Sero-prevalence of brucellosis in sheep and springbok (*Antidorcas marsupialis*) in the Karas Region of Namibia

by

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Submitted in partial fulfillment of the requirements for the degree of Master of Veterinary Medicine (Hygiene) in the Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria.

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I thank God for the strength, wisdom, knowledge, patience and persistence to see this work through. Glory, honor and praise be unto Him forever and ever.

DEDICATION

To my beloved wife Beatitude, son Tinaye and daughters Runyararo and Ruvimbo

DECLARATION

I, Oscar Madzingira, declare that this dissertation, which I hereby submit for the degree M Med Vet (Hyg) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Signed: _____

Date: 21 January 2013

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ABBREVIATIONS

AMOS PCR	Abortus-Melitensis-Oviis-Suis Polymerase Chain Reaction
CFSPH	Centre for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence interval
DEFRA	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
DVS	Directorate of Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FSAI	Food Safety Authority of Ireland
GIS	Geographical information systems
KRC	Karas Regional Council
MZCP	Mediterranean Zoonoses Control Programme
OIE	World Organization for Animal Health/Office International des Epizooties
RBT	Rose Bengal Test
SANCO	Directorate General for Consumer Affairs
WHO	World Health Organisation

SUMMARY

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in 2009 affecting sheep, goats and humans on a farm in the adjacent Hardap Region. Brucellosis outbreaks in sheep have the potential to disrupt Namibia's foreign currency earning as the sheep industry contributes greatly to the economy of the country.

This aim of the study was to estimate the prevalence of *Brucella* (*B. melitensis, B. abortus, B. ovis*).infections in sheep and springbok in the Karas Region and to find out if the outbreak of brucellosis which occurred in the Hardap Region in 2009 had spread to the Karas Region.

Two experimental designs were used in this study. The first was a retrospective analysis of brucellosis testing results from 2008-2010 to indicate probable prevalence and to identify positive farms for follow-up sampling in sheep and springbok. Serological testing results of sera (n=22994) collected from 762 farms between 2008 and 2010 were analyzed and used to estimate apparent brucellosis prevalence. A total of 472 sheep sera and nine springbok sera were collected from eight farms that tested positive for *Brucella* antibodies between 2008 and 2010.

The second part of the study was a prospective serological study in sheep and springbok reared together; sheep in the Tses and Berseba communal areas and in culled ewes at the regional abattoir. Sexually mature sheep and springbok were selected for the prospective serological study because they are more likely to show serological responses than younger animals. Prior to the serological study, eleven questionnaires were completed on the farms (n=11) that reared sheep and springbok

together to gather information about farm management and risk factors for brucellosis. In the serological prevalence study, 332 sheep and 345 springbok sera were collected from the eleven commercial farms and 664 sheep sera were taken from the two communal areas. At the abattoir, 2302 sheep sera were collected from 40 farms in the region using the sample size for determining the absence or presence of disease. All sera were tested for *Brucella* (*B. melitensis, B. abortus*) antibodies using the RBT as a screening test and the CFT as a confirmatory test. *B. ovis* antibodies were tested for in sera from commercial farms only using the CFT test.

Results from the retrospective study revealed an apparent sheep brucellosis prevalence of 0.14% (95% CI: 0.1%-0.2%) over the three years and an annual brucellosis prevalence of between 0.05% and 0.19%. At district level, apparent prevalence was between 0% and 0.49%. The prevalence of positive farms was between 0.72% and 1.82%. When apparent prevalence was adjusted for CFT sensitivity and specificity, the prevalence was zero in all cases, suggesting that the prevalence detected in this study may be due to false positive reactions. However, some of the positives serological reactions were from suspected brucellosis clinical cases which were also confirmed by the PCR test. At district level, brucellosis prevalence was shown to be rising in the Karasburg district and decreasing in the Keetmanshoop and Bethanie districts. However, statistical analysis of the data using Fisher's exact test showed that the differences in brucellosis prevalence between districts was not significant, but that the differences in brucellosis prevalence between the three years was significant. All trace back sera collected in 2011 (using the sample sizes for proving disease freedom) from

sheep (n=472) and springbok (n=9) on previously positive farms (n=8) identified by the retrospective study, tested negative for *Brucella* (*B. melitensis, B. abortus, B. ovis*) antibodies. The negative results provided strong evidence that brucellosis control measures implemented on the farms following the outbreak were effective and that these farms were now free of brucellosis.

Results of questionnaire interviews showed that sheep and springbok were the main species on the farms and that the two species came into close proximity throughout the year especially at watering points in the summer. The interviews also revealed that the study population was naïve because farmers did not vaccinate sheep against brucellosis.

All sera collected in the serological study on commercial farms (sheep and springbok), in the two communal areas (sheep) and at the abattoir (culled ewes) tested negative for *Brucella* antibodies (*B. melitensis, B. abortus*). The prevalence of *B. ovis* antibodies in rams on one farm was 10% (3/30). *B. ovis* antibodies were not detected in springbok. The role of springbok in the epidemiology of sheep brucellosis could not be inferred due to the negative results recorded in both species.

Results of the retrospective and prospective serological studies confirmed that apparent brucellosis prevalence in sheep in the Karas Region was low. These results provided evidence that sheep and springbok reared together on the eleven commercial farms were not infected with *Brucella*. It was surprising that no positive reactors were found in

sheep in the communal areas because the intermingling of sheep from different flocks enhances the spread of brucellosis. The absence of positive reactors at the abattoir confirms that the chances of contracting human brucellosis at the abattoir were low and confirms that the forty farms tested were free of *Brucella* infections.

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CHAPTER 1

INTRODUCTION

1.1 Background

Game is an important source of revenue for Namibia through trophy hunting, game auctions, game meat exports and as a tourist attraction. Trophy hunting contributes US$285 million annually to the country's revenue (Weidlich 2007).

In Namibia, game species are predominantly confined to national parks, game reserves and commercial farms, where they are reared extensively with domestic livestock. In the Karas Region of Namibia, sheep and springbok (*Antidorcas marsupialis*) are the predominant animal species on commercial farms. The consumption of game meat in this region is second only to lamb. Springbok on commercial farms are harvested by trained hunters for local meat consumption and for meat exports. Approximately 500 000 sheep are slaughtered annually at local and export abattoirs in the region. South Africa and the European Union countries are the major markets for lamb. Live sheep and goats are also exported to South Africa. According to the Terrestrial Animal Health Code, *B melitensis* and *B abortus* are diseases that can have a significant impact on trade (OIE 2008).

Game farming has developed rapidly over the years as a result of constraints associated with livestock farming such as diseases, profitability and the cost of inputs. The development of the game farming industry has contributed to the re-emergence of

brucellosis by bringing domestic and wild ruminants into close contact (Godfroid 2002). As a result of the close contact, diseases can be transmitted between wild and domestic ruminants, which may enable zoonotic diseases such as brucellosis to enter the human population through livestock (Böhm *et al*. 2007) and game products. Cross-infection of brucellosis between game species and domestic ruminants may complicate control measures for brucellosis, as game species may serve as reservoirs of infection (Muma *et al*. 2007). Brucellosis is a neglected zoonosis that is transmitted between ruminants and humans in Africa (Marcotty *et al*. 2009).

Brucella melitensis and *B. abortus*, which are both zoonotic diseases and common causes of abortions in domestic ruminants, have been reported in impala (*Aepyceros melampus*) and waterbuck (*Kobus ellipsiprymnus*) respectively (Waghela & Karstad 1986), which had no history of contact with livestock. *Brucella abortus* has also been recovered from a wide variety of wild herbivores raised together with domestic herbivores on ranches (McDermott & Arimi 2002; Gupta *et al*. 2005). *Brucella melitensis* and *B. abortus* have been described in several livestock species (Waghela & Karstad 1986), but the epidemiology of brucellosis transmission between domestic and wild ruminants is not clearly understood. Previous serological studies carried out on black-faced impala (*Aepyceros melampus petersi*) in Namibia yielded no positive reactors (Karesh *et al*. 1997), but these studies were carried out in a national park where there was no contact with domestic ruminants.

Brucella ovis is commonly seen in rams, but it is not zoonotic. It is, however, of economic importance because it causes ram epididymitis and low reproductive rates in affected flocks (Corbel & Brinley-Morgan 1984; Godfroid 2002; Blasco *et al.* 2004).

A serological survey of *Brucella* antibodies in sheep at an abattoir in a region adjacent to the Karas Region in Namibia, yielded an overall prevalence of 2.19% (3/137) and no positive reactors in abattoirs workers (Magwedere, Hoffman & van Schalkwyk 2009). However, anecdotal evidence from the State Veterinary Services indicates that brucellosis due *B melitensis* was subsequently diagnosed in farm workers and sheep on one farm (Personal Communication 2010).

The presence of brucellosis in the adjacent region, and the fact that brucellosis is a neglected and emerging zoonosis worldwide (Marcotty *et al*. 2009) necessitated a serological investigation in the Karas Region. Since 2004, the Directorate of Veterinary Services in Namibia has carried out a voluntary brucellosis testing program on commercial farms in the region, to comply with the export requirement that meat from sheep must be sourced from brucellosis-free flocks. However, there is a lack of information on the prevalence of brucellosis in sheep and game species reared together on commercial farms and in sheep in the communal areas.

It was therefore decided to carry out a serological study of *Brucella* (*B. melitensis, B. abortus B. ovis*) antibodies in sheep and springbok because brucellosis due to *B. melitensis* and *B. abortus* is zoonotic and a significant number of sheep are slaughtered

at abattoirs where workers may be exposed to the disease. *Brucella abortus* was considered as a possible cause of brucellosis in sheep and springbok because on some of the farms in the study area, these species come into contact with cattle and the status of infection with *B. abortus* is not known. Although it is not a zoonosis, *B. ovis* is a disease of economic importance in sheep because of its association with reproductive failure.

The aim of the study was to find out if *Brucella* infections occur in sheep and springbok reared together in the Karas Region; to estimate the prevalence of such infections; to find out the role of springbok in the epidemiology of brucellosis of sheep and to make inferences about the possibility of acquiring human brucellosis through contact with and consumption of meat and other products from sheep and springbok.

1.2 Research problem

Brucella melitensis infection was diagnosed in 2009 in sheep and farm workers on a farm in a region of Namibia that is adjacent to the Karas Region. The presence and extent of the disease in sheep in the Karas Region is not known. Moreover, springbok and other wild ruminant species are reared together with sheep, under extensive ranching conditions in the study area and may remain a reservoir of infection, after the disease has been controlled in ruminants. *Brucella ovis* (in sheep) and *Brucella abortus* (in cattle) are endemic diseases in Namibia, thus could potentially infect wild antelope with which they come into contact.

1.3 Research hypothesis

Null hypothesis (H_o): There is no difference in brucellosis sero-prevalence between sheep reared with springbok and sheep that are not reared with springbok.

Alternative Hypothesis (H_A): There is a difference in brucellosis sero-prevalence between sheep reared with springbok and sheep that are not reared with springbok.

1.4 Benefits arising from the research

- Results of the study will provide an estimate of the prevalence of *Brucella* infections in sheep and springbok and provide information about the possible role of springbok in the epidemiology of sheep brucellosis.
- Serological results will provide an indication as to the effectiveness of brucellosis control measures implemented by state veterinary services.
- Serological studies at the abattoir will provide information as to the likelihood of occupational exposure of workers to brucellosis and an indication of the absence or presence of the disease at farms of origin.

1.5 Objectives

- To analyze sheep brucellosis testing results for the Karas Region from 2008 to 2010 to estimate prevalence and identify positive farms for trace back sampling in sheep and springbok.
- To collect serum samples from sheep and culled springbok (on commercial farms) and sheep in the communal areas and test for *Brucella* antibodies.
- To collect sera from old culled ewes slaughtered at the regional export abattoir and test for *Brucella* (*B. melitensis, B. abortus*) antibodies so as to determine the presence or absence of brucellosis at the abattoir and in the flocks of origin.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, the literature on brucellosis will be reviewed and discussed in relation to the epidemiology of the disease and the current situation in Africa.

2.2 Brucellosis

Brucellosis is a world-wide zoonotic disease that is endemic in most African countries (Mangen *et al*. 2002) and affects humans, domestic and wild animals. It is caused by Gram-negative cocci, coccobacilli or short rods of the genus *Brucella*. *Brucella* species are facultative intracellular pathogens which can cause chronic diseases in mammals (Vemulapalli *et al*. 2004). DNA-DNA hybridization has shown that there is a great genomic similarity between the species, which makes it difficult to differentiate the species (Matope *et al*. 2009).

The main species within the genus *Brucella* are shown in Table 2.1. There is a general host preference within the genus. The main species causing zoonotic disease in descending order of severity are *B. melitensis, B. suis, B. abortus* and *B. canis* (Seleem, Boyle & Sriranganathan 2010; Surendran *et al*. 2011). *B. ovis* is not a zoonotic agent and does not cross-react with *B. melitensis* or *B. abortus* in serological tests. *Brucella abortus* and *B. melitensis* are responsible for late abortions and the birth of weak and sickly newborns in cattle, sheep and goats. *Brucella melitensis* is the common cause of

disease in adult male and female sheep and goats (SANCO 2001; Robinson 2003). Sporadic cases of ovine and caprine brucellosis caused by *Brucella abortus* infections have been reported, but clinical disease is rare (McDermott & Arimi 2002; FAO 2003). Cattle and camels are increasingly being reported as reservoirs of *B. melitensis* (FAO 2010). Bovine brucellosis is caused mainly by *B. abortus* and less frequently by *B. melitensis* and *B. suis* (Robinson 2003). *B. melitensis* was isolated for the first time in Southern Africa in Karakul sheep in Namibia in 1953 and has since been reported in other countries (Godfroid *et al*. 2004). Three biovars (1, 2 and 3) are recognized for *B. melitensis*, seven for *B. abortus* and five for *B. suis* (SANCO 2001).

Brucella suis and *B. canis* are responsible for infections in swine and canines respectively. *B. ovis* is commonly associated with ram epidydimitis and orchitis in Southern Africa and is a rare cause of abortions and new born lamb mortality (Blasco *et al.* 2004). *B. ceti* (cetaceans), *B. pinnipedialis* (seals), *B. microti* (common voles, foxes) and *B. inopinata* (breast implant) have also been reported (Matope *et al*. 2009; Godfroid, Nielsen & Saegerman 2010), but are not important in livestock farming. All *Brucella* spp. can infect wild animal species (Godfroid, Nielsen & Saegerman 2010).

Table 2.1 The main species of *Brucella* and their hosts

Species	Smooth/rough	Hosts
Brucella abortus	smooth	cattle, camels, wild ungulates, humans
Brucella melitensis	smooth	sheep, goats, cattle, camels, humans
Brucella ovis	rough	sheep, red deer (New Zealand)
Brucella suis	smooth	swine, cattle, humans
Brucella canis	rough	dogs, humans
Brucella neotomae	smooth	wood rats

Sources: Corbel & Brinley-Morgan 1984; Godfroid 2002

Brucella can be classified into smooth (*B. abortus, B. melitensis, B. suis* and *B. neotomae*) and rough (*B. ovis* and *B. canis*) strains (SANCO 2001; FAO 2003; Godfroid *et al*. 2004). Smooth strains are generally more virulent than rough strains (Bandara *et al*. 2009). *Brucella* bacteria are aerobic, but some species except *B. melitensis,* grow optimally in an atmosphere containing 5-10% carbon dioxide (Alton *et al*. 1988). Growth of cultures is enhanced in the presence of serum or blood, a pH of 6.6-7.4 and at temperatures of between 36^{o}C and 38^{o}C (SANCO 2001).

2.3 The epidemiology of brucellosis

Risk factors for brucellosis can be categorized into those determinants necessary for the transmission and maintenance of the disease within herds (herd immunity, type of housing, stocking density, use of maternity pens) and those factors that are required for the transmission of the disease between herds (lack of biosecurity, intermingling with other herds, sources of water) (Nicoletti 1980). Factors related to the host, the agent,

the environment and management practices determine the extent of exposure, spread and maintenance of brucellosis in a geographical area (Godfroid 2002).

2.3.1 Transmission

Natural transmission of brucellosis is by ingestion of *Brucella* bacteria that are present large numbers in fetal membranes, milk, contaminated feedstuffs, aborted fetuses and post parturient uterine and vaginal discharges of infected animals especially at the time of parturition (Garin-Bastuji *et al*. 1998; Mangen *et al*. 2002). *Brucella* infection enters the host through mucus membranes, conjunctivae, wounds or intact skin (Ibironke *et al*. 2008; Kahn 2008). Congenital infections affect a limited number of lambs, kids and calves born of infected dams. The majority of *B. melitensis* infections in lambs and kids are acquired through ingestion of infected milk or colostrums (Grilló, Barberán & Blasco 1997). The mixing of colostrums from different dams to feed new born animals can transmit the disease into this age group (FAO 2006). Artificial insemination with infected semen has been implicated as a source of infection (Amin, Hamdy & Ibrahim 2001; FAO 2006). *B. melitensis* may occur in the semen of infected rams (Amin, Hamdy & Ibrahim 2001) and it has been suggested that the venereal route may have a greater role in the transmission of this agent than previously thought (DEFRA 2002; WHO 2006). Embryo transfer is considered to be safe in all species, provided recommended procedures for embryo harvesting, preservation and transfer have been followed (WHO 2006). Other routes of infection include inhalation of contaminated aerosol or dust particles (Godfroid *et al*. 2004), contaminated pastures, feed, water, equipment, clothing and udder inoculation from infected milk cups (CFSPH 2007). The penning of sheep

and goats at night is known to provide an ideal crowded environment for the spread of brucellosis within the flock (Alton 1990; Anonymous 1996).

Passive venereal transmission via the ewe is the primary route of transmission of *B. ovis* in sheep, but ram to ram transmission through mucus membranes, homosexual activity and ingestion are common routes of transmission (OIE 2008). *Brucella ovis* infected ewes can excrete the bacteria in vaginal discharges and milk, and transmit the infection to rams and lambs respectively (OIE 2008).

2.3.2. Agent factors

The virulence of *Brucella* varies markedly with the species, strain and infective dose. In livestock species, *Brucella* bacteria tend to be host specific and produce typical clinical signs of the disease. *B. melitensis, B. abortus*, *B. canis* and *B. suis* are the main pathogenic species for sheep and goats, cattle, dogs, and pigs respectively (SANCO 2001). Although humans can be infected by other *Brucella* species, *B. melitensis* is the most pathogenic and causes severe disease (SANCO 2001).

2.3.3 Host factors

Host susceptibility and establishment of *Brucella* infection is variable and dependent on the ruminant species infected, reproductive status, age, immune status, virulence and the infection dose (SANCO 2001). *B. melitensis* can infect all age groups of sheep and goats, but susceptibility is greatest in sexually mature sheep and goats (FAO 2006; CFSPH 2007). Goats are generally susceptible to *B. melitensis* and are severely

affected by the disease, but sheep show a breed variation in susceptibility, with milk breeds more susceptible than meat breeds (Corbel & Brinley-Morgan 1984).

In pregnant animals, the infection establishes in the uterus because of the presence of erythritol (FAO 2006; Seleem, Boyle & Sriranganathan 2010) and causes a placentitis that leads to abortions and other sequelae. Erythritol is a sugar produced during the later stages of pregnancy by the placenta and promotes the growth of some *Brucella* species strains. The effect of erythritol on *Brucella* growth occurs mainly in the presence of iron (Jain, Boyle & Sriranganathan 2012). However, pathogenic *Brucella* species have also been recovered from animals in the absence of erythritol, putting doubts into the exact role of erythritol (SANCO 2001).

Congenitally infected lambs or kids have latent infections; do not show detectable antibodies before their first gestation (FAO 2010) or show a weak and transient serological response (Grilló, Barberán & Blasco 1997; SANCO 2001) and are therefore not appropriate subjects for serological surveillance for brucellosis. According to Grilló, Barberán & Blasco (1997), a self-cure mechanism may occur in infected lambs.

Infected adult sheep and goats develop antibody titers that fluctuate during lambing, kidding or abortion (FAO 2010). Sheep that have recovered from *B. melitensis* infection are very resistant to re-infection (Alton 1990). Infected animals remain infectious after an abortion or normal parturition (CFSPH 2007).

The excretion of *Brucella* bacteria from the vagina in goats is copious and prolonged lasting up to three months, whereas in sheep excretion ceases within three weeks of abortion or parturition (Alton 1990; CFSPH 2007). *The e*xcretion of *Brucella* bacteria in milk in sheep that have aborted does not normally exceed two months and the disease in sheep tends to be self-limiting (Alton 1990).

2.3.4 Survival of *Brucella* in the environment

Brucella bacteria survive well in the host and in the environment as shown in Table 2.2. Within the host's body, they survive and multiply inside leucocytes and monocytes, which enable the bacteria to evade cell and humoral mediated immune mechanisms and enables the bacteria to be disseminated throughout the body (SANCO 2001). In the presence of organic matter in the environment, *Brucella* can withstand desiccation and is able to survive for longer periods at lower temperatures (CSFPH 2007). The bacteria do not survive for long periods in hot dry weather. Wet conditions prolong survival and increase the probability of transmission to the next host. In farm slurry, *Brucella* bacteria can survive for up to seven weeks at ambient temperatures (SANCO 2001).

Table 2.2 *Brucella* survival times in the environment (SANCO 2001)

Environment	Conditions	Survival time
Water	20°C	2.5 months
Water (lake)	37°C, pH= 7.2	< 24 hours
Water (lake)	8°C, pH =6.5	2 months
Soil	Dried at 18°C	69-72 days
Soil	Dried in laboratory	< 4 days
Urine	37°C, pH =8.5	16 hours
Manure/dung	Summer	3 months
Manure/dung	Winter	6 months
Pasture	Sunlight	< 5 days
Wool	In warehouse	4 months
Hay		Several days to months
Street dust		3-44 days

The survival of *Brucella* in dairy products depends on the level of humidity, type and age of product, temperature, pH, moisture content, presence of other bacteria and storage conditions. *Brucella* cannot survive pH levels below 3.5 (WHO 2006), which may occur in some products such as ripened cheese. Survival of the bacteria in chilled meat is of extremely short duration because of the drop in pH which occurs as part of the maturation process, but the bacteria can survive for many years in frozen meat (SANCO 2001). Muscle tissue generally contains low numbers of *Brucella* in infected animals and

therefore poses a lower risk for human brucellosis than lymph nodes, uterine fluids and the infected fetus (WHO 2006).

The major risk factor for introducing disease in a previously non-infected flock is the introduction of new sheep and goats into a flock without implementing biosecurity measures (SANCO 2001; McDermott & Arimi 2002). Husbandry practices and environmental conditions determine the survival and the extent of spread of brucellosis. Large mobile flocks that frequently intermingle with other flocks from different owners (SANCO 2001; McDermott & Arimi 2002; WHO 2006) and the mingling of animals at markets favors the transmission of brucellosis. Lambing or kidding in crowded dirty pens and the use of the same pens year after year, are risk factors for the spread of brucellosis. Lambing and kidding are periods associated with a high risk of infection in sheep, goats, cattle and humans. Seasonal increases in the incidence of brucellosis in sheep and goats have been associated with peak times of kidding and lambing (WHO 2006).

In Zambia, geographical location, husbandry practices, herd size, breed and contact with wild animal species have been reported as risk factors for brucellosis in cattle (Muma *et al*. 2007). In fact, the development of the game farming industry has been implicated as the cause of the re-emergence of brucellosis in livestock and wildlife, because of the lack of pre-movement screening and an increase in the density of infected game species (Godfroid 2002).

2.3.5 Prevalence of brucellosis

The serological prevalence of brucellosis on sheep and goat farms depends on many factors including husbandry and management practices (FAO 2006; FAO 2010). McDermott and Arimi (2002) reported an average brucellosis sero-prevalence of between 5.6% and 14.5% in sheep and goats in Sub-Saharan Africa. The sero-prevalence of brucellosis in sub-Saharan Africa has been reported to be greater than 10% in many surveys (Hesterberg *et al*. 2008). In KwaZulu-Natal, the prevalence of brucellosis in sheep was between 1.23% and 4.02% (Emslie & Nel 2002) and in Syria the serological prevalence on commercial farms was between 9.94% and 13.9% (Darwesh & Benkirane 2001). A serological survey of *Brucella* antibodies in sheep at an abattoir in the Hardap Region in Namibia yielded an overall prevalence of 2.19% (3 out of 137) (Magwedere, Hoffman & van Schalkwyk 2009).

2.4 Pathogenesis

The major routes of entrance of *Brucella* to the body are mucus membranes of the alimentary tract, conjunctiva and respiratory tract, damaged skin and male and female genital tracts (SANCO 2001; Neta *et al*. 2010). After penetration, the bacteria are phagocytosed by macrophages and neutrophils and carried to regional lymph nodes where they proliferate and cause a lymphadenitis. The infection may resolve or proceed to a bacteremia (which is detectable for up to 20 days and is associated with a fever) and disseminated to other organs especially the udder, pregnant uterus, spleen, supramammary lymph nodes, testes and male accessory sex glands. Bacteremia may recur particularly during pregnancy (Godfroid *et al*. 2004; Neta *et al*. 2010).

The establishment of infection depends on the species infected, age, sex, pregnancy status, virulence and number of infecting bacteria. The preference of *Brucella* spp. for the uterus and genital organs of sheep, goats and cattle is related to the affinity for erythritol present in these organs (Sangari *et al*. 2000; Godfroid *et al*. 2004). Invasion of the pregnant uterus leads to an ulcerative endometritis of the inter-cotyledon areas and destruction of villi. The severity of the placentitis, villi destruction or infection of the fetus, determines whether abortions or birth of weak newborns will occur. The bacteria frequently localize in other organs especially the liver and spleen in large numbers and in other sites such as joints, heart, kidneys and the central nervous system in lesser numbers (Baldwin 1994).

Brucella infections stimulate both humoral and cell mediated immune responses, but it is the later which are important for the clearance of the intracellular pathogens (Schurig, Sriranganathan & Corbel 2002). Smooth *Brucella* organisms such as *B. abortus* and *B. melitensis* have lipopolysaccharide (LPS) molecules containing a polysaccharide O-chain, which is absent in the rough strains. The O-chain is the dominant antigen that is able to stimulate the production of bactericidal antibodies that result in the clearance of *Brucella* bacteria from the circulation (Schurig, Sriranganathan & Corbel 2002). Cell mediated immune responses are effected through activated macrophages and cytotoxic T cells and these are best stimulated by live vaccines (SANCO 2001; Schurig, Sriranganathan & Corbel 2002). The detection of antibodies against the O-chain is the basis of serological diagnostic tests for brucellosis. Serological responses may be seen 2 to 4 weeks following a natural infection. Uterine invasion causes a much more rise in

antibodies than localized udder infections (SANCO 2001). Although the serological responses in sheep and goats have not been fully studied, it is presumed that they follow the pattern as in cattle, that is, a predominance of IgM antibodies in the early stages of the infection and IgG antibodies in the later and chronic stages of the disease. The serological response is transient in sexually immature animals.

B. ovis penetrates mucus membranes and enters the circulation, where a bacteremia develops. This is followed by localization of the infection in lymph nodes and body organs, mainly the epididymis, seminal vesicles, ampullae, bulbourethral glands, spleen, liver and kidneys. Excretion of the bacteria in the semen is common 31 to 45 days post exposure (Blasco *et al*. 2004). The bacteria in the epididymis cause degeneration and necrosis of the epithelium, which results in the leakage of semen into the interstitial tissues, eliciting a severe inflammatory reaction and the formation of spermatic granulomas. The same inflammatory changes may occur concurrently in the vas deferens, ampullae, seminal vesicles, bulbourethral glands and testes (Kimberling *et al*. 1986). These lesions lead to the presence of neutrophils in the semen, decreased production and quality of sperms, leading to reduced fertility and ultimately infertility. The infertility may be due to the cessation of sperm production or obstruction of the epididymis by granulomas. Semen quality is related to the degree of epididymal lesions and the number of leukocytes present in semen. Poor quality semen is associated with a low spermatozoa count and many defective spermatozoa. The defects are mainly of the head and neck (Blasco *et al*. 2004). In pregnant ewes, *B. ovis* infection also results

in a bacteremia and placentitis which may lead to abortions or birth of weak lambs. The capacity of *B. ovis* to cause abortions is very low (FAO/WHO 1986).

2.5 Clinical signs in sheep and goats

The clinical presentation of brucellosis depends on many factors including age, sex, breed, vaccination status and herd management factors such as flock size and density. Ruminants generally abort once in the mid third of gestation, but reinvasion of the uterus in subsequent pregnancies occurs resulting in the shedding of bacteria in uterine fluids and retained fetal membranes (CSFPH 2007; Neta *et al*. 2010). Abortion storms in the latter part of gestation are the first indicator of brucellosis in sheep and goats. In nanny goats, abortions are associated with retained placentas and fetal membranes, as well as reduced milk production and quality (Anonymous 1986; Alton 1990; Theon, Enright & Cheville 1993). The percentage of females that abort is less in areas where brucellosis is enzootic because of early exposure and “immunization” against the pathogen (SANCO 2001). In fact, the prevalence of abortions is high in unvaccinated populations, in which the shedding of bacteria is also high. In sheep and goats, abortions generally occur in the last two months of pregnancy (Alton 1990). After the first abortion storm, the number of ewes or nanny goats aborting declines in subsequent breeding seasons and eventually abortions may cease to occur, although the flock is persistently infected (Alton 1990). Kids and lambs carried to term may be born weak or asymptomatic persistent latent carriers of the infection that will shed *Brucella* at their first parturition (Grilló, Barberán & Blasco 1997).

The udder is a predilection site for *B. melitensis* in ewes and nanny goats. Persistent infection of the udder is a feature in nanny goats and is associated with intermittent shedding of bacteria in milk and reduced milk yield (Godfroid *et al*. 2004; WHO 2006; Seleem, Boyle & Sriranganathan 2010). In non-pregnant goats, the bacteria localize in the secretory tissue of the mammary gland leading to the excretion of the bacteria in milk during subsequent lactations (Alton 1962; Alton 1985). The reduction in milk yield is estimated at 10% (SANCO 2001). *Brucella* infections in goats cause a greater reduction in milk yield than in cattle (Alton 1985). Udder infection is not commonly associated with mastitis in sheep (CSFPH 2007).

Arthritis affects both sexes of sheep and goats and is one of the consequences of brucellosis which has a negative bearing on production and reproduction (Anonymous 1986; Alton 1990; CSFPH 2007). Hygromas are well documented in cattle, but are not a typical feature of sheep or goat brucellosis (Mangen *et al*. 2002).

Limited numbers of sheep, goats and heifers with latent infections may abort or give birth to infected newborns, which are central to maintaining the disease in the herd. These animals may seroconvert at their first parturition only, excrete and contaminate the environment with *Brucella* bacteria (FAO 2006; FAO 2010). The course of brucellosis may be self-limiting, with the infection being eliminated from the body or persistent in the mammary glands, supramammary and genital lymph nodes with intermittent or constant shedding in milk and genital secretions (Fensterbank 1987).

In males, *B. melitensis* may localize in the testes, epididymis and accessory sex glands, causing orchitis and epididymitis and ultimately infertility and the shedding of bacteria in semen (Godfroid *et al*. 2004; OIE 2008).

B. ovis has an incubation period of between 50 and 250 days (Blasco *et al*. 2004). Clinically, various degrees of unilateral or bilateral enlargement of the tail of the epididymis have been observed with *B. ovis* infections. Involvement of the entire epididymis, the head or the body alone is less common. In acute cases, the affected testis may be swollen, hot and edematous, or have only a localized swelling of the epididymis in less severe cases. In chronic cases, in addition to testicular enlargement, there is an increase in the consistency of the affected parts and reduced mobility of the testis in the scrotum. The testis may be atrophied and have a softer consistency or in chronic cases, the testis may be smaller and feel firm (Blasco *et al*. 2004). Examination of semen usually reveals the presence of the bacteria, decreased sperm count and the presence of inflammatory cells. *Brucella ovis* infections have also been reported to cause abortions and lamb mortalities (OIE 2008).

2.6 Pathology

B. melitensis provokes a regional lymphadenitis and lymphadenitis of other body lymph nodes as the disease progresses. The lymphadenitis is associated with hyperplasia of reticuloendothelial and lymphoid cells and infiltration of mononuclear cells, neutrophils, eosinophils and plasma cells (Bishop, Bosman & Herr 1994).

The type of lesions observed will vary with the extent and duration of inflammation. With progression of the disease, endometrial lesions change from acute to chronic. However, edema and greyish-white areas of necrosis of the placenta and the presence of a brownish-red exudate between the allantochorion and the endometrium are consistent findings. Affected cotyledons are necrotic (Godfroid *et al*. 2004).

Some aborted fetuses have subcutaneous oedema and blood tinged fluid in the thoracic and abdominal cavities. No gross lesions are commonly observed in the udder, but the supramammary lymph nodes may be enlarged (Godfroid *et al*. 2004).

Microscopically, granulomatous or necrotic foci are present in lymphoid tissues, reproductive organs, associated lymph nodes and synovial membranes. Endometritis, vasculitis and extensive necrosis of the chorioallantoic membrane with large numbers of bacteria in necrotic villi, have been reported. In addition to microgranulomas in various tissues, multifocal bronchopneumonia may occur in aborted fetuses (Godfroid *et al*. 2004).

B. ovis is accompanied by single or multiple spermatocoeles and spermatic granulomas filled with creamy or caseous material and fibrosis of the tunica albuginea and interductal connective tissue. Fibrous adhesions frequently occur between the tail of the epididymis, the parietal tunica vaginalis and the distal pole of the testis (Blasco *et al*. 2004). Atrophy of the testes is also associated with *B. ovis* infections. Lesions in the vas deferens and accessory sex glands are similar to those in the epididymis. Abnormalities

observed in semen include the presence of leucocytes especially neutrophils, decreased sperm density, reduced sperm motility and an increase in the proportion of spermatozoa with defects (Blasco *et al*. 2004).

Fetuses aborted due to *B. ovis* infection are dehydrated and have a fibrinous peritonitis. Placentitis caused by *B. ovis* produces yellowish fibrinous exudate especially in the areas between the cotyledons. Histologically, the lesions are suppurative. The placentitis is characterized by a multifocal suppurative inflammation, whilst aborted fetuses have a suppurative bronchitis, bronchiolitis and bronchopneumonia (Blasco *et al.* 2004).

2.7 Diagnosis

Brucellosis should be considered in all cases of abortions in sheep and goats because the signs are not specific. Diagnostic tests used for brucellosis have been extensively reviewed by SANCO (2001), the OIE (2004) and FAO (2006) and can be broken down into tests for isolating and identifying *Brucella*; tests for detecting antibodies against *Brucella* and tests based on allergic reactions (Alton 1988; OIE 2004).

Presumptive diagnosis is done by using the subjective microscopic examination of modified Ziehl-Neelsen stained smears of vaginal swabs, placentas or abomasum from aborted fetuses. Using this stain, *Brucella* stains red against a blue background, but *Coxiella burnetii* and *Chlamydophila abortus* may confuse the diagnosis (FAO 2003; Godfroid *et al*. 2004). Lymph nodes, spleen, udder, uterus, epididymes and testes are

the recommended samples for microscopic examination and culture from dead animals (FAO 2003; FAO 2010). The ideal samples from live animals are milk and vaginal swabs because the mammary gland is the target organ in small ruminants (Marín *et al*. 1996) and vaginal excretion of *B. melitensis* persists for several weeks after abortion (Alton 1990).

Culture, isolation and typing of *Brucella* from blood and other tissue is still the gold standard test, but is time consuming, characterization is complicated and is done only by reference laboratories (Mangen *et al*. 2002; FAO 2010; Godfroid, Nielsen & Saegerman 2010). Another disadvantage of culture and isolation as a diagnostic tool is that it may fail to detect *Brucella* when the numbers are low and the bacteria are slow growing (Godfroid *et al*. 2004). For culture, purposes, the use of both Farrell's selective media and the less selective Thayer-Martin modified medium may help isolate *B. melitensis* (Marín *et al.* 1996; Godfroid, Nielsen & Saegerman 2010). Colonies may appear after 3 days, but cultures may only be considered negative after 2-3 weeks (Godfroid, Nielsen & Saegerman 2010).

Serological tests for the diagnosis of smooth *Brucella* species (*B. abortus, B. melitensis* and *B. suis*) have been developed to detect antibodies against A or M epitopes which are shared by all naturally occurring biovars of the three species. Diagnostic antigens are usually prepared from smooth strains of *B. abortus* (strain 1119-3 or strain 99). Cross reactions in serological tests are therefore a common occurrence amongst smooth species. No cross reactions have been reported between *B. ovis* and *B. canis*,

because species specific antigens are used to detect antibodies to *B. ovis* and *B. canis* in serological tests (Godfroid *et al*. 2004).

The Rose Bengal Test (RBT) is the internationally recognized screening test for brucellosis in sheep and goats (Garin-Bastuji & Blasco 1997; FAO 2010), whilst the Complement Fixation Test (CFT) is widely used for serological confirmation of brucellosis in livestock (SANCO 2001; FAO 2003) and these two are the only prescribed tests for international trade in these species (Nielsen 2002; OIE 2004; FAO 2010). However, the RBT is highly sensitive and false positives are a possibility, particularly in vaccinated animals, while the CFT is more specific and used in series on animals reacting positively to the RBT (OIE 2004). Other tests used for brucellosis diagnosis include the Milk Ring Test (MRT), competitive ELISA, Coombs Test, Immunocapture Test, Flourescence Polarisation Assays (FPA), Radial Immunodifussion (RID) test, Counter Immunoelectrophoresis, Serum Agglutination Test (SAT), Brucellin Test and Interferon-gamma-test (Diaz-Aparicio *et al*. 1994; Bercovich 1998; Lucero *et al*. 1999; Orduna *et al*. 2000).

The antigenic suspensions used in the diagnosis of *B. melitensis* in the RBT and CFT tests, are made with *B. abortus* biovar 1(an A-dominant strain) (Alton *et al*. 1988). This implies that *B. melitensis* biovar 1 (M-dominant strains) may be misdiagnosed, but this is not the case in practical terms, because no significant difference has been found in the sensitivity of RBT antigen made with *B. abortus* biovar 1 in sheep infected with *B. melitensis* biovar 1 or 3 (Blasco 1997). This practice however, still accounts for the

conflicting results between the RBT and the CFT. In addition, the RBT and CFT have been standardized for *B. melitensis* based on similar tests used for the diagnosis of *B. abortus* in cattle, which may explain the relatively low sensitivity of some RBT antigens in sheep (Godfroid *et al*. 2004).

According to Bercovich (1998) and Diaz-Aparicio *et al*. (1994), the RBT has a specificity of between 71% and 80% and a sensitivity of between 78-100%. Bercovich (1998) found a specificity of 98% and a sensitivity of 81% for the CFT. The RBT is useful for the detection of infected flocks, but has a low specificity especially in low prevalence areas and a low sensitivity in sheep (FAO 2010). The relatively low sensitivities of the RBT and CFT tests mean that discrepancies can occur between results from both tests. These serological tests cannot distinguish between Rev.1 induced antibodies and those due to natural infection – interpretation of results must take into account the vaccination status of a flock. Parallel use of the RBT and CFT tests increases the sensitivity of the diagnosis compared to series application, but is more expensive (FAO 2010). Cross-reactions may occur between *B. melitensis* and *Yersinia enterocolitica* O: 9 (OIE 2004; OIE 2008) as a result of identical antigenic determinants in the O-polysaccharide (OPS) molecule (Nielsen *et al*. 2006). Species and biovars are identified using phage lysis and by cultural, biochemical and serological methods (FAO 2003).

Diagnosis of *B. ovis* infections should always start with the clinical examination of the external genitalia of rams because this is a primary site of infection. After the clinical examination, other tests such as microscopic examination of stained smears,

bacteriological culture of semen and serology can then be applied. The presence of neutrophils and *B. ovis* in semen stained by the modified Ziehl-Neelsen method is highly suggestive of infection. However, the presence of neutrophils alone is not pathognomonic (Blasco *et al*. 2004). Lesions caused by *B. ovis* should always be differentiated from those caused by *Haemophilus* spp., *Actinobacillus seminis* and *Corynaebacterium pseudotuberculosis* among others. Following abortions, *B. ovis* can be demonstrated in ewes in smears made from uterine discharges or by bacteriological culture of milk and uterine exudates. In aborted fetuses, cultures of the spleen, liver, lungs and abomasal contents can be used to isolate the organism (Blasco *et al*. 2004).

A number of serological tests have been used to detect *B. ovis*. The CFT, agar gel immunodiffusion (AGID) test and indirect-ELISA are the most efficient and widely used tests for the detection of *B. ovis* infections, but the CFT is the only test prescribed for international trade by the OIE and the European Union, although the AGID test has similar sensitivity (Blasco *et al*. 2004; OIE 2008). The ELISA is more specific and sensitive than the CFT or AGID test. *Brucella* antigen used in serological tests contains rough lipopolysaccharide (R-LPS) antigens which are specific for *B. ovis,* making the tests highly diagnostic of the agent. However, some antigenic components are shared with smooth *B. melitensis*, thus accounting for the cross-reactions that are observed with *B. melitensis* or *B. melitensis* Rev. 1 vaccinated sheep (Marín *et al*. 1998).

In cattle, the Rose Bengal test, buffered plate agglutination tests, CFT, ELISA or the fluorescent polarization assay are suitable screening tests. The MRT is effective for screening and monitoring brucellosis in dairy herds. *Brucella* infections in wild ruminants

follow a course similar to that of cattle, sheep or goats. Serological tests used in domestic species may be used in these species, but each test will need to be validated for use in the particular wild animal species (OIE 2008).

The Polymerase Chain Reaction (PCR) is a highly sensitive and specific reliable test, which is used to detect small numbers of *Brucella* (dead or alive) in samples in the shortest possible time. PCR methods for the diagnosis have been described by Bricker (2002). A number of PCR based assays have been developed to identify the bacteria up to genus level and to differentiate between species, biovars and strains. The best validated assays are those that are based on the detection of specific genes of *Brucella* spp., such as the 16S-23S genes, the *IS*711 insertion sequence or the bcsp31 gene encoding a 31-KDa protein (Godfroid, Nielsen & Saegerman 2010). PCR based assays and other tests are also used to differentiate *B. melitensis* Rev. 1 from natural infection (Bricker 2002; Godfroid *et al*. 2004). The AMOS PCR allows for discrimination between *Brucella* spp., vaccine strains and wild-type strains, but not among biovars of a given *Brucella* species. Detection of *Brucella* spp. DNA using PCR and culture and isolation are the only methods that allow certainty of diagnosis (Godfroid, Nielsen & Saegerman 2010).

The recently developed Multiple Locus Variable Analysis (MLVA) test can differentiate isolates within a biovar and is considered a test for the future for molecular typing (Godfroid, Nielsen & Saegerman 2010).

2.8 Control and prevention

When brucellosis is detected in a flock, region, or country, international veterinary restrictions may be imposed on animal movements and trade, which results in huge economic losses. This is the reason why brucellosis control or eradication programs have been implemented worldwide in domestic livestock (Godfroid, Nielsen & Saegerman 2010). All cases of abortions must be thoroughly investigated by a veterinarian, the cause identified and appropriate actions taken. Aborted fetuses and associated fetal membranes must be properly disposed off and contaminated areas disinfected (WHO 2006).

Continuous surveillance for brucellosis provides information about the presence or absence of the disease and gives information necessary for evaluating and improving control and preventive measures (FAO 2010). Surveillance in humans is particularly important as it may be the first indicator of infection in domestic animals. Data from surveillance studies must be analyzed and reported to the relevant stakeholders if it has to have a positive impact on control measures (WHO 2006). Serological tests are commonly used to identify positive flocks of sheep and goats and to establish brucellosis prevalence. In addition to serological tests, surveillance involves following up on reported and suspected cases of abortions. Clinical surveillance by veterinary personnel complements serological surveillance of brucellosis (FAO 2006). These techniques must be applied in a regular systematic manner for the results to have a meaning. Surveillance for brucellosis in sheep and goats in Namibia is done using

clinical surveillance and voluntary serological testing. Positive farms are quarantined and all sheep on the farm tested. Positive sheep may be destroyed or sent for slaughter at a designated abattoir in a sealed truck, taking into consideration the measures that are necessary to prevent infection of slaughter men. Quarantine is lifted only after two consecutive negative serological results (DVS 2009).

The FAO strategy for the control of brucellosis comprises a five point action plan comprising a baseline sero-prevalence survey of animals based on statistical methods; development and implementation of a risk-based vaccination control strategy; an effective surveillance system to ensure early warning against spread of disease to new areas; monitoring results for progress and changes in infection/disease incidence; and reviewing and updating control strategies, to reflect the results obtained (FAO 2010).

Treatment of brucellosis is futile in animals because of the chronic nature of the disease and the intracellular location of the bacteria. The primary objective of brucellosis control measures is to reduce infection in the animal population to such a level that the impact of the disease on human and animal health, as well as animal production is minimized (SANCO 2001). Various strategies have been used to control brucellosis, but all strategies require a well functioning active surveillance system backed by valid data collected from the field to determine disease prevalence. The surveillance system must be able to detect early changes in incidence and prevalence (Thrusfield 1995; WHO/MZCP 1998) to enable control strategies to be realigned. The most common methods employed to control brucellosis include prevention strategies, test and

slaughter, animal movement controls, vaccination, eradication and disease surveillance (McDermott & Arimi 2002; Godfroid *et al*. 2004; CFSPH 2007). These approaches are applied in varying combinations.

2.8.1 Biosecurity

Brucellosis is introduced into free flocks by infected animals or semen. Replacement animals such as rams must be purchased from brucellosis-free accredited flocks. Before new animals are introduced into the rest of the flock, they must undergo a period of quarantine during which their brucellosis status is confirmed by serological tests.

Farm boundaries must be secure to prevent animals of unknown brucellosis status such as wild or feral reservoirs (CFSPH 2007) from straying onto the farm. Disinfection facilities must be available at farm entrances to prevent people introducing brucellosis onto the farm through fomites (FAO 2006).

Rearing of own replacement animals is a viable option against the introduction of brucellosis from outside. Import risk analysis is an important tool that can be used to identify, quantify and to manage the risk of introducing brucellosis into a country through imported animals and animal products (FAO 2010).

Vermin and pests such as rodents and flies can mechanically tramsit brucellosis and should therefore be controlled by use of appropriate rodenticides, fly traps and baits. Waste management should be such as to prevent the build-up of flies on the farms or

premises as these can be carriers of infectious agents including *Brucella* (SANCO 2001).

2.8.2 The test-and-slaughter approach

The test-and-slaughter approach has been applied in herds or flocks where the prevalence of brucellosis is very low, that is, less than 2% (Nicoletti 1993; FAO 2003). For successful implementation, the flock or herd must be under strict surveillance for a period of time; movement controls must be in place; animals must be individually identified; a pool of replacement animals must be available; full cooperation of farmers must be sought; adequate compensation must be provided and the laboratory must be of a high standard (Nicoletti 1993; FAO 2010). This approach is applicable to sheep, goats and cattle, but has been most successfully used to control cattle brucellosis.

In South Africa, brucellosis positive cattle are identified with a 'C' brand on the right side of the neck and sent for slaughter at a quarantine abattoir in an officially sealed vehicle under a red-cross movement permit issued by a state veterinarian and after notification of the veterinarian at the destination abattoir. Lesotho has also used this approach to declare brucellosis freedom in 1997 (McDermott & Arimi 2002).

2.8.3 Control of animal movements

The control of animal movements within a territory and across international boundaries is essential to prevent the spread of brucellosis between farms, regions and countries with different brucellosis statuses. Movements must be permitted only between areas with the same certified brucellosis status. For movement controls to be effective, animal

identification must include brucellosis status and area of origin using tags, brands or tattoos. Individual animal identification helps in quick identification of restricted animals. Imported animals must be certified brucellosis free before imports are authorized (OIE 2004; WHO 2006).

2.8.4 Vaccination as a means of control

As mentioned previously, vaccination of cattle, sheep and goats is a recognized method of controlling brucellosis. This will be discussed in depth below.

2.8.4.1 Sheep and goats

Vaccination of sheep and goats is the only practical and effective way of reducing the overall incidence of brucellosis caused by *B. melitensis* (Alton 1990; SANCO 2001; FAO 2003) especially when applied on a long term basis (FAO 2010). Vaccination is an important component of control, preventive and eradication measures, especially when an effective vaccine is strategically used. The live attenuated *Brucella melitensis* Rev. 1 is considered the best available vaccine for use in sheep and goats. This vaccine may cause transitory infection, but the period of bacterial secretion from the udder or vagina is short. However, abortion storms, milk secretion and human infections associated with the use of *B. melitensis* Rev. 1 vaccines have been reported (Vemulapalli *et al*. 2004; FAO 2010). To avoid these drawbacks, it is recommended that *B. melitensis* Rev. 1 be administered subcutaneously before the first gestation at 3-7 months of age (Godfroid *et al*. 2004).

Reduced dose vaccination has been successfully utilized to reduce abortions in sheep (Godfroid *et al*. 2004). Adequate protection against *B. melitensis* is achieved if at least 80% of the animals at risk are *B. melitensis* vaccinated (Garrido 1992). *B. melitensis* Rev. 1 vaccine confers lifelong immunity, when administered subcutaneously (Alton 1990), which may interfere with the interpretation of serological tests. However, when administered conjunctivally, the immunity conferred is comparable to the subcutaneous route, but the serological response is markedly reduced (Godfroid *et al*. 2004).

Discrepancies in the safety of *B. melitensis* Rev. 1 vaccines produced in different countries have been observed and these have been ascribed to differences in residual infectivity and immunogenicity (Blasco 1997). Mass vaccinations of all ages and sexes of sheep and goats every two to three years, using the conjunctival route, is ideal when the prevalence of brucellosis is high and under extensive management conditions, whilst targeted vaccination of replacement females is appropriate when herd immunity is high (Blasco 1997; FAO 2010).

B. melitensis Rev. 1 vaccination of young sheep and the test and slaughter approach have been successfully used in Israel to reduce the prevalence of *B. melitensis* infections in sheep and humans (FAO, 2010). The live attenuated *B. melitensis* Rev. 1 vaccine also stimulates protective immunity against *B. ovis* in rams and is administered between 3 and 6 months of age (Schurig, Sriranganathan & Corbel 2002; FAO 2010).

B. melitensis Rev. 1 however, interferes with interpretation of serological tests and may therefore interfere with control programs based on serological testing (OIE 2008). It is well documented that *B. melitensis* can be recovered occasionally in ram semen. The vaccination of males therefore increases herd immunity and reduces the excretion of the organism in semen (FAO 2010). Although *B. abortus* RB51 has been demonstrated to be protective against all *Brucella* spp. in a mouse model, but there is little evidence to support the same activity against *B. melitensis* in sheep (Godfroid *et al*. 2004).

2.8.4.2 Cattle

In cattle, *B. abortus* strain 19 and RB51 are widely used, although strain 19 has been reported to be superior (FAO 2006). Vaccination with *B. abortus* strain 19 is limited to sexually immature heifers (4-8 months) in order to reduce post-vaccine antibodies, which may confuse the interpretation of diagnostic tests and to prevent possible abortions due to the vaccine (Schurig, Sriranganathan & Corbel 2002; FAO 2006). For these reasons, *B. abortus* strain 19 is not suitable for simultaneous use in test and slaughter approaches for the control of brucellosis (Schurig, Sriranganathan & Corbel 2002). In South Africa, all heifers between 4-8 months are required by law to be vaccinated once against brucellosis. Cows older than 8 months require the written approval of a state veterinarian before vaccination. *B. abortus* strain RB51 is a rough mutant strain that is devoid of the *O*-polysaccharide chain. The vaccine produced from this strain does not stimulate the production of persistent antibodies which interfere with serological tests and thus enables the differentiation of vaccinated and naturally exposed animals (Schurig, Sriranganathan & Corbel 2002; Vemulapalli *et al*. 2002).

Brucella abortus strain RB51 allows for the vaccination of adult animals and repeated vaccinations without interfering with serological reactions (Oberem *et al*. 2006). *B. abortus* RB51 does not induce placentitis or abortions in pregnant animals (Schurig, Sriranganathan & Corbel 2002). Vaccination results in the development of herd immunity, elimination of clinical signs of brucellosis and a reduction in the number of bacteria shed through the milk and vagina (Nicoletti 1993). Insufficient information is available to support the use of *B. melitensis* Rev. 1 in cattle and other reservoir species (FAO 2010).

2.8.5 Eradication of brucellosis

Eradication entails elimination of disease causing organisms from a country or region. Eradication programs have been more successful for *B. abortus* than for *B. melitensis* in most countries (FAO 2010). Eradication programs require adequate planning and good decision making as well as an effective reliable surveillance system with adequate laboratory support as a foundation (FAO 2006; FAO 2010). Before deciding on whether to implement control or eradication measures, the prevalence of brucellosis in the epidemiological unit of concern must be defined first. If the prevalence is less than 2%, an eradication campaign using the test-and-slaughter approach alone is recommended. Where the prevalence of brucellosis is around 5%, a combination of the test-and-slaughter approach in adult sheep and goats and vaccination of young replacement animals is recommended to eradicate the infection in the medium to long term (SANCO 2001).

Where the collective prevalence is very high, mass vaccinations of all sheep and goats involved in the epidemiological cycle of brucellosis is a practical strategy to control the disease (FAO 2010). Mass vaccinations can be carried out in two ways. The first approach entails mass vaccinating sheep and goats of all sexes every two years. The second approach requires vaccinating and identifying all animals in the first year, followed by vaccinations of replacement animals in subsequent years (FAO 2010).

Another factor that is necessary for the success of brucellosis eradication programs is a shared understanding of the eradication program by decision makers, farmers and all relevant stakeholders. Without this, there will no concerted effort and the programs will fail. Eradication programs require a significant investment in human and financial resources. Ovine and caprine brucellosis were eradicated from Botswana in 1995, Zimbabwe in 1996 and South Africa in 1999 (McDermott & Arimi 2002).

According to the FAO (quoting OIE), eradication of *B. melitensis* can only be achieved through a combination of the test and slaughter approach, movement control and preventive measures (FAO 2010). In a test-and-slaughter program, two consecutive negative CFT results six months apart in all animals in a flock are accepted as adequate proof of eradication of the disease (Kolar 1984; Alton 1987). For a successful eradication campaign, all susceptible animal species must be identified; movements of all susceptible animals controlled and the market value of animals be paid as compensation. In resource poor countries, vaccinations (FAO 2010), movement controls and public health education are the only effective approaches.

2.9 Brucellosis in humans

2.9.1 Aetiology

Human brucellosis is caused by *B. abortus, B. melitensis, B canis or B. suis. Brucella ovis* is of no significance in relation to human disease (WHO 2006; Seleem, Boyle & Sriranganathan 2010). The clinical disease produced by the four species is indistinguishable, but *Brucella melitensis* causes a much more serious acute form of undulant fever (WHO 2006; Kahn 2008). *B. abortus* accounts for most cases of human brucellosis globally (Godfroid *et al*. 2004; WHO 2006).

2.9.2 Epidemiology

Humans are accidental and dead end hosts of brucellosis (Ibironke *et al*. 2008). Infection is acquired via direct or indirect contact with infected material such as aborted fetuses, vaginal discharges and the consumption of infected unpasteurized milk and dairy products (Neta *et al*. 2010). The disease is an occupational risk for professions such as veterinarians, abattoir workers, farmers, laboratory technicians and others who work with animals and their products (SANCO 2001; Godfroid 2002; WHO 2005). In the Czech Republic, in a survey of 479 veterinarians, 32.4% were serologically positive, whilst 17.5% showed clinical symptoms of brucellosis (Kouba 2003). *B. abortus* and *B. suis* have been reported as frequently affecting occupational groups (Seleem, Boyle & Sriranganathan 2010). Human brucellosis may also occur as a result of accidental inoculation of live *Brucella abortus* strain 19 or *B. melitensis* Rev. 1 vaccines (Seleem, Boyle & Sriranganathan 2010). The infection in humans is often the first indicator of the disease in animal populations (WHO 2006). Ineffective animal and public health

programs have been implicated as the reason for increasing brucellosis cases in humans (FSAI 2009). Lambing, kidding and parturition are periods associated with a high risk of infection in humans as a result of increased exposure to infected material (SANCO 2001).

Brucella enters the human body through the ocular or oral mucosa, inhalation and direct inoculation into the blood stream through abrasions on the skin or accidental inoculation of live vaccines. Although not a common route of infection, inhalation of *Brucella* is an important route of infection for people working in abattoirs and laboratories (Seleem, Boyle & Sriranganathan 2010). Among dairy products, the consumption of cheese presents a greater risk for human brucellosis in the European Union (Westrell *et al*. 2009; Neta *et al*. 2010). This is because sheep and goat cheese is preferably prepared from unpasteurized milk; rennet may originate from infected lambs or kids and the fact that the cheese making process tends to concentrate *Brucella* (SANCO 2001; Seleem, Boyle & Sriranganathan 2010). Soft fresh cheeses present a greater risk for human brucellosis than hard matured cheeses, because the latter have acids and a low pH that kills *Brucella* bacteria. Unpasteurized milk poses a high risk for food-borne brucellosis because of the large volumes of milk that can be consumed at one time (FAO 2010).

2.9.3 Clinical signs and pathology

Brucellosis in humans is normally missed because there are other common human diseases such as malaria, typhoid, paratyphoid and influenza with similar clinical manifestations (Renukaradhya, Isloor & Rajasekhar 2002; Seleem, Boyle &

Sriranganathan 2010). The disease manifests as an acute to subacute febrile illness with intermittent (undulant) fever accompanied by malaise, anorexia, headache, night sweats, chronic fatigue, weight loss, polyarthritis, meningitis, pneumonia, endocarditis, back pain and prostration which in the absence of treatment may become chronic (SANCO 2001; Godfroid 2002; Neta *et al*. 2010; Seleem, Boyle & Sriranganathan 2010; Surendran *et al*. 2010).

It may take 2-4 weeks or several months before clinical symptoms of brucellosis become apparent (WHO 2005; Seleem, Boyle & Sriranganathan 2010). *B. melitensis* causes mainly acute disease, whereas other species are associated with sub-acute to chronic disease (Seleem, Boyle & Sriranganathan 2010).

Human brucellosis is associated with an undulant fever with body temperature reaching 40^{o}C. Clinical symptoms are non-specific but the most common are chronic malaise, headache, arthralgia, night sweats, sacroilitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis and tenosynovitis (Seleem, Boyle & Sriranganathan 2010). Systemic involvement of body organs may result in the enlargement of the spleen, lymph nodes and liver, meningitis, endorcaditis, orchitis and the presence of small granulomas (Seleem, Boyle & Sriranganathan 2010). Bacteremia may result in abortions in the first and second trimesters despite the absence of erythritol in the human placenta (FAO 2006; Seleem, Boyle & Sriranganathan 2010). Human brucellosis tends to be persistent, as a result of frequent relapses, chronic localized infection or delayed convalescence (FAO 2006). Unpublished data indicates that five cases of

human brucellosis associated with sheep handling and consumption of milk were reported on a farm in the Mariental district of Namibia (Personal communication 2010).

In acute brucellosis, isolation of *Brucella* spp. from the blood or other tissues gives a definitive diagnosis. However, in chronic cases, cultures are often negative. Presumptive diagnosis can be made by using the Rose Bengal Test and the Standard Agglutination Test (WHO 2005). In addition to blood cultures, confirmation can be done using the Coombs Antiglobulin Test, Complement Fixation Test, ELISA (WHO 2005; FAO 2006), Counter-immunoelectrophoresis test (CIEP) and the radioimmunoassay test (Diaz *et al*. 1989). Molecular assays are a more sensitive diagnostic tool, but are yet to be validated (Seleem, Boyle & Sriranganathan 2010). Methods which differentiate between IgM and IgG can distinguish between acute and chronic infections, because IgM antibodies predominate in early stages of infections, whilst IgG antibodies predominate in chronic infections (FAO 2006).

2.9.4 Control and prevention

In humans, control and prevention of brucellosis involves two main strategies, that is, prevention of exposure to infected animals and fomites; and maintaining food safety in animal products.

2.9.4.1 Prevention of exposure

Control of brucellosis in livestock results in control of the disease in humans (Vemulapalli *et al*. 2004) because transmission between persons and from persons to the environment is rare (Seleem, Boyle & Sriranganathan 2010). The ultimate objective

of preventive measures against human brucellosis is to avoid direct and indirect contact with infected livestock or material. In this regard, brucellosis can be prevented in occupationally exposed professions by putting on adequate effective protective gear. Protective gear includes overall or coats, rubber or plastic aprons, rubber gloves and boots and eye protection (face mask, goggles) which reduce the risk of accidental infection (CFSPH 2007). The protective clothing should be reserved for their intended use, be cleaned and disinfected after use to prevent spread of brucellosis. Protective clothing used during activities such as milking cows must not come into contact with personal clothing. All wounds and cuts must be treated, and dressed, preferably with an impervious bandage or wound dressing (FAO 2006; CFSPH 2007). Exposure to aerosols of infected blood or secretions is an occupational risk in abattoirs that can be prevented by routine vaccination and testing of herds. Protective clothing should be worn when slaughtering known positive animals (WHO 2006).

2.9.4.2 Brucellosis and food safety

The production of dairy products such as soft cheeses and cream from *Brucella* contaminated milk tends to concentrate the *Brucella* organisms making the products a serious hazard to human health. Therefore, all dairy products must be produced from pasteurized milk. In situations where pasteurization of milk is not possible, milk can be boiled or heated to at least 80^{o}C and held for several minutes at this temperature. Prolonged boiling of milk is generally assumed to kill *Brucella* (Godfroid *et al*. 2004). Soft cheeses produced from unpasteurized milk may be stored for six months (matured) to make them safe for human consumption. Hard cheeses which undergo fermentation

processes during production are less of a risk to humans. Rennet used in the cheese making process needs to be sourced from *Brucella* free animals to prevent human infections. Butter, sour milk, sour cream and yoghurt all undergo acidification processes which reduce the *Brucella* content. For effective killing of *Brucella*, the pH must be less than 3.5, but it must be remembered that it is an intracellular organism and thus well protected, so a low pH does not guarantee absence of infective organisms in unpasteurized dairy products (FAO 2006).

Brucella infected meat contains low numbers of organisms and if the meat is properly stored (matured), it is unlikely to be a source of human brucellosis because the organism has a short survival in meat unless it is frozen. *Brucella* organisms survive well under refrigeration and deep freeze conditions – meat from infected animals will remain infectious under these conditions. Salting, smoking and drying are unreliable methods of killing *Brucella* bacteria. Thorough cooking of meat is the best way of preventing human brucellosis, however, slaughter men, butchers and cooks can be infected when cutting up raw meat if wounds are contaminated by infective blood, mammary secretions or uterine fluids (FAO 2006).

2.9.4.3 Decontamination of infected material

All brucellosis infected material and products of abortion should be collected with care and disposed off by incineration or deep burial with lime away from water courses. Contaminated grounds and farm implements must be washed with a disinfectant such as an iodophor, 20% freshly slaked lime, phenol, 2-3% caustic soda, 2% formaldehyde,

70% ethanol or 2.5% hypochlorite (CFSPH 2007). Dung should be cleared and stored in a secluded area until rendered safe by natural decomposition, a process which may take up to 12 months. The addition of xylene to liquid dung hastens the destruction of *Brucella*. Dung, dust and soil on dairy farms may be contaminated with *Brucella* organisms (CFSPH 2007).

2.9.4.4 Public health education

Targeted public health education to improve awareness of the disease especially in resource poor communities helps to prevent human brucellosis (Marcotty *et al*. 2009; FAO 2010). Education of people directly involved in the animal and food industry empowers them with the knowledge to take up responsibility in preventing brucellosis within their environment. There is a lack of adequate information among medical personnel such as nurses and physicians who are at the fore front of human disease surveillance (Hesterberg *et al*. 2008). This can be addressed through training and adequate intersectoral collaboration between the relevant governmental and intergovernmental organizations (FAO 2010). Studies in Asia found that human populations that are aware of the mode of transmission or the need for pasteurization of milk and other dairy products have significantly reduced risk of *Brucella* infection (FAO 2010).

2.9.4.5 Intersectoral collaboration

For effective brucellosis control, the public health and animal health sectors must share control activities, administrative structures and arrangements to facilitate cross-

notification of cases, as well as coordination of joint investigations, control, and public health education programs. The main sectors of the community must be involved if control measures are to be successful (WHO 2005).

2.9.4.6 Treatment of brucellosis in humans

Treatment of brucellosis in humans is feasible using various antibiotic combinations and has been described by the World Health Organisation (2005) and Seleem *et al*. (2010). A number of antibiotic classes are used to treat uncomplicated brucellosis in human beings. In the tetracycline group, doxycycline is the drug of choice as it can be given orally twice per day for 6 weeks, whilst tetracycline needs to be given every six hours for the same duration and has more gastrointestinal side effects. A relapse rate of between 10-20% has been recorded with tetracyclines, necessitating the addition of an aminoglycoside for the first 2-3 weeks of therapy. The preferred aminoglycosides are streptomycin and gentamicin. The most effective therapy involves giving 100mg of doxycycline twice a day for 45 days and 1g daily of streptomycin for the first 15 days of treatment especially in cases of localized and acute brucellosis (Seleem, Boyle & Sriranganathan 2010) . The main alternative therapy is doxycycline 100mg twice a day for 45 days and rifampicin 15mg/kg/day for 45 days. Other alternative therapies are doxycycline-flouroquinolone and Trimethoprim-sulphamethoxazole combinations. Cotrimoxazole has been used to treat brucellosis in pregnant women with success. Rifampicin and gentamycin are other alternatives for treatment of brucellosis in pregnant women (WHO 2005; FAO 2006). In cases of accidental inoculation with live

Brucella vaccines, in addition to wound management and tetanus toxoid injection, a six week course of doxycycline must be taken (FAO 2006).

There are currently no safe, effective and reliable vaccines for the immunization of humans against brucellosis (Surendran *et al*. 2011), although different types of vaccines have been developed world-wide. Vaccination is therefore not recommended (FAO 2006), but live *B. abortus* strain 19-BA and 104M are in use in the former Soviet Union and China respectively (Seleem, Boyle & Sriranganathan 2010).

2.10 Brucellosis in wild ruminants

The development of the game farming industry has resulted in the re-emergence of brucellosis as a global concern for livestock and wildlife because of the lack of pre-movement screening and an increase in the density of infected game species (Godfroid 2002).The rearing of domestic ruminants and game together in close contact may result in cross-infection of diseases.

Cross-infection is easily possible between taxonomically related species. Buffalo and bovine antelope have caused disease outbreaks in cattle because of their close phylogenetic relationship to ancestral cattle (Bengis, Kock & Fischer 2002). Contact between wildlife species and livestock at watering points and at locations with good forage resources facilitates the transmission of brucellosis (Jiwa *et al*. 1996; Reviriego, Moreno & Dominguez 2000). The degree of contact is influenced by feeding habits. Animals with the same feeding habits are likely to share habitat and diseases. Wild

animals, however, usually avoid domestic livestock spatially and temporally unless habituated (Bengis, Kock & Fischer 2002). The disease interface between wildlife and domestic livestock is not usually a direct physical interaction or sharing of the same space at the same time, but usually indirect through the soil, forage, and water with which another animal has recently been in contact and has left bodily discharges, such as faeces, urine, saliva, or ocular or nasal discharge, or through shared insect vectors or intermediate hosts (Fenner 1982). In a study of brucellosis, spillover infections need to be distinguished from sustainable infections in wild animals which introduce infections into livestock (Godfroid 2002). Buffalo sera (14 of 29) collected from an area where domestic livestock were excluded in Zimbabwe, tested positive for *Brucella* antibodies and it was concluded that the infection in buffalo was sustainable (Godfroid 2002). However, *B. melitensis* biotype 3 was isolated from sable antelope (*Hippotragus niger*) reared together with sheep and goats on a ranch in South Africa, with tested small stock showing negative results. The sable antelope showed signs of systemic disease such as abortions, orchitis and hygromas (OIE 2004). Late term abortions, the birth of weak young ones, arthritis and bursitis and testicular infections have also been reported wildlife (Fyumagwa *et al*. 2009). Spillover of *B. melitensis* infection from sheep to chamois and ibex has been documented in France and Italy (Garin-Bastuji 1996; Ferroglio *et al*. 1998). The probability of brucellosis becoming established and being sustainable in a species depends on a combination of factors including host susceptibility, infectious dose, contact with infected animals, management and environmental factors (Godfroid 2002).

B.ovis infection has been demonstrated in farmed red deer in New Zealand and causes symptoms similar to those found in sheep (CFSPH 2007). The organism is endemic in sheep in South Africa and Namibia, but has not been reported in wild ruminants (Blasco *et al*. 2004).

Wild ruminants that come into contact directly or indirectly with *Brucella* infected sheep or goats may acquire and maintain *B. melitensis* infection in the environment (SANCO 2001). *Brucella abortus* has been reported in a wide variety of wild herbivores raised together with domestic herbivores on ranches (McDermott & Arimi 2002). *Brucella* seropositivity has been reported in bushbuck (*Tragelaphus scriptus*), common eland (*Taurotragus oryx*), impala (*Aepycros melampus*), greater kudu (*Tragelaphus strepsiceros*), common duiker (*Sylvicapra grimmia*), Thomson's gazelle (*gazelle thomsonii*), Kafue lechwe (*Kobus leche* kafuensis), Oryx (*Oryx beisa*) and wildebeest (*Connochaetes taurinus*) (Paling *et al*. 1988; Thorne 2001; Godfroid 2002; Muma *et al*. 2007). A study in Zambia found that seropositivity in cattle was associated with contact with wild animal species (Muma *et al*. 2007). *Brucella abortus* biotype 1 infection has been reported in buffalo, hippopotamus and water buck (*Kobus ellipsiprymnus*) (Condy & Vickers 1972; Gradwell *et al*. 1977). *Brucella melitensis* has been isolated from impala (*A. melampus*) and *B. abortus* from waterbuck (Waghela & Karstad 1986). Brucellosis was identified as a problem of gregarious wildlife species (Rottcher 1978; Pandey *et al*. 1999). A serological surveillance for *Brucella* spp. antibodies in impala in Namibia did not yield positive reactors (Karesh *et al*. 1997). The control of *B. melitensis*

in wild ruminants has not been extensively studied, although vaccination of wild ruminants for *B. melitensis* in the USA has been reported (CSFPH 2007).

Brucellosis serological tests in wildlife are performed using the same antigens used in domestic animals and the tests are usually directly transposed to wild species without validation (Godfroid, Nielsen & Saegerman 2010).

Surveillance and control of brucellosis is costly. It is estimated that US$150 million was spent each year in the United States of America for surveillance and control of brucellosis (Boschiroli, Foulongne & O'callaghan 2001). In many African countries, responsibilities and the legal frameworks that are necessary for wildlife disease surveillance are not clearly defined and free ranging wild animals are therefore not included in disease surveillance and monitoring (Bengis *et al*. 2004). Brucellosis may pose a potential barrier to international trade as potential trade partners may impose stringent sanitary and control measures prior to trade (Godfroid 2002). The economic impact of brucellosis includes losses due to decreased fertility, abortions, still births, weak offspring, decreased milk production, discarded milk, early culling of affected animals and replacement costs, costs related to research, eradication, control and prevention strategies and reduced human working capacity (Mangen *et al*. 2002; FAO 2002).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study area, the sampling strategy and the methods used to collect and test the samples. The last section describes how the data was handled and processed.

3.2 Study area

The Karas Region of Namibia is located at the extreme southern end of the country and shares borders with South Africa to the east and south, the Hardap Region to the north and the Atlantic Ocean to the west. The region is divided into four magisterial districts. This study was conducted in the Keetmanshoop, Karasburg and Bethanie districts which have farms that rear sheep and springbok together. The Luderitz district has predominantly mining activities and was therefore not included in the study. The study area is shown as the shaded area in Figure 3.1.

There are a total of 700 commercial sheep farms in the region rearing approximately 1 million sheep and 250 000 goats (Personal communication). Sheep and cattle farming activities are distributed throughout the region, while goat farming is the main farming activity in the rural populations of the region (KRC 2011). There are six communal areas in the region namely Berseba, Vaalgras, Tses, Bethanie, Warmbad and Bondelswarts.

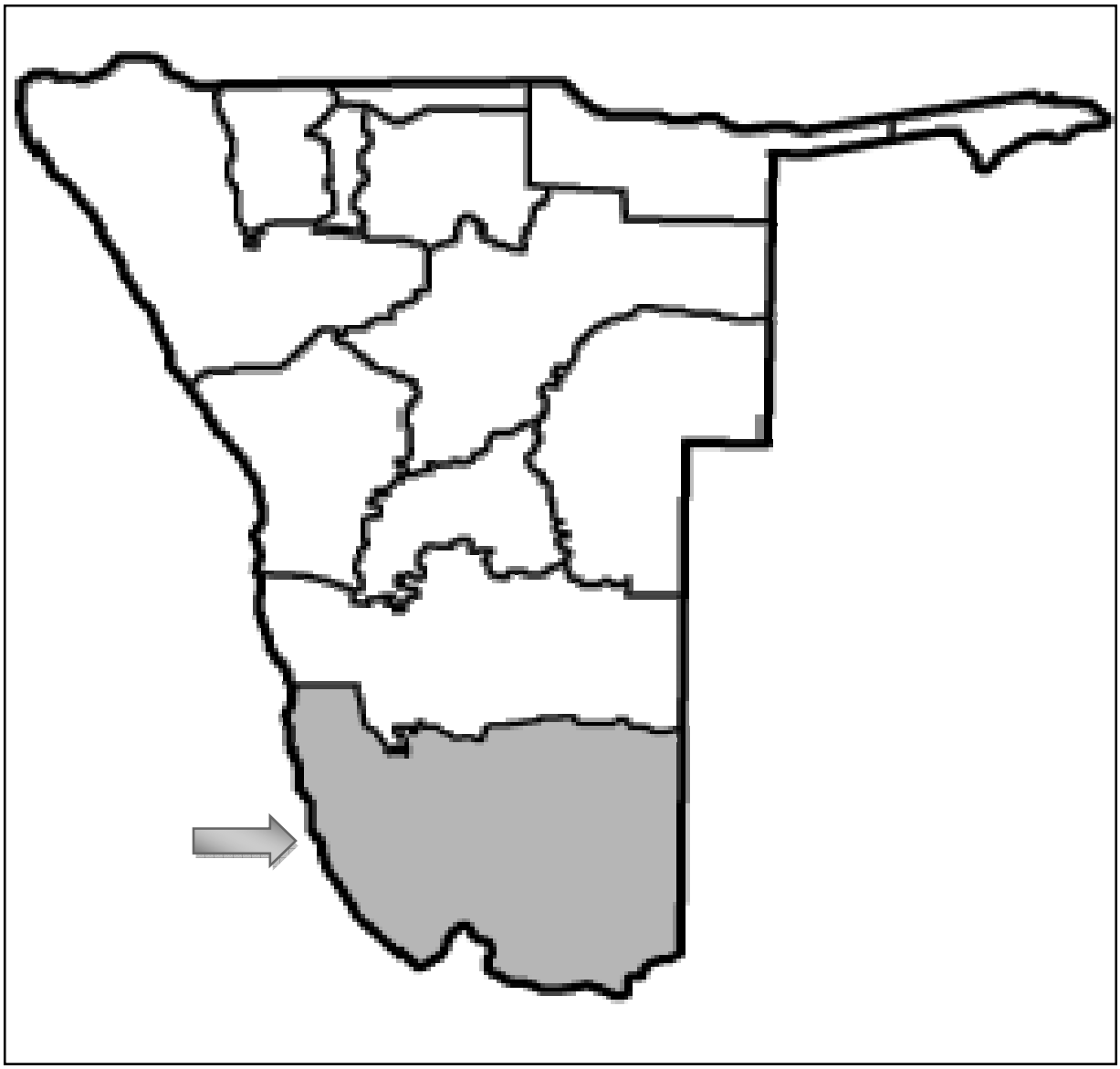

Figure 3.1 Map of Namibia showing the Karas Region as the shaded area (Source: http://www.map-of-namibia.com)

The Karas Region is the driest region of the country and has a hot and dry climate, with unpredictable average summer rainfalls (October to March) of between 142-152mm. In the hottest months, temperatures reach 40°C, whilst in winter temperatures frequently drop below freezing point at night (KRC 2011).

The regional abattoir is the only place where sheep from the Karas region are slaughtered for export purposes and for local meat consumption in large numbers. The abattoir employs approximately 130 workers who come into direct contact with live sheep or their products on a daily basis.

3.3 Experimental design

The first part of the study comprised a retrospective analysis of brucellosis (*B. abortus, B. melitensis*) testing results for the Karas Region from 2008 to 2010. This study was aimed at identifying positive or exposed sheep commercial farms for trace back serological testing in sheep and springbok to determine the presence or absence of brucellosis. Brucellosis prevalence estimated using this data was compared with the prevalence determined by this study.

The second part of the study comprised serological sampling and testing of sheep and culled springbok on eleven randomly selected commercial farms to estimate brucellosis prevalence. Interviewer administered questionnaires were used to gather information about the study population on the eleven farms before the study commenced.

In the third part of the study conducted in two communal areas, sera were collected from sheep at randomly selected watering points. The study ended with a serological study of randomly selected culled ewes (more likely to carry *Brucella* infection) at the abattoir, to confirm the presence or absence of brucellosis on the farms of origin and to find out if abattoir workers are exposed to *Brucella* infections.

3.3.1 Study population

The study population comprised sexually mature sheep and springbok of both sexes because they are more likely to show serological reactions. Sheep and springbok on commercial farms were reared together extensively on natural pastures. The dorper was the predominant sheep breed in the study area. The study farms and communal areas are indicated in Plate 3.1.

3.3.2 Sampling frame

3.3.2.1 Sampling

The sub-population selected for serological surveillance was sexually mature sheep and springbok of both sexes because they are more likely to show serological responses than younger animals (FAO 2006; CFSPH 2007). Serological testing was done on commercial farms (sheep and springbok), communal areas (sheep) and at the abattoir (culled ewes).

3.3.3 Sample size determination

The sample size for estimating disease prevalence in sheep and springbok populations was determined using the formula described by Martin *et al*. (1987), that is:

$$n = \frac{4PQ}{L^2}$$

Where:

n = sample size,

P = expected prevalence in proportion of one,

Q= 1-P

L = allowable error or precision in proportion of one, assuming a brucellosis prevalence

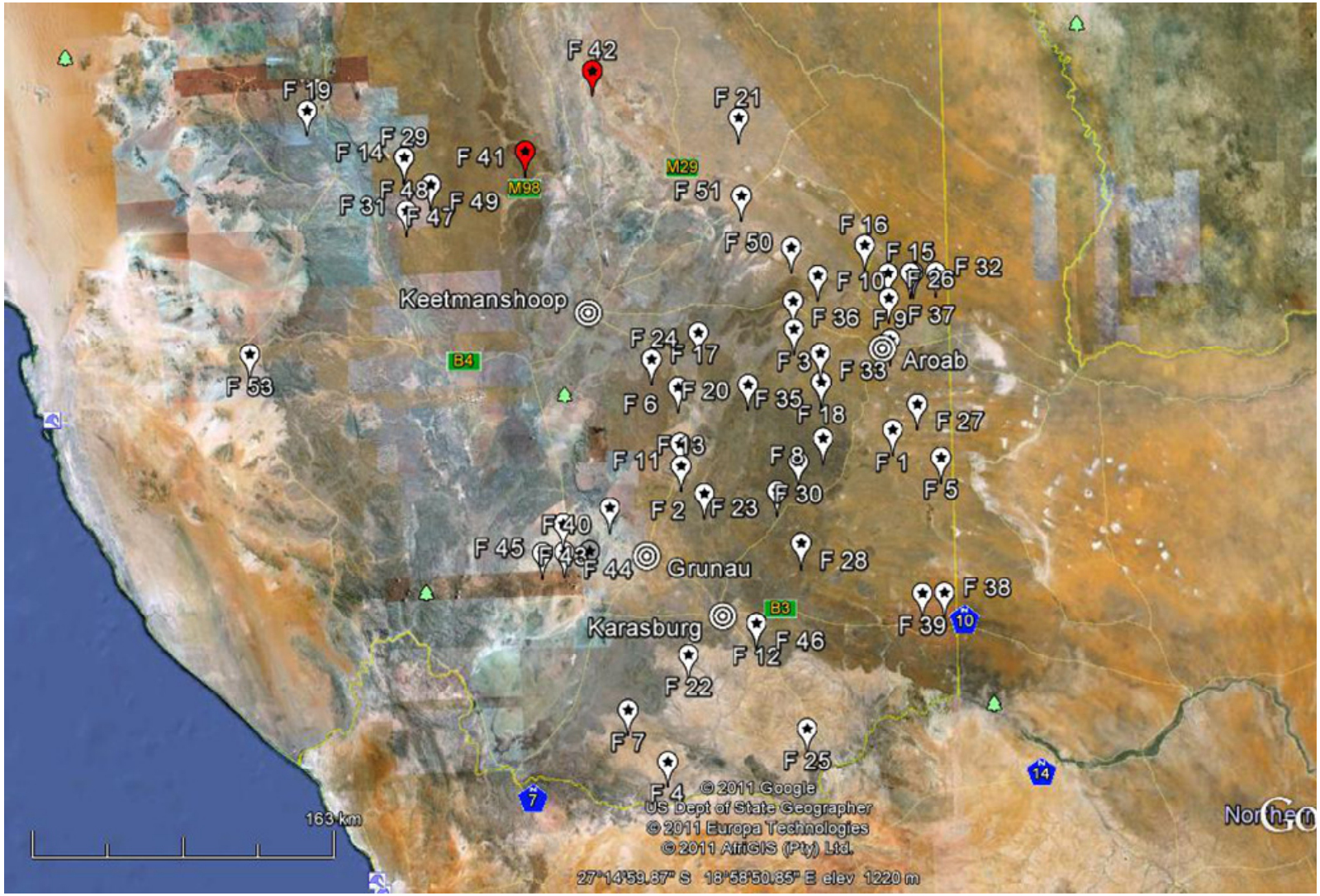

Plate 3.1 Map of the Karas Region showing the spatial location of the farms sampled (F) and communal areas (F41 and F42)

of 5% in sheep and springbok populations at 95% level of confidence using a precision of 0.025. Sample size determination was done in consultation with an epidemiologist in the Epidemiology Section of the Directorate of Veterinary Services and taking into account the existing brucellosis surveillance program. Using this formula, 304 sera (from sheep and springbok) were required to determine brucellosis prevalence. However, 332 sheep and 345 springbok sera were collected from the commercial farms and 664 sheep sera from the communal areas. The extra samples were collected as cover for possible breakages and spoilages of samples during handling and transport.

The number of samples required to detect the presence or absence of brucellosis during trace back sampling on sheep and springbok farms and at the abattoir was determined as described by Martin *et al*. (1987), that is:

$n = \{1-(1-a)^{1/D}\} \{N-(D-1)/2\}$ where:

n is the required sample size

a = probability of detecting at least one diseased animal in a sample when the disease affects at least D/N in population

D = number of diseased animals in population

N = population size.

Assuming an expected prevalence of 5% in the sheep and springbok populations, at 95% confidence level and a population size of 1000 sheep or springbok per farm, it was calculated that 58 sera were to be taken from each farm to be 95% certain of detecting at least one positive sheep or springbok. Sampling of ewes was done over a nine month

period to cover the sheep breeding and non-breeding seasons. Over this period, a total of 40 sheep farms (and n=2302 sera) were sampled. Simple random sampling was used to collect sera.

3.4 Retrospective data analysis

Results of the annual national brucellosis voluntary testing program and disease investigation testing in sheep in the Karas Region for the years 2008, 2009 and 2010 were collated and analyzed. The prevalence of *Brucella* antibodies was estimated per farm, district and year, and comparisons made.

Farms that tested positive for *Brucella* antibodies *(B. melitensis or B. abortus)* between 2008 and 2010 (n=8) were identified, traced back and follow up sera taken from adult sheep (n=472) and springbok (n=9) on the farms (see Table 3.1) to determine the presence or absence of the disease in sheep and springbok to evaluate the effectiveness of control measures that were implemented following the positive results. The sample size for the trace back study was determined as described by Martin *et al*. (1987) as described above.

Table 3.1 Number of farms followed up after the retrospective study

District	Farms tested	Sheep sera sampled	Springbok sera sampled
Keetmanshoop	5	292	5
Karasburg	2	119	2
Bethanie	1	61	2
Total	8	472	9

3.5 Serological study on sheep and springbok farms

3.5.1 Interviewer administered questionnaires

Prior to the serological study on sheep and springbok rearing farms, interviewer administered questionnaires (n=11) (see Annexure 1) were used to gather information on brucellosis vaccination status, history of brucellosis, intermingling of sheep and springbok, farm management and husbandry practices and environmental factors that are necessary for the survival of *Brucella* bacteria. Interviews were carried out before the start of sera collection to ensure that only farms where sheep and springbok come into close contact are sampled and to acquire information about the population at risk before sampling. Farm owners or their responsible representatives were interviewed. The questionnaire comprised five sections, A to E as outlined below.

- Section A was used to gather information relating to the farm owner, location of the farm, farm size and the general management of the farm.

- Section B was meant to collect information relating to the size of the population at risk, the different species of animals on the farms, *Brucella* vaccination status and the history of the farm with respect to brucellosis.

- Section C was meant to find out if any positive cases of brucellosis had been reported on the farm and if so, which *Brucella* species was implicated and what actions were taken with regards to the farm and the animals.

- Section D was meant to investigate if environmental factors for the survival and spread of brucellosis existed on the farms by gathering information about the lambing areas, lambing season, cleaning and disinfection of lambing areas as well as the disposal of aborted materials.

- Section E was meant to assess the potential risk to humans by finding out which animal products are consumed on the farm and marketed outside of the farm.

3.5.2 Sampling of sheep and springbok on commercial farms

Seventeen farms approved and registered for springbok culling for the purposes of exporting meat through the export abattoir in 2009 were grouped according to magisterial district. A total of eleven farms were then selected by simple random sampling from each district and serum samples were collected from sheep and springbok using simple random sampling (Thrusfield 1995). Sampling for brucellosis in sheep was done in accordance with the instructions issued by the Directorate of Veterinary Services in Namibia (DVS 2009). It was confirmed before the study commenced through an interviewer administered questionnaire, that sheep and springbok on these farms were reared in close contact and that none of the sheep were vaccinated against *B. melitensis* or *B.ovis*. The number of sheep and springbok sera that were taken from the eleven commercial farms between April and August 2009 are shown in Table 3.2.

Table 3.2 Number of sheep and springbok serologically tested on the eleven commercial farms

Farm	Sheep sampled	Springbok sampled
1	30	28
2	31	31
3	30	35
4	30	32
5	31	30
6	30	31
7	30	30
8	30	29
9	30	34
10	30	32
11	30	33
Total	**332**	**345**

3.6 Serological study in sheep in the communal areas

Sheep in the communal areas are unlikely to come into close contact with springbok. A serological study was therefore undertaken in two randomly selected communal areas of Berseba and Tses (see Plate 3.1) to estimate brucellosis prevalence and compare prevalence between sheep in commercial and communal areas and between sheep reared with springbok and sheep that are not reared with springbok. From May to July 2011, sera (n=664) were taken from sexually mature male and female sheep at 11 randomly selected water points (see Table 3.3) using simple random sampling.

Table 3.3 Number of sheep sampled in Berseba and Tses communal areas

Communal area	Number of water points sampled	Number of owners sampled	Number of sheep sampled	Ewes	Rams
Berseba	5	12	360	338	22
Tses	6	10	304	294	10
Total	11	22	664	632	32

3.7 Serological study in ewes at the regional abattoir

To confirm the presence or absence of brucellosis on the farms of origin and to find out the possibility of occupational exposure to human brucellosis at the abattoir, sera (n=2302) were taken from culled ewes (sub-population most likely to be positive for *B. melitensis* or *B. abortus*) from 40 farms between July 2010 and April 2011. Simple random sampling was used because the number of ewes slaughtered per day could be determined well in advance of slaughter. The number of samples taken at the abattoir per district is shown in Table 3.4.

Table 3.4 Number of ewes sampled at the abattoir by district

District	Number of farms sampled	Total number of ewes sampled
Keetmanshoop	25	1452
Karasburg	11	618
Bethanie	4	232
Totals	40	2302

3.8 Methods used to collect and transport sera

The methods used to collect and dispatch serum samples are described for sheep and springbok in detail below.

3.8.1 Collection of sera from sheep

Each sheep sampled had 10ml taken from the jugular vein using individual sterile 20G needles and sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). All animals were humanely restrained. At the abattoir, blood was taken after the jugular veins and carotid arteries were severed, using simple random sampling by taking blood from selected batches of culled ewes.

The collected blood was identified with respect to the farm and animal species, date of sampling and given an individual identification number to prevent the mixing of samples from different animals and farms. It was then placed in identified metal containers and packed in such as way as to prevent damage and leakage during transport to the regional laboratory. Ice packs were added to transport containers to preserve the samples during transportation from the place of collection to the regional laboratory. At the regional laboratory, sera were separated from the clotted blood and placed in sterile serum tubes. Cooled sera were dispatched in refrigerated containers (4^{o}C) to the Central Veterinary Laboratory in Windhoek for serological testing.

3.8.2 Collection of sera from springbok

Blood samples were collected from the jugular vein immediately after shooting using individual sterile plain vacuum tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). Blood sampling was done only from springbok populations that were known to come into contact with sheep. The blood tubes were identified with respect to the farm and animal species, date of sampling and given an individual identification number to prevent the mixing of samples from different animals and farms. The tubes

were then placed in identified metal containers and packed in such as way as to prevent damage and leakage during transport to the regional laboratory. Ice packs were added to transport containers to preserve the samples during transportation from the place of collection to the regional laboratory. At the regional laboratory, springbok blood was handled as described for sheep blood.

3.9 Serological testing of sheep and springbok sera

Testing for *Brucella* antibodies (*B. melitensis or B. abortus)* was done using the Rose Bengal Test (RBT) as a screening test and confirmation of all samples was done with the Complement Fixation Test (CFT) as described in the OIE Manual (2004). The Rose Bengal Test (RBT) is the internationally recognized screening test for brucellosis in sheep and goats (Garin-Bastuji & Blasco 1997; FAO 2010), whilst the Complement Fixation Test (CFT) is widely used for serological confirmation of brucellosis in livestock (SANCO 2001; FAO 2003). The RBT has a specificity of between 71%-80% and a sensitivity of between 78-100% (Bercovich 1998; Diaz-Aparicio *et al*. 1994), and the CFT has a specificity of 98% and a sensitivity of 81% (Bercovich 1998). The antigenic suspensions used in the detection of *Brucella* antibodies (*B. abortus* and *B, melitensis*) in the RBT and CFT tests were obtained from *B. abortus* strain 99 as prescribed for by the OIE Manual (2004).

Other serological tests such as the competitive ELISA have not yet been standardized for use in small ruminants at the Central Veterinary Laboratory, Namibia and were

therefore not used. The Polymerase Chain Reaction (PCR) test is an expensive test to apply to a lot of samples; hence the test was not used in this study.

3.9.1 Rose Bengal test (RBT)

The Rose Bengal test was performed as described by the OIE Manual (2004). Equal volumes (25µl) of antigen suspension and test serum were thoroughly mixed in haemagglutination plate wells. The mixture was gently agitated at room temperature on a rocker for 4 minutes and results read thereafter. Any visible agglutination was considered as test positive.

3.9.2 Complement Fixation test (CFT)

Preparation of the diluent (Veronal buffer/CFT buffer), sensitized sheep red blood cells (SRBC), approved smooth *Brucella* antigen, complement and sera was done according to the OIE Manual (2004). Diluted test sera and working standards were placed in small tubes and incubated at 56^{o}C for 30 minutes to inactivate the native complement. The CFT test was carried out in standard microtitre plates with round bottoms.

Volumes of 25µl of diluted inactivated serum were added to the wells in the first, second and third rows. The first row was designated as an anti-complimentary control for each test serum. Diluent (25µl) was placed in the first row wells to make up for the lack of antigen. Another 25µl of diluent was added to all wells, but those in the second row. Serial two-fold dilutions were carried out and 25µl discarded at the end. Volumes of 25µl

of *Brucella* antigen were added to all wells, except those of the first row. Complement (25µl) was then added to each well. Control wells with diluent only, complement and diluent, complement, diluent and antigen were set up. The plates were incubated at 37°C for 30 minutes, 25µl of sensitized SRBC added to all wells including control wells and reincubated at 37°C for 30 minutes after thorough mixing by agitation. Results were read after the plates were left to stand for one hour to allow unlysed cells to settle. Titres of 1:8 and above were recorded as positive based on the presence or absence of haemolysis.

3.10 Data analyses

Data from the study was stored and processed in Microsoft Excel®[(a)] (Microsoft Corporation, 2007). Brucellosis prevalence estimates were calculated per farm, district and region. Overall brucellosis prevalence for the study area was calculated per species using the overall number of positive animals and the overall number of sampled animals in the study area. To account for the clustering effect of sampling at the abattoir and on farms, the 95% confidence intervals around the mean prevalence were adjusted according to Reiczigel *et al*. (2010). Fisher's Exact Test [(b)] was used to calculate the *p*-value and test the significance of differences in brucellosis prevalence between various groups.

[(a)] Microsoft Corporation (2007, Microsoft, Redmond, Washington: USA

[(b)] http://aoki2.si.gunma-u.ac.jp/exact/fisher/fisher.cgi

CHAPTER 4

RESULTS

4.1 Introduction

This chapter presents the results of the study and statistical analysis in tabular and graphical formats.

4.2 Results for *Brucella* serology in sheep between 2008 and 2010

4.2.1 *Brucella* testing results for sheep in the Karas Region in 2008

Results of brucellosis testing in sheep in the Karas Region for 2008 are shown in Table 4.1. A total of 6719 sera from 220 farms were serologically tested for brucellosis in 2008. The overall prevalence of brucellosis was 0.19% (95% CI: 0.11-0.33) and the prevalence of positive farms in the region was 1.82% (95% CI: 0.62-4.58). No positive sera were detected in the Bethanie district in 2008. In the Karasburg and Keetmanshoop districts, prevalence estimates of 0.07% (95% CI: 0.02-0.24) and 0.32% (95% CI: 0.18-0.57) were recorded respectively. On positive farms, the prevalence was 8.23% (95% CI: 4.47%-13.42%).

Table 4.1 Results for sheep sera collected in the Karas Region in 2008

District	Number of farms tested	Total sera tested	Number of positive farms	Number of positive sera	% positive farms	% positive sera
Bethanie	8	241	0	0	0	0
Karasburg	100	3045	1	2	1 (0.05-5.14)	0.07 (0.02-0.24)
Keetmanshoop	112	3433	3	11	2.68 (0.73-7.29)	0.32 (0.18-0.57)
Total	220	6719	4	13	1.82 (0.62-4.58)	0.19 (0.11-0.33)

Values in brackets are 95% confidence limits (Reiczigel, Földi & Ózsvari 2010)

4.2.2 *Brucella* testing results for sheep in the Karas Region in 2009

Table 4.2 shows the results of *Brucella* antibody testing in 2009. A total of 8078 sera from 266 farms were tested. Overall prevalence of brucellosis in the Karas Region was 0.05% (95% CI: 0.02-0.13). The prevalence of positive farms in the region was 0.75% (95% CI: 0.13-2.66). In Keetmanshoop and Bethanie districts, prevalence was found to be 0.02% (95% CI: 0%-0.11%) and 0.21% (95% CI: 0.07-0.61) respectively. No positive reactors were identified in the Karasburg district. The Bethanie district had the highest prevalence of *Brucella* positive sheep. On positive farms, the prevalence was 2.25% (95% CI: 0.77%-5.46%).

4.2.3 *Brucella* testing results for sheep in the Karas Region in 2010

Table 4.3 shows the brucellosis testing results for 2010. A total of 8197 sera from 276 farms were tested in 2010. The prevalence of brucellosis in the Karas Region was 0.18% (95% CI: 0.11-0.30) and the prevalence of positive farms was 0.72% (95% CI: 0.12-2.56). In Keetmanshoop and Karasburg districts, the prevalence was 0.05 % (95%

CI: 0.01-0.17) and 0.49% (95% CI: 0.28-0.83) respectively. No positive reactors were detected in the Bethanie district. The Karasburg district had the highest prevalence of positive reactors. On positive farms, the prevalence was 30% (95% CI: 18.49%-43.90%).

Table 4.2 Results for sheep sera collected in the Karas Region in 2009

District	Number of farms tested	Total sera tested	Number of farms positive	Number of sera positive	% positive farms	% positive sera
Bethanie	48	1445	1	3	2.08 (0.10-10.74)	0.21 (0.07-0.61)
Karasburg	44	1343	0	0	0	0
Keetmanshoop	174	5290	1	1	0.57 (0.02-2.95)	0.02 (0.00-0.11)
Totals	266	8078	2	4	0.75 (0.13-2.66)	0.05 (0.02-0.13)

Values in brackets are 95% confidence intervals (Reiczigel, Földi & Ózsvari 2010)

Table 4.3 Results for sheep sera collected in the Karas Region in 2010

District	Number of farms tested	Total sera tested	Number of farms positive	Number of sera positive	% positive farms	% positive sheep
Bethanie	40	1139	0	0	0	0
Karasburg	76	2672	1	13	1.32 (0.06-6.77)	0.49 (0.28-0.83)
Keetmanshoop	160	4386	1	2	1.25 (0.22-4.42)	0.05 (0.01-0.17)
Totals	276	8197	2	15	0.72 (0.12-2.56)	0.18 (0.11-0.30)

Values in brackets are 95% confidence limits (Reiczigel, Földi & Ózsvari 2010)

Table 4.4 A summary of brucellosis prevalence (%) in 2008, 2009 and 2010

Year	Keetmanshoop	Karasburg	Bethanie	Overall Prevalence
2008	0.32 (0)	0.07 (0)	0 (0)	0.19 (0)
2009	0.02 (0)	0 (0)	0.21 (0)	0.05 (0)
2010	0.05 (0)	0.49 (0)	0 (0)	0.18 (0)

Values in brackets represent true prevalence calculated according to Reiczigel, Földi & Ózsvari (2010)

4.2.4 Summary of brucellosis testing results for 2008, 2009 and 2010

A summary of brucellosis prevalence for 2008, 2009 and 2010 is given in Table 4.4. The prevalence of *Brucella* antibodies at district level was between 0% and 0.49%. The annual prevalence in the region was between 0.05% and 0.19%. The year 2009 had the lowest brucellosis prevalence compared to 2008 and 2010. In the Bethanie district, no sera tested positive for *Brucella* antibodies in 2008 and 2010. The overall brucellosis prevalence over the three years was 0.14% (32/22994).

True prevalence was calculated from apparent prevalence according to Reiczigel, Földi & Ózsvari (2010) using CFT test sensitivity and specificity of 81% and 98% (Bercovich, 1998) respectively. Using this method, the prevalence of brucellosis was found to be zero in the region in 2008, 2009 and 2010.

4.2.5 Brucellosis prevalence trend between 2008 and 2010

Figure 4.1 shows the trend of brucellosis apparent prevalence in the districts of the Karas region. The prevalence of brucellosis showed a decreasing trend in the

Keetmanshoop and Bethanie districts, but an increasing trend in the Karasburg district, but remained below 0.5% in all districts.

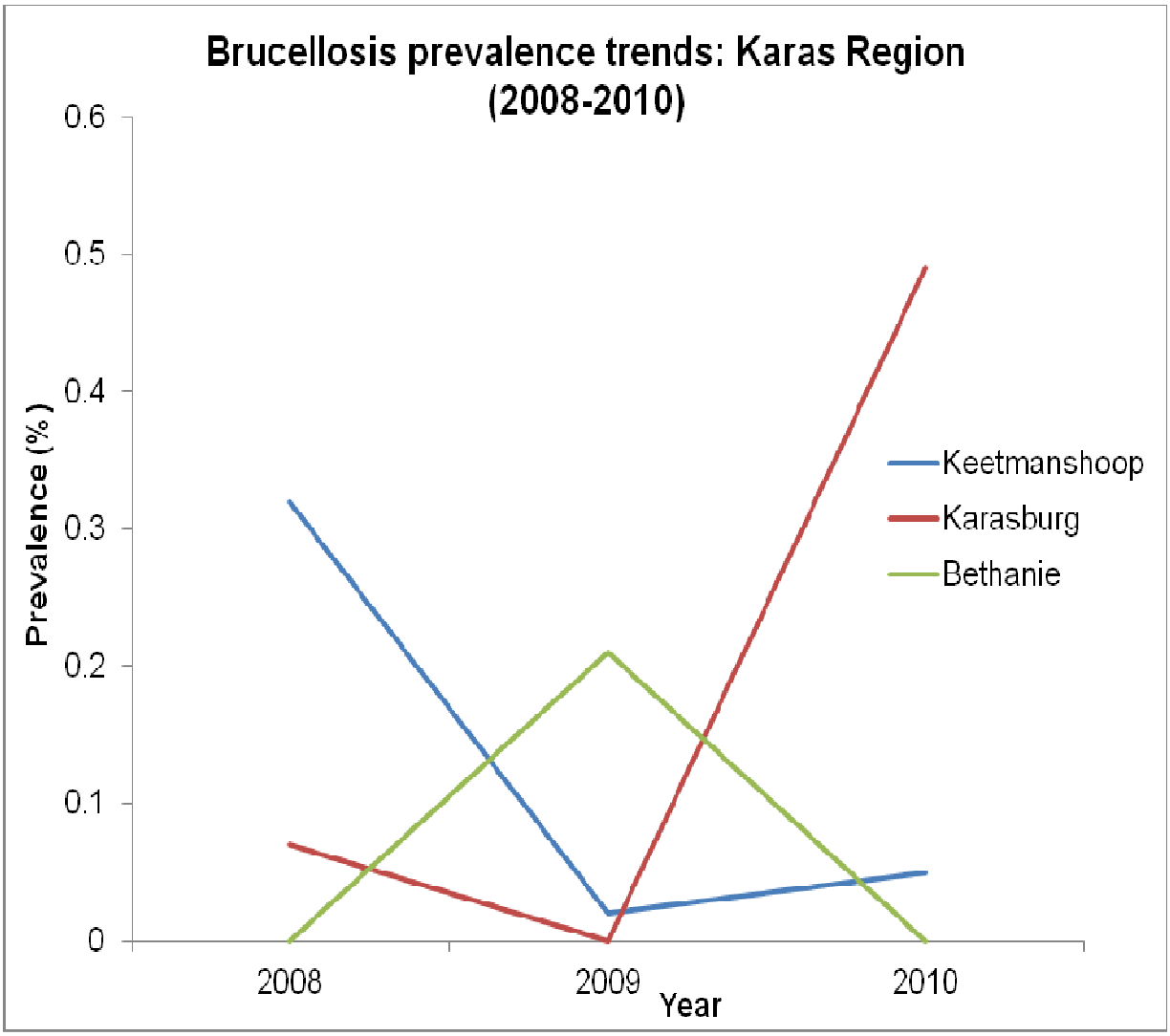

Figure 4.1 The trend of brucellosis prevalence between 2008 and 2010

4.2.6 Serology results of the eight previously positive farms

Eight farms tested positive for *Brucella* antibodies between 2008 and 2010. Five of these farms were in Keetmanshoop; two in Karasburg and one in the Bethanie district.

Follow up sera collected from sheep (n= 472) and springbok (n=9) on these farms tested negative for *Brucella* antibodies.

4.2.7 Statistical analysis of retrospective study results

Analysis of brucellosis prevalence data using Fisher's exact test showed that the differences in prevalence between districts in 2008 were not statistically significant ($p=0.063$), but the differences in prevalence between districts in 2009 ($p=0.041$) and 2010 ($p=0.0001$) were statistically significant at $p<0.05$. Statistical analyses also revealed that there were statistically significant differences ($p=0.038$) between annual brucellosis prevalence for the three years at $p<0.05$.

4.3 Results of questionnaire interviews

4.3.1 Population structure

The number and proportion of sheep, goats, cattle, springbok and other game species reared on the eleven commercial farms is shown in Table 4.5 and in Figure 4.2 respectively. Sheep and springbok were the main species reared on the farms representing 69% and 21% of the total animal population respectively. The average size of the sheep breeding population (ewes and rams) was 1231 ± 802 (range: 248-2634) sheep per farm. The dorper breed was the most common breed of sheep on all the farms. The mean size of the springbok herd per farm was 370 ± 269 (40-1000). The ratio of springbok to sheep was 1:2 on 55% (n=6) of the farms and higher on 45% (n=5) of the farms. Goats and cattle were present on 55% (n=6) and 64% (n=7) of the farms

respectively in fewer numbers (26-313). Oryx were recorded on five farms and blesbok one farm (number 9). The farm sizes ranged from 4812 ha to 20600 ha.

Table 4.5 Number of domestic and game species on the 11 commercial farms

Farm Number	Farm size (ha)	Sheep		Goats		Cattle		Springbok	Other
		ewes	rams	does	bucks	cows	bulls		oryx
1	9000	900	70	0	0	0	0	300	0
2	10324	2604	30	128	12	104	2	650	0
3	4812	890	15	25	2	25	1	435	0
4	6543	754	10	0	0	0	0	345	0
5	20600	2300	70	0	0	70	2	1000	60
6	7493	500	6	300	7	0	0	40	9
7	13347	643	20	0	0	76	3	300	70
8	12831	240	8	0	0	0	0	150	0
9	10365	1880	40	80	10	101	2	400	60
10	9561	1700	70	250	4	61	2	100	23
11	5237	740	48	281	32	52	22	350	0
Totals	110113	13151	387	1064	67	489	34	4070	222

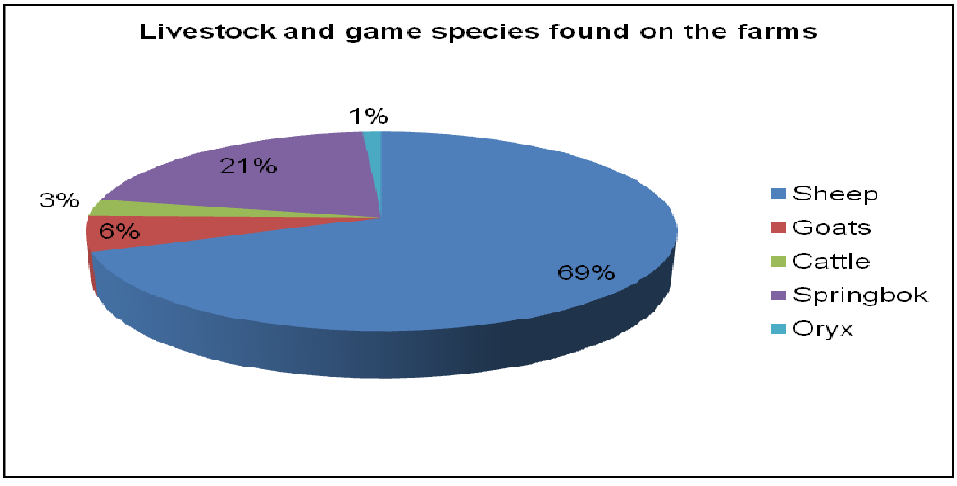

Figure 4.2 The proportion of livestock and game species on the eleven farms

4.3.2 Management of sheep and springbok

Sheep and springbok were managed extensively on natural pastures on all the farms. On nine farms, there were no designated paddocks or camps for keeping springbok. However, two farms had designated camps for springbok which are separate from sheep camps. On all the farms, sheep and springbok were observed close to each other at watering points. In addition, three farms observed that sheep and springbok also came into close proximity in certain camps, but they could not provide reasons for such an occurrence. Results of the questionnaire interviews also showed that close interaction happened throughout the year on eight farms; during the summer months (October-April) on two farms and after the first summer rains on one farm. Supplementary rock salt was provided to sheep on seven farms. The remainder of the farms did not provide supplementary feed during the dry winter season.

4.3.3 Vaccination status

All eleven farms did not vaccinate sheep against brucellosis (due to *B. melitensis* or *B. ovis*). Three farmers reported that they had vaccinated heifers between 4 and 8 months once against *B. abortus* between 2008 and 2009 using the *B. abortus* S19 vaccine. No cases of previous abortions, stillbirths, infertility or poor reproductive performance, aborted fetuses and lambs mortalities were reported by the farmers during the interviews. All farms had no history of positive serological results for brucellosis as confirmed during interviews and by records at the local State Veterinary Office.

4.3.4 Environmental management

Lambing occurred throughout the year in all camps with no designated lambing camps. The cleaning and disinfection of lambing paddocks was not practiced on all the farms. No farmer had seen aborted fetuses or dead lambs on the farm in the recent past. Borehole water was the primary source of drinking water for sheep and springbok, although springbok had access to water in salt pans (n=11), dams (n=2) and natural water collections during the rainy season.

4.3.5 Marketing of sheep and springbok from the farms

All eleven farmers investigated traded live sheep via auctions and through direct exports. Sheep and springbok meat was consumed on the farms and also marketed through export and local abattoirs. On 27% (n=3) of the farms, sheep milk was consumed raw or used for making tea. Four farms sourced replacement rams from other farms, without first confirming their brucellosis status. No springbok purchases from other farms had occurred in recent times on all the farms.

4.4 Serology results

4.4.1 Results for sera collected from sheep and springbok reared together

Serological testing results for sheep sera are shown in Table 4.6. *Brucella* antibodies (*B. melitensis* and *B. abortus*) were not detected in sheep on the 11 farms. However, on one farm, 10% (95% CI: 2.78%-26%) of the sampled sheep tested positive for *Brucella ovis* antibodies. *Brucella* antibodies *(B. melitensis, B. abortus* and *Brucella ovis)* were

not detected in 345 sera collected from a total population of 4070 springbok reared with sheep on the eleven commercial farms.

Table 4.6 Serology results for sheep on the11 commercial farms

Farm number	**Sheep population**	**Number tested**	**Number positive for:** *B. melitensis/B. abortus* antibodies	*B. ovis antibodies*	**% positive for *B. ovis***
1	970	30	0	0	0
2	2634	31	0	0	0
3	905	30	0	0	0
4	764	30	0	0	0
5	2370	31	0	0	0
6	506	30	0	0	0
7	663	30	0	0	0
8	248	30	0	0	0
9	1920	30	0	0	0
10	1770	30	0	0	0
11	788	30	0	3	10% (2.78-26.0)*
Totals	12386	332	0	3	

*95% confidence intervals (Reiczigel, Földi & Ózsvari 2010)

4.4.2 Results for sheep in the communal areas

A total of 664 sheep sera collected from Tses and Berseba communal areas tested negative for *Brucella* antibodies.

4.4.3 Results for sera collected from ewes at the abattoir

Serum samples (n=2302) collected from old culled ewes from 40 commercial farms at the abattoir tested negative to *Brucella* antibodies (*B. melitensis* and *B. abortus*). No positive farms were therefore identified for follow up sampling in springbok.

CHAPTER 5

DISCUSSION

5.1 Introduction

This study estimated the prevalence of brucellosis on commercial (sheep and springbok) and communal farms (sheep) and confirmed the absence of *Brucella* antibodies in ewes at the abattoir. These results are discussed in relation to similar studies in Southern Africa.

5.2 Retrospective data analysis

The estimated sheep brucellosis prevalence over the three years was 0.14% (95% CI: 0.1%-0.2%). McDermott and Arimi (2002) have reported the prevalence of brucellosis in Southern Africa as between 5.6% and 14.5%. In the KwaZulu-Natal province of South Africa, brucellosis prevalence in sheep was found to be between 1.23% and 4.02% (Emslie & Nel 2002). The prevalence in this study was lower than in other studies in Southern Africa. This may be explained by the differences in climatic conditions and by the fact that the data used in this study was from voluntary sampling. The Karas Region is characterized by very hot and dry climatic conditions which are not favorable for the survival and transmission of *Brucella* species bacteria (SANCO 2001). Furthermore, the extensive management of sheep on natural pastures and the fact that there was no mixing of flocks from different farms may have reduced the infection rate (Hesterberg 2008). According to McDermott & Arimi (2002), sheep flocks that are restricted on farms have lower brucellosis prevalence than mobile pastoral flocks.

The prevalence of brucellosis in 2008, 2009 and 2010 was 0.19% (95% CI: 0.11%-0.33%), 0.05% (95% CI: 0.02%-0.13%) and 0.18% (95% CI: 0.11%-0.30%) respectively. The prevalence recorded in 2009 (0.05%) was lower than the prevalence in 2008 and 2010, although the number of sera and farms tested were comparable to the other years. It is presumed that the preventive and control measures that were implemented country wide in 2009 by the Directorate of Veterinary Services following an outbreak of brucellosis on a farm in the adjacent Hardap Region, played a big part in reducing brucellosis prevalence. When a sheep flock tests positive for brucellosis, the farm is placed under quarantine and all sheep above six months of age are serologically tested for *Brucella* antibodies. All sheep that test positive on the CFT test are eliminated. Quarantine restrictions on the remaining sheep are only removed after two consecutive negative CFT serological results at least three months apart (DVS 2009). Kolar (1984) and Alton (1987) recommend the use of two consecutive negative serological results six months apart to confirm the absence of *Brucella* infections in a herd.

At district level, brucellosis prevalence varied between 0% and 0.49%. The prevalence of brucellosis found in this study in all districts was lower than the prevalence of between 0.9% and 20% reported at district level in Namibia (Depner 1993). The differences can be attributed to the fact that the data used in the current study was drawn from commercial farms only, whilst the study by Depner (1993) encompassed commercial and communal farming systems. Although Depner's study was based on a smaller sample size (n=1517), the higher prevalence reported can be ascribed to the

higher sensitivity and specificity of the competitive enzyme immunoassay that was used for serology.

Although the prevalence of positive farms was low (0.72% - 1.82%), brucellosis prevalence on such farms was relatively high (2.25% to 30%). These findings show that positive reactors were concentrated on a few farms. Therefore, if control measures were to be focused on these farms, it should be possible in the short term to reduce brucellosis prevalence significantly and eventually eradicate brucellosis from the region. Follow up investigations on positive farms also revealed that these farms had a history of introducing sheep from other flocks and countries. The introduction of new sheep without implementing biosecurity measures is known to be a major risk factor for introducing brucellosis in clean flocks (SANCO 2001; McDermott & Arimi 2002).

Analysis of brucellosis prevalence trends at district level revealed that the Karasburg district had a rising trend of brucellosis prevalence compared to other districts. Therefore, control programs for brucellosis in the Karas Region must focus more on this district in order to curb the rising prevalence. The decreasing trend in other districts and the low brucellosis prevalence recorded in this study reflect the effectiveness of control and prevention measures implemented by the Directorate of Veterinary Services. The Bethanie district had no positive reactors in 2008 and 2010. However, this apparent low prevalence must be interpreted in light of the small number of sheep sera tested in Bethanie in 2008 (n=241) and 2010 (1139) compared to other districts.

The low prevalence of brucellosis recorded in the retrospective study indicates that brucellosis is present in the region and confirms the effectiveness of brucellosis control programs implemented in the region. When the prevalence of brucellosis is less than 2%, as in the current study, the test-and-slaughter approach is recommended for the control of brucellosis (Nicoletti 1993; FAO 2003). This is the approach currently used for sheep brucellosis in Namibia. In addition to the test and slaughter approach, farmers on *Brucella* positive farms are encouraged to implement measures that prevent the transmission of brucellosis such as keeping a closed flock and purchasing replacement sheep from brucellosis-free flocks (DVS 2009).

True prevalence was found to be zero in all districts over the three years. The apparent low brucellosis prevalence recorded between 2008 and 2010 could therefore be attributed to false positive results because only a limited number of sera (32/22994) and farms (8/762) tested positive for *Brucella* antibodies or to cross reactions with other organisms such *Yersinia enterocolitica* O: 9 (OIE 2004; Nielsen *et al*. 2006; OIE 2008). However, the low prevalence cannot be attributed to vaccine induced antibodies because it was confirmed through telephonic interviews with the farm owners and through the checking of farm records at the regional State Veterinary Office that the farms which tested serologically positive over the study period had no history of vaccinating sheep against brucellosis. Although true prevalence was recorded as zero, some of the positive serological reactions that were recorded over the three years were from clinical cases which were confirmed by the Polymerase Chain Reaction (PCR) test, thus confirming the presence of *Brucella* infections in the region.

The major limitation of the retrospective study was that the data was drawn from a voluntary testing program. Interpretation of the results must therefore be made with caution. However, the sample size was considerably large enough (n = 22994) to provide a representation of the brucellosis situation in the Karas Region.

5.3 Brucellosis on eight exposed farms identified by the retrospective study

Serum samples collected from sheep and springbok on the eight positive (exposed) farms identified by the retrospective study, tested negative for *Brucella* antibodies, confirming the absence of brucellosis on these farms and the effectiveness of brucellosis control measures implemented on these farms following positive serological results. It was only possible to collect nine springbok sera from the eight farms because the farmers were not willing to cull more springbok during 2011. Although the nine sera tested negative for *Brucella* antibodies, the sample size was deemed too small to make inferences about the brucellosis status of springbok herds reared with brucellosis exposed sheep.

5.4 Brucellosis prevalence on eleven sheep and springbok farms

5.4.1 Questionnaire interviews

Results of questionnaire interviews showed that the study farms were large and managed extensively because of the limited grazing. Sheep (dorper breed) and springbok were the predominant animal species on the farms, because they are well adapted to the semi-arid conditions on the farms (Estes 1992). Although Oryx (*Oryx gazelle*) and blesbok (*Damaliscus lunatus*) were reported by farmers as the only other

game species on the farms, it is well known that kudu (*Tragelaphus strepsiceros*) were present in small numbers on the farms. There were small numbers of cattle and goats on the farms because grazing and browse were too little to support these species. As expected, the stocking densities on all the farms were low because of limited grazing and browse.

Sheep and springbok were allowed to move in all camps on the farms with no separation of the two species, although two farms stated that they had designated camps for springbok. The observation of sheep and springbok in close proximity at watering points can be explained by the hot dry weather conditions and the presence of few and unevenly distributed watering points on the farms, which compelled springbok to drink water at sheep watering points. Contact between wild and domestic ruminants at watering points and in areas with good foraging resources has been documented and implicated as the point at which cross-infection of diseases may occur (Jiwa *et al*. 1996; Reviriego, Moreno & Dominguez 2000). Wild animals generally stay away from domestic animals spatially and temporally unless habituated (Bengis, Kock & Fischer 2002). On 72.7% (n=8) of the farms, it was observed that sheep and springbok came into close proximity throughout the year, which suggests that the springbok were habituated to sheep or that good grazing was concentrated on certain parts of the farm. The competition for grazing and water resources may explain the observation of sheep and springbok in close proximity during the dry summer months. On one farm, springbok were seen grazing close to sheep especially after the first summer rains. This observation is not surprising because the early summer rains are associated with a

flush of green grass in certain areas of farms, which brings grazing animals into close proximity.

The degree of close contact between sheep and springbok is influenced by available grazing and browse (Bengis, Kock & Fischer 2002); frequency of hunting activities and the distribution of watering points on the farm. Sheep are grazers, while springbok are mixed feeders. Therefore, the probability of sheep and springbok coming into close proximity of each other as a result of competition for grazing resources was high, because most of the farms had more grazing than browse. The researchers did not have the opportunity to observe sheep and springbok grazing next to each other because the times of the visits coincided with the time when springbok had been disturbed by hunting activities.

The fact that the study farms did not vaccinate sheep against brucellosis using *B. melitensis* Rev. 1 is evidence that the farmers did not view the disease as important in their flocks; that the flocks were naïve and that any positive serological results would not have been due to vaccine induced antibodies. In Namibia, farmers are not obliged by law to vaccinate sheep against brucellosis. It was surprising to find that all the eleven farmers had not seen any symptoms suggestive of brucellosis such as abortions, weak lambs and poor reproductive performance in their flocks. This finding may reflect a lack of knowledge about the disease. Although information from the literature suggests that abortions may stop in persistently infected flocks (Alton 1990), it is presumed that the size of the farms, lack of proper record keeping and the role of jackals in removing

aborted fetuses may have influenced these findings. Examination of records from the local State Veterinary Office confirmed that the farms did not have a history of positive serological and clinical cases of brucellosis.

Lambing and kidding in sheep and springbok occurred throughout the year potentially contaminating all camps and providing opportunities for the transmission of brucellosis in either direction. However, the absence of designated lambing areas may have helped in reducing the risk of transmission of brucellosis within the flock (WHO 2006). In springbok, lambing occurred in early spring, at about the time close contact was reported to occur between sheep and springbok on some farms, thus increasing the risk of disease transmission. Environmental conditions in the study area were hot and dry and not favorable for the transmission of brucellosis. The cleaning and disinfection of lambing areas was considered by farmers as unpractical and unnecessary under these conditions. Sheep were not penned at night, thus removing the possibility of overcrowding, which is a risk factor for the spread of brucellosis within flocks (Alton 1990; Anonymous 1996; McDermott & Arimi 2002). Drinking water was drawn from underground (borehole) water and was considered unlikely to pose a direct risk for the transmission of brucellosis unless contaminated at the surface.

The marketing of live sheep, sheep and springbok meat from Namibia without prior brucellosis testing has the potential of disseminating brucellosis to other areas if the farm of origin has the infection. In Namibia, imported sheep are required to be tested for brucellosis before they are introduced into the flock. Rams from other farms were

introduced onto four flocks with no prior testing to ascertain brucellosis status, thus risking the introduction of brucellosis. Farm workers on three farms drank raw sheep milk. There was therefore a risk of human brucellosis on these farms as raw unpasteurized milk is a major source of human brucellosis (FAO 2010). Public health education campaigns are required to advocate for the boiling of raw milk before consumption and to prevent occupational exposure. No springbok purchases onto farms had been recorded in the past 20 years – it was therefore unlikely that brucellosis could have been brought onto the farms through these species.

Questionnaire interviews confirmed that sheep were naïve and that they grazed within close proximity of springbok. It was therefore possible for *Brucella* infections to be transmitted between the two species.

5.4.2 Brucellosis prevalence in sheep and springbok

The serological study on the eleven sheep and springbok farms yielded no positive reactors, although sampling was done from sexually mature animals which have a higher risk of carrying permanent *Brucella* infections (FAO 2006; CFSPH 2007). These results are consistent with the results of the questionnaire interviews which showed that the farms had no history of serologically and clinically positive cases of brucellosis and no reports of abortions, infertility and poor reproductive performance. The extensive management of sheep and springbok in hot and dry conditions prevailing on the farms may have played a part in reducing the survival and transmission of *Brucella* bacteria on pasture. *Brucella* bacteria do not survive for long periods in hot dry weather (SANCO

2001). Jackals and other carnivores which are prevalent on farms, feed on aborted fetuses and fetal membranes reducing the possibility of infectious material coming into contact with the next susceptible animal. According to Godfroid (2002), the establishment and sustainability of brucellosis in a species depends on host susceptibility, infectious dose, contact with infected animals, management and environmental factors (Godfroid 2002). In the present study, the animals were naïve, but environmental factors were not ideal for the transmission and sustainability of *Brucella* infections. The absence of *Brucella* (*B. melitensis, B. abortus*) antibodies in springbok is in agreement with results of a previous study by Karesh *et al*. (1997) in impala in Namibia in which no positive reactors were found. In fact, there is no record of springbok testing positive for *Brucella* antibodies in the literature. Although cases of other wild ruminant species testing positive for *Brucella* antibodies have been documented in Namibia (Depner 1993; Karesh *et al*. 1997) and in Southern Africa (Paling *et al*. 1988; Thorne 2001; Godfroid 2002; McDermott & Arimi 2002; Muma *et al*. 2007), it is widely acknowledged that the transmission of brucellosis between wild and domestic ruminants is not easy even after prolonged direct and indirect contact (Ferroglio *et al*. 2007). Although, the cross-infection of brucellosis from domestic ruminants to wild ruminants has been proven, the sustainability of such infections and the role of wild ruminants as reservoirs has not been confirmed.

The disease interface between wild ruminants and domestic livestock is not commonly a direct physical interaction or sharing of the same space at the same time. It is usually indirect through contaminated soil, forage, and water with which another animal has

recently been in contact and has left contaminated bodily discharges, such as faeces, urine, saliva, or ocular or nasal discharge (Fenner 1982). Information from previous studies by Paling *et al* (1988); SANCO (2001); Thorne (2001); Godfroid (2002); Muma *et al*. (2007) suggests that if brucellosis was present in sheep, it would have been detected in sympatric springbok. The role of springbok in the epidemiology of sheep brucellosis in the present study could not be inferred because of the negative results that were recorded.

B. ovis, a common cause of ram epidydimitis, orchitis and infertility in Southern Africa (Blasco *et al.* 2004), was not detected in springbok. These results are consistent with the fact that *B. ovis* has not been reported in wild ruminants in Southern Africa (Blasco *et al*. 2004), although it has been reported in farmed red deer in New Zealand as producing the typical clinical picture in males (CFSPH 2007). Antibodies against *B. ovis* were detected in three sheep rams on one farm confirming that this agent is endemic in rams in the region (Blasco *et al*. 2004). The prevalence of *B. ovis* antibodies detected in rams was high considering that the rams are not housed, but extensively managed on pasture. Group penning of rams facilitates the transmission of *B. ovis* infections (OIE 2008). Interviews with farmers revealed that they did not vaccinate rams against *B. ovis*. Therefore, the serological reactions observed in this study were due to active or past infections. The detection of *B. ovis* in sheep is of economic rather than public health significance as it does not cause zoonotic disease, but affects the reproductive capacity of the both rams and ewes. Rare cases of abortions and mortalities in lambs associated

with *B. ovis* infections in ewes have been reported (FAO/WHO 1986; Blasco *et al.* 2004).

5.5 Brucellosis prevalence in sheep in the communal areas

A total of 664 sheep sera collected from Tses and Berseba communal areas yielded no positive reactors on the RBT and CFT tests. Sheep in the communal areas are highly mobile; intermingle with and share grazing and watering points with livestock from other flocks and are penned at night – factors which favor the transmission of brucellosis between and within flocks (Alton 1990; Anonymous 1996; SANCO 2001; McDermott & Arimi 2002; WHO 2006). It was expected that *Brucella* antibodies would be detected in sheep in the communal areas because of the existence of risk factors for brucellosis such as the rearing of sheep and goats together. Goats are very susceptible to brucellosis caused by *B. melitensis* (Corbel & Brinley-Morgan 1984). The absence of antibodies in the current study is in agreement with results of Depner's study which found no positive reactors in sheep in unspecified regions of the country. The prevalence of *Brucella* antibodies in sheep in Namibia has been reported as 8.6% (0.9%-20%) (Depner 1993). The findings of this study are similar to the results of a study in sheep in Mozambique (Manhica 2009) where no positive reactors were detected despite the presence of risk factors for brucellosis. The failure to detect positive reactors in communal areas provides evidence that *Brucella* infections are absent in these areas and that abortions occurring in these areas may be due to other agents such as *Chlamydophilla abortus*.

5.6 Absence or presence of brucellosis in ewes at the abattoir

A serological survey to detect the absence or presence of brucellosis that was undertaken at a sheep export abattoir targeting old culled ewes (n=2302) did not yield positive reactors. A similar prevalence investigation at an export abattoir in the adjacent Hardap Region of Namibia in 2009 which was based on a smaller sample size yielded a prevalence of 2.19% (3/137) (Magwedere, Hoffman & van Schalkwyk 2009). Although, the current study was based on culled ewes selected by farmers for slaughter, sampling was done using random methods to remove bias. Culled ewes are sexually mature and thus more susceptible to *B. melitensis* than other age groups of sheep (FAO 2006; CFSPH 2007). Therefore, these results confirm the absence of *Brucella* antibodies (and infections) at the abattoir and on the farms of origin. It can therefore be inferred that the chances of occupational exposure of workers to brucellosis at the abattoir were low and that *Brucella* infections were absent from the 40 farms of origin.

5.7 Serological tests used in the study

The RBT and CFT tests were used in this study. The RBT has a specificity of between 71%-80% and a sensitivity of between 78-100% (Bercovich 1998; Diaz-Aparicio *et al*. 1994), and the CFT has a specificity of 98% and a sensitivity of 81% (Bercovich 1998). The antigenic suspensions used in the detection of *Brucella* antibodies (*B. abortus* and *B, melitensis*) in the RBT and CFT tests were obtained from *B. abortus* strain 99 as prescribed for by the OIE Manual (2004).

The serological tests used in this study are internationally recognized tests (SANCO 2001). However, the RBT has been reported to have a low specificity in low prevalence areas and a low sensitivity in sheep (FAO 2010), which may explain the failure to detect antibodies in this study. The RBT and CFT tests and the antigens used in the tests were directly transposed to springbok sera without validation. Therefore, the interpretation of these results must be made with caution as this may have limited their effectiveness in this species (OIE 2004; Godfroid, Nielsen & Saegerman 2010).

Other serological tests such as the competitive ELISA have not yet been standardized for use in small ruminants at the Central Veterinary Laboratory and were therefore not used. The Polymerase Chain Reaction (PCR) test is a very sensitive but an expensive test to apply to a lot of samples - hence the test was not used in this study. It is recommended that further studies on brucellosis prevalence be carried out using more sensitive and specific tests such as the indirect ELISA so as to confirm the brucellosis status of the region.

5.8 General discussion

The serological study yielded no positive reactors on commercial and communal farms and at the abattoir. However, a retrospective analysis of voluntary brucellosis testing results from 2008 to 2010, revealed an estimated prevalence of 0.14%. The discrepancy between the results of the serological and retrospective studies can be explained by the different sample sizes. The sample size for the retrospective study was

massively larger than that of the prospective study and included suspect clinical cases; hence it was able to pick up antibody reactions in a population of very low prevalence.

The absence of positive reactors in the serological study can also be explained by the fluctuation of antibody titers during lambing, kidding or abortion in infected adult sheep (FAO 2010) and by the self-limiting nature of brucellosis in sheep (Alton 1990). However, serological sampling in this study was done over a nine month period to cater for possible fluctuations. Although fluctuations of titers may occur, *Brucella* infections generally persist for life (Alton 1990).

B. melitensis is the main species infecting sheep in Southern Africa and has been reported in Namibia since 1953 (Godfroid *et al*. 2004). Based on this fact and the fact that *Brucella* species are generally host specific (SANCO 2001; Robinson 2003), it can be deduced that the species infecting sheep in this study was *B. melitensis,* although *B. abortus* has also been reported to cause sheep brucellosis (McDermott & Arimi 2002; FAO 2003). It is recommended that the infecting *Brucella* species be identified in clinical cases, so as to institute appropriate and effective control and prevention measures.

5.9 Public health risk mitigation

The retrospective and prospective studies showed that the prevalence of brucellosis was very low in the region. Therefore, the risk of acquiring human infections through the handling of sheep and springbok meat at the abattoir and in the field in this region is low. Presently, all brucellosis positive sheep are required by law to be slaughtered at

the abattoir only after taking the necessary precautions. However, because brucellosis testing is voluntary, sheep of unknown status are also slaughtered at the abattoir without taking the necessary precautions for protecting abattoir workers from possible infection.

Education and awareness campaigns are required to ensure that raw milk is boiled before consumption, because raw milk is a well known source of human infection (FAO 2010) and to prevent occupational exposure with infected material. These campaigns can be carried out through pamphlets in official languages of the region summarizing the disease, its zoonotic aspects and prevention. Animal health technicians can also disseminate this information during farm inspection visits.

For effective control of human brucellosis, a concerted effort and collaboration between the public health and animal health sectors is required especially with regards to cross-notification of cases, the coordination of joint investigations and public health education programs (WHO 2005).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The hot and dry climate prevailing in the Karas region and the low stocking densities of sheep and springbok were not favorable for the survival and transmission of *Brucella* bacteria between and within sheep and springbok populations. However, the sharing of pastures and water resources between sheep and springbok and the lack of immunity within sheep herds were risk factors for the spread of *Brucella* infections. Retrospective analysis of brucellosis testing results revealed that the prevalence of brucellosis in sheep (0.05%-0.19%) and on positive farms (0.72%-1.82%) was low.

No evidence of *Brucella* infections was detected in sheep and springbok on the eleven commercial farms and in sheep raised in the two communal areas of Tses and Berseba. Serological results of ewes at the abattoir revealed that sheep on the 40 farms of origin and at the abattoir were free of *Brucella* infections. Therefore, occupational handling and exposure to sheep at the abattoir and at the farms of origin is unlikely to pose a risk for human brucellosis. There was insufficient evidence to make conclusions about the role of springbok in the epidemiology of sheep brucellosis because there were no positive reactors in either species. Serological reactions to *B. ovis* confirmed the fact that this agent is endemic in sheep in the region, but absent in springbok.

The low brucellosis prevalence recorded in this study validated the effectiveness of existing sheep brucellosis control measures implemented by the Directorate of Veterinary Services that are based on testing and the elimination of positive sheep. These measures prevented the spread of brucellosis from the Hardap Region to the Karas Region in 2009.

6.2 Recommendations

The current measures for brucellosis control are effective and need to be maintained. However, annual serological surveillance must be based on a statistically calculated number of farms, instead of using the voluntary testing program as the only basis for surveillance. In addition, sample sizes for prevalence studies must take into account the low prevalence of brucellosis in the region. All confirmed cases of brucellosis need to be accompanied by bacterial culture and identification, to ensure there is knowledge of the *Brucella* species affecting the region. Farmers are advised not to vaccinate sheep against brucellosis because the prevalence is very low and *B. melitensis* Rev. 1 vaccine may interfere with serological surveillance (Godfroid *et al*. 2004).

Although the RBT and CFT tests used in this study are internationally recognized tests, it is advised that serological tests with higher sensitivity and specificity such as the indirect ELISA be used in the future, because the prevalence is low in the region. To improve the validity of serological results in springbok, tests for brucellosis must be validated before being used on springbok sera.

Raw sheep milk consumed on some farms is a potential source of human brucellosis. Veterinary personnel need to educate the farmers about the zoonotic aspects, prevention and control of brucellosis and in particular the need to boil raw milk before drinking.

Future studies on the assessment of the risk of acquiring human brucellosis must include serological testing of abattoir workers for *Brucella* antibodies.

CHAPTER 7

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APPENDICES

APPENDIX 1

BRUCELLOSIS SURVEY QUESTIONNAIRE

SECTION A: FARM DETAILS

1. Farm Name	
2. Address	
3. Telephone no:	
4. GPS Reading	
5. Farm Size (ha):	
6. Farming system:	
7. Flock identification number:	

8. Farm management:	Farming system	Extensive	Intensive	Semi-intensive
	Source of feed	Internal	External	
	Other (please specify):			
Separate camps for sheep and springbok?		Yes	No	
Where do sheep and springbok herds meet?		Grazing	Waterholes	
		Other (specify):		

How often do sheep and springbok flocks interact? (please tick)	All the time	Weekly	Monthly	Seasonally
	Other (specify):			

Supplementary feeding in winter? (please tick)		Yes	No
Type of supplementary feed			
Watering points in each camp? (please tick)		Yes	No
Source of replacement animals? (please tick)	Internal		External
Exact source of animals (please tick)	Imports	Auctions	Other farms

Comments:	

SECTION B: HOST FACTORS

	Sheep				Springbok		
1. Number of sheep and springbok on:	Ewes >18 m	Ewes 6-18m	Lambs < 6m	Rams > 6m	Does	Buck	Lambs
2010							
2009							
2008							

2. Breed (s):	Sheep		Goats		Cattle	

3. Total number of:	Cattle	Goats	Gemsbok	Springbok	Wildebeest	Kudu

4. Number vaccinated:	Sheep	Cattle	Goats

5. Which vaccine do you use in (please tick):	Sheep	Rev.1	Cattle	RB51
		None		S19

6. Which age groups do you vaccinate? (Please tick)	Sheep	Rams	Cattle	Cows
		Ewes		Heifers
		Lambs		

7. Do you vaccinate :	Yearly	twice/year	Other (please specify)
Sheep			
cattle			

8. What time of the year do you vaccinate against brucellosis?	Summer	Winter	Other (please specify)
Sheep			
Cattle			

9. Have you observed:	Stillbirths	Abortions	Infertility	Other (please specify)
2008				
2009				
2010				

SECTION C: AGENT FACTORS

1. Have you ever had a positive brucellosis test?	Yes	No	If positive, last date:	

2. If so, which species were detected?	*B. melitensis*	*B. abortus*	*B. ovis*

3. If positive, what happened?	

SECTION D: ENVIRONMENTAL FACTORS

1. Where do sheep lamb? (please specify)	In one camp	All camps	One pen	Other, please specify

2. What time of the year is the lambing season?	Summer	Autumn	Winter	Spring

3. Are lambing areas cleaned?	Yes		No	

4. If cleaned, how often?	After lambing	Weekly	Monthly	Annually

5. How are aborted materials disposed off?	

6. Source of drinking water for sheep?	Tap water	Dam	Underground/borehole

SECTION E: OTHER

1. What animal products are marketed from the farm? (please tick)	Sheep milk	Mutton/lamb
	Goat milk	Goat meat
	Cow milk	Beef

2. Where are animals marketed?	Auctions	
	Abattoirs	
	Live exports	
Other markets (please specify):		

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following:

My promoter, **Professor C M E McCrindle** for her guidance, encouragement and support at all stages of this thesis. Many thanks for believing in me.

The **Chief Veterinary Officer**, Namibia, for authorizing the research.

Dr C Kaseke (State Veterinarian), **Mr A Koberzig** (Chief Animal Health Technician), **Mr B Mieze, Mr M Uuandara** and **Mr A Mbuze** (Animal Health Technicians), **Mr Fredericks**, **Mr Matengu** and **Mrs Windstaan** for their assistance during blood sample collection, sera separation, preservation and dispatch to the Central Veterinary Laboratory.

Mrs G Tjipura-Zaire and all staff in the Serology Section of the Central Veterinary Laboratory in Windhoek, for performing serological tests on the samples.

Ms Segomotso Lebogo for assistance with administrative issues related to this work.

I thank God for the strength, wisdom, knowledge, patience and persistence to see this work through. Glory, honor and praise be unto Him forever and ever.

DEDICATION

To my beloved wife Beatitude, son Tinaye and daughters Runyararo and Ruvimbo

DECLARATION

I, Oscar Madzingira, declare that this dissertation, which I hereby submit for the degree M Med Vet (Hyg) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

.

Signed: _____

Date: 21 January 2013

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ABBREVIATIONS

AMOS PCR	Abortus-Melitensis-Oviis-Suis Polymerase Chain Reaction
CFSPH	Centre for Food Security and Public Health
CFT	Complement Fixation Test
CI	Confidence interval
DEFRA	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
DVS	Directorate of Veterinary Services
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organisation
FSAI	Food Safety Authority of Ireland
GIS	Geographical information systems
KRC	Karas Regional Council
MZCP	Mediterranean Zoonoses Control Programme
OIE	World Organization for Animal Health/Office International des Epizooties
RBT	Rose Bengal Test
SANCO	Directorate General for Consumer Affairs
WHO	World Health Organisation

SUMMARY

Sero-prevalence of brucellosis in sheep and springbok (*Antidorcas marsupialis*) in the Karas Region of Namibia

by

Oscar Madzingira

Promoter: Professor C M E McCrindle

Department: Paraclinical Sciences

Degree: M Med Vet (Hyg)

Game farming developed in Namibia over the years as a result of constraints associated with livestock farming such as diseases and profitability. The development of this industry has brought livestock and game species into close contact. In the Karas Region, a major sheep producing area, sheep and springbok are reared together on commercial farms. The rearing of these species in close proximity may result in cross-transmission of zoonotic diseases such as brucellosis, enabling such diseases to enter the human population through meat and other livestock products. Game species may complicate the control of brucellosis by acting as reservoirs of infection after the disease has been controlled in sheep. Brucellosis due to *B. melitensis* has been reported in Namibia as a cause of reproductive failure in sheep. An outbreak of brucellosis occurred

in 2009 affecting sheep, goats and humans on a farm in the adjacent Hardap Region. Brucellosis outbreaks in sheep have the potential to disrupt Namibia's foreign currency earning as the sheep industry contributes greatly to the economy of the country.

This aim of the study was to estimate the prevalence of *Brucella* (*B. melitensis, B. abortus, B. ovis*).infections in sheep and springbok in the Karas Region and to find out if the outbreak of brucellosis which occurred in the Hardap Region in 2009 had spread to the Karas Region.

Two experimental designs were used in this study. The first was a retrospective analysis of brucellosis testing results from 2008-2010 to indicate probable prevalence and to identify positive farms for follow-up sampling in sheep and springbok. Serological testing results of sera (n=22994) collected from 762 farms between 2008 and 2010 were analyzed and used to estimate apparent brucellosis prevalence. A total of 472 sheep sera and nine springbok sera were collected from eight farms that tested positive for *Brucella* antibodies between 2008 and 2010.

The second part of the study was a prospective serological study in sheep and springbok reared together; sheep in the Tses and Berseba communal areas and in culled ewes at the regional abattoir. Sexually mature sheep and springbok were selected for the prospective serological study because they are more likely to show serological responses than younger animals. Prior to the serological study, eleven questionnaires were completed on the farms (n=11) that reared sheep and springbok

together to gather information about farm management and risk factors for brucellosis. In the serological prevalence study, 332 sheep and 345 springbok sera were collected from the eleven commercial farms and 664 sheep sera were taken from the two communal areas. At the abattoir, 2302 sheep sera were collected from 40 farms in the region using the sample size for determining the absence or presence of disease. All sera were tested for *Brucella* (*B. melitensis, B. abortus*) antibodies using the RBT as a screening test and the CFT as a confirmatory test. *B. ovis* antibodies were tested for in sera from commercial farms only using the CFT test.

Results from the retrospective study revealed an apparent sheep brucellosis prevalence of 0.14% (95% CI: 0.1%-0.2%) over the three years and an annual brucellosis prevalence of between 0.05% and 0.19%. At district level, apparent prevalence was between 0% and 0.49%. The prevalence of positive farms was between 0.72% and 1.82%. When apparent prevalence was adjusted for CFT sensitivity and specificity, the prevalence was zero in all cases, suggesting that the prevalence detected in this study may be due to false positive reactions. However, some of the positives serological reactions were from suspected brucellosis clinical cases which were also confirmed by the PCR test. At district level, brucellosis prevalence was shown to be rising in the Karasburg district and decreasing in the Keetmanshoop and Bethanie districts. However, statistical analysis of the data using Fisher's exact test showed that the differences in brucellosis prevalence between districts was not significant, but that the differences in brucellosis prevalence between the three years was significant. All trace back sera collected in 2011 (using the sample sizes for proving disease freedom) from

sheep (n=472) and springbok (n=9) on previously positive farms (n=8) identified by the retrospective study, tested negative for *Brucella* (*B. melitensis, B. abortus, B. ovis*) antibodies. The negative results provided strong evidence that brucellosis control measures implemented on the farms following the outbreak were effective and that these farms were now free of brucellosis.

Results of questionnaire interviews showed that sheep and springbok were the main species on the farms and that the two species came into close proximity throughout the year especially at watering points in the summer. The interviews also revealed that the study population was naïve because farmers did not vaccinate sheep against brucellosis.

All sera collected in the serological study on commercial farms (sheep and springbok), in the two communal areas (sheep) and at the abattoir (culled ewes) tested negative for *Brucella* antibodies (*B. melitensis, B. abortus*). The prevalence of *B. ovis* antibodies in rams on one farm was 10% (3/30). *B. ovis* antibodies were not detected in springbok. The role of springbok in the epidemiology of sheep brucellosis could not be inferred due to the negative results recorded in both species.

Results of the retrospective and prospective serological studies confirmed that apparent brucellosis prevalence in sheep in the Karas Region was low. These results provided evidence that sheep and springbok reared together on the eleven commercial farms were not infected with *Brucella*. It was surprising that no positive reactors were found in

sheep in the communal areas because the intermingling of sheep from different flocks enhances the spread of brucellosis. The absence of positive reactors at the abattoir confirms that the chances of contracting human brucellosis at the abattoir were low and confirms that the forty farms tested were free of *Brucella* infections.

APPENDIX 2

INFORMED CONSENT FORM

I, the undersigned farm owner/authorised representative, hereby agree that the animal(s), as specified below, may be used by the researcher(s), as specified below, in the procedures as explained below:

1. To be completed by the researcher(s)

- **NAME OF THE RESEARCHER(S):** *Dr. O. MADZINGIRA*

NAME OF RESEARCH PROJECT: Sero-prevalence of brucellosis in sheep and springbok (*Antidorcas marsupialis*) in the Karas Region of Namibia

- **PURPOSE OF RESEARCH PROJECT:** To find out if sheep and springbok in the Karas Region have brucellosis infections; to estimate the prevalence of such infections and to find out if there is a risk for brucellosis infection for people who handle sheep and springbok meat**.**

- **DETAILED PROCEDURE(S) TO BE PERFORMED:**

Collection of information related to the possible occurrence, spread and control of brucellosis and farm management using an interviewer administered questionnaire. Blood samples shall be collected from sheep and springbok from the jugular vein using standard procedures. Collection of blood samples from springbok shall be done immediately after shooting by trained and registered professional hunters. Small pieces of organs may be taken from dead animals for further analysis.

- **RISK(S) INVOLVED IN SPECIFIED PROCEDURE:**

Collection of blood shall be done by trained personnel (veterinarian and Animal Health Technicians) using separate syringes and needles for each animal and under adequate physical restraint for sheep. No significant risks are expected.

- **IDENTIFICATION OF ANIMAL TO BE USED:**

Numbers shall be placed on the back of live sheep using paint. Springbok shall be identified using numbers attached to the carcass.

2. To be completed by the animal's owner or person duly authorized to sign on his/her behalf:

- **Name of owner/authorised representative:** _____
- **Farm Name, Number and address:** _____

_____

- **Have you received detailed information regarding the proposed study? (Yes/No)** _____

  Have all the risks involved in the procedure been explained to you and do you fully understand these risks? (Yes/No) _____

- **Do you grant full consent for the procedures to be performed? (Yes/No)** _____

_____ _____

Signature: Farm owner/representative **Signature: Researcher (s)**

_____ **DATE:** _____

Signature: Witness

REPUBLIC OF NAMIBIA

MINISTRY OF AGRICULTURE, WATER AND FORESTRY

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19 January 2011

State Veterinarian

PO Box 30

Keetmanshoop.

Dear Dr. O. Madzingira

RE: **SERO-SURVEILLANCE OF BRUCELLOSIS IN SHEEP, GOATS AND SPRINGBOK IN THE KARAS REGION**

Your application to undertake the above stated research in partial fulfillment of the requirements of a Masters degree in Veterinary Public Health at the University of Pretoria has reference.

Approval is hereby granted to:

1. carry out the research as part of your routine work as a State Veterinarian and for the samples to be tested at the Central Veterinary Laboratory in Windhoek;

2. collect blood samples from springbok and livestock at the abattoir and on commercial and communal farms. The

results of the research must be shared with the Directorate of Veterinary Services and all publications must get prior approval from the Chief Veterinary Officer.

I wish you the best in your studies.

Dr. C. Bamhare, BVSc, M. Sc.
Acting Chief Veterinary Officer

Ref: V017/11

23 May 2011

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Prof CME McCrindle
Department Paraclinical Sciences
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Dear Prof McCrindle

PROTOCOL V017/11: SERO-PREVALENCE OF BRUCELLOSIS IN SHEEP AND SPRINGBOK (*ANTIDORCAS MARSUPIALIS*) IN THE KARAS REGION OF NAMIBIA – *O Madzingira*

I am pleased to inform you that the abovementioned protocol was approved by the Research Committee.

Kindly note that, if there are animal ethical issues involved in the project, the protocol needs to be approved by the Animal Use and Care Committee as well before you may commence with the project.

Please take note of the attached document.

Kind regards

NIESJE TROMP
SECRETARY: RESEARCH COMMITTEE

Copy: O Madzingira, Researcher (madzing@iway.na)
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29 June 2011

Prof CME McCrindle
Department of Paraclinical Sciences
Faculty of Veterinary Sciences
(cheryl.mccrindle@up.ac.za)

Dear Prof McCrindle

V017-11 : Sero-prevalence of Brucellosis in Sheep and Springbok (Antidorcas Marsupialis) in the Karas region of Namibia (O Madzingira)

The application for ethical approval, dated on 9 June 2011 was approved, by the Animal Use and Committee at its meeting held on 27 June 2011.

Kind regards

Elmarie Mostert

AUCC Coordinator

Copy Dr O Madzingira