term abortion (Cunningham, 1977). However, in infected herds, a certain proportion of infected cows may not abort (Brinley Morgan, 1977; Matope, 2009) and this could distort association between history of abortion and sero-positivity (Matope, 2009). In Chad, Schelling *et al*. (2003) found out that a total of 19% of brucellosis-seropositive cows had a history of abortion and brucellosis seropositivity of cattle was significantly correlated to history of abortion. Schelling *et al*. (2003) also found an association between history of abortion and seropositivity of cows, an association often described in literature (McDermott and Arimi, 2002).

The results of the present study showed no significant difference of positive reactors between males and females, suggesting that the risk of infection with *Brucella* spp. is independent of cattle sex and results of this study agree with (McDermott *et al*., 1987).

However, the association between sex and the risk of brucellosis has been reported to vary with different cattle populations (Kadohira *et al*., 1997; Omer *et al*., 2000).

From this study, it was evident that older animals had increased chances of testing Brucella positive which is consistent with other reports (Omer *et al*., 2000; Muma *et al*., 2006; Matope, 2009). The preponderance of seropositive reactors in older cattle is consistent with what is generally known about the biology of *Brucella* spp. infection (Kadohira *et al*., 1997; Omer *et al*., 2000; Matope, 2009). The onset of sexual maturity is associated with a significant increase in the risk of infection with *Brucella* spp. and results of this study agree with (Walker, 1999) and such animals are likely to seroconvert. However, the age at which sexual maturity is attained varies with breeds of cattle and this is likely to influence the observed relationship between age and positive reactors in different sub-populations (Matope *et al*., 2010)

Brucellosis has been reported to be prevalent in areas of livestock-wildlife interactions (Nicoletti, 1980; Jiwa *et al*., 1996; Muma *et al*., 2006; 2007). Although not statistically significant, the results of the present study showed that cattle at the interface and having a history of grazing in the park had increased chances of testing *Brucella* positive. At the interface, cattle share grazing pastures and watering points with African buffalos, greater kudus and impalas. Although not detected in this study (except one giraffe) earlier studies in Zimbabwe, showed the presence of brucellosis in wild animals such as the African buffalo, eland antelope, giraffe (*Giraffa camelopardalis*), impala, hippopotamus, black rhinoceros (*Diceros bicornis*), Burchell's zebra and waterbuck (Condy and Vickers, 1969; 1972; 1976; Madsen and Anderson, 1995). The absence of

Brucella spp. positive cases in wildlife during the present study could be attributed to the small sample size or that the sampled herds/animals were free from Brucella infection. However, Madsen and Anderson (1995) working in the same study area, reported a seroprevalence of 8.2% (4/49) and 8.8% (3/34) in African buffaloes sampled from the GNP and surrounding hunting areas, respectively and a seroprevalence of 3.7% (2/54) in giraffes, 3.7% (8/218) in eland and 1.9% (1/53) in impala sampled from two mixed cattle and game ranches in the southeast lowveld. According to Madsen and Anderson (1995), contact with livestock was likely in the cases of seropositive eland antelope, impala and giraffe. However, about half of the positive samples from African buffaloes were obtained from animals that had no contact with cattle or other livestock. This probably demonstrates an independent Brucella infection cycle in the African buffalo, which consequently should be considered as a possible source of infection to domestic stock (Madsen and Anderson, 1995). Hence, considering the contagious nature of *Brucella* spp. sharing grazing land and watering points between cattle and wildlife at the studied interface is likely to facilitate transmission of the disease in both directions.

5.2 Bovine tuberculosis

The tuberculin test is a widely used test to identify *M. bovis*-infected cattle herds in bTB control programs (Thoen and Ebel, 2006). The test can be carried out under different settings (O'Reilly, 2005); in herds with unknown *M. bovis*-infection history or in which non-specific reactions can occur, the SCITT is recommended. Cattle that develop more induration in response to the bovine rather than to the avian PPD are considered to be infected with *M. bovis* (Thoen and Ebel, 2006) while those developing more induration in response to avian PPD are considered to be exposed to atypical mycobacteria (Hope

et al., 2005). Therefore, bovine-reacting cattle were identified as those with bovine tuberculosis and avian-reacting cattle as those with atypical mycobacterioses.

During the present study only five cattle reactors were recorded. However, based on the SCITT, observation of a positive reaction is not proof of disease (Thoen and Ebel, 2006). During the present study no conclusive evidence could be obtained for bTB in cattle: the bovine SCITT reactors were negative to the gamma interferon test, none of them showed clinical signs of the disease and two of the slaughtered reactors were also negative on culture and histopathology. Therefore, the present results based on the SCITT may probably reflect previous exposure in the animal, and not necessarily a disease. However, false positive reactions have been reported to occur as a result of exposure to bacterial species that have some identical antigens to those found in *M. bovis* (Tschopp *et al*., 2010). These reactions can be caused by a range of different mycobacterial species such as members of the *Mycobacterium avium* complex, but in most cases the cause of false positive response is not determined (De Lisle *et al*., 2002). During the present study 11 (2.3%) cattle avian reactors were recorded. Hence, the five cattle reactors could probably be false positive responses. In addition, the five cattle reactors were all found to be negative on the confirmatory gamma-interferon test, which has been shown to have a sensitivity of 85% and a specificity of 93% in cattle that reacted positively to the skin test 8-28 days previously (Ryan *et al*., 2000). However, the sensitivity of the gamma-interferon test has been demonstrated to decrease in cattle experimentally or naturally co-infected by *M. bovis* and *M. avium paratuberculosis* (Amadori *et al*., 2002; Hope *et al*., 2005; Alvarez *et al*., 2009). In a group of cattle with

dual infection, 50% (8/16) of the animals were false negative reactors in the gamma interferon assay (Alvarez *et al*., 2009). Hence, the present negative gamma interferon results could have been due to a dual infection by *M. bovis* and *M. avium* complex. In summary, the results of the present study failed to conclusively demonstrate the presence of bTB in the sampled cattle.

Based on the SCITT, the overall cattle prevalence of bTB was lower compared to reports from other African countries such as Nigeria, Uganda, Ethiopia and Zambia (Cadmus *et al*., 2004; 2010; Oloya *et al*., 2006; Ameni *et al*., 2007; Munyeme *et al*., 2009). In Ethiopian cattle production the prevalence ranged from 0.8% to 13.5% in rural areas (Ameni *et al*., 2007; Berg *et al.,* 2009; Tschopp *et al.,* 2010). Semi-arid conditions have been demonstrated to reduce the survival of the bacilli in the environment (Menzies and Neill, 2000; Phillips *et al*., 2003) and its transmission (King *et al*., 1999; Phillips *et al*., 2003). Dry conditions also affect the critical size of aerosol droplets necessary to establish an infection (Chambers *et al*., 2001) and diminish the efficiency of infection by the respiratory route (O'Reilly and Daborn, 1995), which is of most importance in bTB transmission. Since the first case was diagnosed in 1908, bTB infections in cattle have been sporadically reported in Zimbabwe, with a very low prevalence (Huchzermeyer *et al*., 1994). Due to the small number of reactors, the effect of location could not be demonstrated, although all the five bovine reactors came from the interface area. However, other studies in Zambia have demonstrated a significantly higher prevalence of bTB in cattle at a wildlife-livestock interface (Munyeme *et al*., 2009).

The results of the present study demonstrated the presence of bTB in African buffaloes for the first time in Zimbabwe's GNP. Its presence in buffaloes in GNP raises many questions on its source of origin and transmission. Bovine tuberculosis was first diagnosed in African buffaloes in South Africa's KNP in 1990 (Bengis *et al*., 1996). African buffaloes can act as maintenance hosts of *M. bovis* and propagate bTB in large ecosystems in the absence of cattle (De Vos *et al*., 2001). Their social behaviour provides favourable conditions for aerosol transmission of *M. bovis* to members of the same herd (Michel *et al*., 2006). In addition, males frequently disperse between herds via bachelor groups while females and juveniles move to different herds via splinter groups (Halley *et al*., 2002; Cross *et al*., 2005a). Drought conditions may favour spatial spread of the disease by prompting herds to explore new areas and mix with previously un-associated herds (Cross *et al*., 2005b). These events promote the spatial spread of the disease (Cross *et al*., 2004; 2005a). Hence, following the first recorded cases in KNP, follow-up surveys have demonstrated a gradient of infection from south to the north of the Park (Rodwell *et al.,* 2000). Although transboundary movements of buffaloes between South Africa's KNP and Zimbabwe's GNP have not been specifically documented, it is likely that such movements across the Limpopo do occur. Since bTB cases have been recorded in a northern KNP buffalo population located less than 45 km from the unfenced KNP-GNP boundary (Michel *et al*., 2006), the source of infection of the GNP African buffaloes is probably likely to be a buffalo-to-buffalo transmission across KNP-GNP boundary. However, lack of strain typing data in the present study makes it difficult to make a definite conclusion on this route of transmission (de-Garine-Witchaskt *et al.,* 2010).

In KNP, a *M. bovis* strain genetically unrelated to the one characterized in buffaloes was associated with tuberculosis in kudu (*Tragelaphus strepsiceros*), strongly suggesting the maintenance host potential for this species (Michel *et al*., 2006). In addition, warthogs (*Phacochoerus aethiopicus*) could also play a role in the spread of bTB infections in African ecosystems (Michel *et al*., 2006). Hence, these two potential maintenance hosts could also be a source of infection of the GNP buffaloes. However, a study based on molecular typing of the strains involved would provide more valid evidence for this route of transmission.

Retrospective outbreak investigations suggested that bTB was transmitted to buffaloes in KNP from domestic cattle in the southeast corner of KNP between 1950 and 1960 (Rodwell *et al*., 2000). Since the first case was diagnosed in 1908, bTB infections in cattle have been sporadically reported in Zimbabwe, with a very low prevalence (Huchzermeyer *et al*., 1994), and the last case was officially reported to OIE in 1996. However, the current lack of funds and resources has reduced the capacity of the Zimbabwean Veterinary Services to undertake reliable bTB surveillance in wildlife and livestock. The introduction of the agrarian reform programme in Zimbabwe in 2000 led to increased uncontrolled and illegal transboundary movements of cattle from Zimbabwe to South Africa and Mozambique through the GNP. Thus, bTB infected buffaloes in GNP could result from a cattle-to-buffalo contact although, results of the present study failed to conclusively demonstrate the presence of bTB in cattle in the study area.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

The results of the present study established the presence of brucellosis from cattle in communal lands in the south-east lowveld of Zimbabwe. The zoonotic risk to small-scale farmers and other people involved in livestock production should be a cause of concern, although no human brucellosis information was available when this study was conducted. Hence, public awareness and prevalence surveys of human brucellosis in the study area are of great importance for public health. From this study, it was evident that older cattle had increased chances of testing Brucella positive, but the results suggest that the risk of infection with *Brucella* spp. is likely to be independent of sex. The significant association between abortion history and seropositivity observed in this study illustrates the potential economic significance of *Brucella* in cattle, and possibly wildlife productivity. Hence, educational awareness and control measures such as vaccinations against the disease in cattle populations in the study area must be explored and instituted. The results of the present study showed that cattle at the interface and having a history of grazing in the park had increased odds of testing Brucella positive. Hence, the sharing of grazing land and watering points between cattle and wildlife at the studied interface is likely to facilitate transmission of the disease in both directions, posing a threat to livestock and wildlife productivity and public health. However, molecular typing of *Brucella* spp. from both livestock and wildlife is required to determine whether livestock-wildlife interaction has causal implications. In addition, further studies on wildlife brucellosis in the study area are required to determine its

prevalence and epidemiology. One of the recommendations for controlling infection of wildlife with *Brucella* spp. from cattle is by re-electing fences to prevent sharing of grazing land or water points and to control brucellosis in cattle through vaccination and or test and slaughter policy at wildlife-livestock interface.

The results of the present study established the presence of exposure to *M. bovis* in GNP buffaloes but failed to conclusively demonstrate its presence in cattle. Its presence in buffaloes in GNP could probably be due to contact with buffaloes in South Africa's KNP or contact with other potential wildlife maintenance hosts such the kudu and warthogs or via cattle-buffalo contact. Both isolates were typed by analysis of variable number of tandem repeat (VNTR) sequences at 6 loci (exact tandem repeat A–F) and compared with the VNTR profiles of ≈75 isolates from KNP (de Garine-Wichatisky *et al*., 2010). All isolates showed an identical VNTR profile (7544*52.3), which suggests an epidemiologic link between the *M. bovis* infections in the GNP and KNP (de Garine-Wichatisky *et al*., 2010). A typing regimen comprising different typing methods and markers will be useful for more accurately determining the genetic relationship between the isolates from the 2 parks, GNP and KNP (de Garine-Wichatisky *et al*., 2010). In addition, further studies on livestock, wildlife and human bTB in the study area are required to determine its prevalence and epidemiology. People who are frequently exposed to either *M. bovis* infected livestock or wildlife or their infected products such as unpasturized milk, should be considered at risk of contracting zoonotic tuberculosis. This risk increases considerably in individuals with an immune-suppression induced by HIV infection as documented previously (Raviglione *et al*., 1995). Hence, public awareness of bTB in the study area is of great importance. The use of questionnaires to

obtain epidemiological data could highlight the zoonotic risk of diseases such as brucellosis and bTB and at the same time appraise the communities' awareness to these zoonotic diseases. The potential impact of bTB in buffalo population biology is unknown, but recent studies in South Africa have demonstrated that bTB may affect population growth, resilience and fecundity (Jolles *et al*., 2005). In addition, the diagnosis of bTB in game species has severe implications on the national and international trade in wildlife due to movement restrictions and revenue loss. Furthermore, transmission of the disease to communal cattle in the study area could be detrimental to the livelihood of the communal households. Hence, improvements in diagnostics, surveillance, control and prevention of this disease in wildlife are of paramount importance.

CHAPTER 7

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