<u>Research project</u>

The prevalence of Bovine Brucellosis in the Mopani district South Africa, from 2010 to

2013

Ramaselela Ngoako Godfrey

J30/29473/2009

Bachelor of Veterinary medicine

Year 5 2015

University of Nairobi

Supervisor: **Prof Kangethe, E.K. (BVM,MSC,PhD)**

i

Declaration

I declare that the project entitled õ THE PREVALENCE OF BRUCELLOSIS IN THE

MOPANI DISTRICT FROM 2010 TO 2013ö submitted by me to the University of Nairobi

for my Bachelor of Veterinary Medicine degree is a record of bonafide project work carried

out by me under the supervision of Prof Kangethe E.K. I further declare that the project has

not been submitted for the award of any degree in this institution.

Ramaselela Ngoako Godfrey

Signature í í í í í í í í í í í í í í í í í .. date í í í í í í í í í í í ..

Prof Kangethe, E.K. (BVM, MSC, PhD)

Signature í í í í í í í í í í í í í í í í í í date í í í í í í í í í í í ..

Table of Contents

Acknowledgement

I would like to express my deepest appreciation to my supervisor Prof Kangethe E.K. for giving me the opportunity to work with him and supervise me with my project. I would like to thank the University of Nairobi for giving me the opportunity to work on my project and gain experience.

In addition, a thank you to Dr Letsoalo K.V. for allowing me work in her office and for centralizing all the data that I needed for the project. I also thank my family and friends for being there all step of the way till I finished my research.

Abstract

Bovine brucellosis (contagious abortion) is a bacterial disease of cattle and may infect other domestic animals and humans. The disease is caused by *Brucella abortus* , a gram-negative, coccobacillus bacteria which is anaerobic and intracellular bacteria. In South Africa the disease was first discovered in 1906 by Gray, and up to date Brucellosis is a controlled disease in South Africa. The national *Brucella* prevalence is 3.8% in South Africa. The retrospective study was done in the Mopani district in the Limpopo province in South Africa, with data collected from 2010 to 2013. The prevalence of brucellosis reduced from 4.16% in 2010 to 0.845% in 2013. The use of S19 vaccine in cattle in South Africa interferes with the interpretation of results, tested by rose Bengal test and complement fixation test.

List of abbreviations

CFT- Complement Fixation Test

EA- Expected Agreement

ELISA- Enzyme linked Immunosorbent Assay

IgG- Immunoglobulin G

IgM- Immunoglobulin M

K- Kappa

OA- observed Agreement

OIE- World Organisation for Animal Health

RBT- Rose Bengal Test

S19- Strain 19 vaccine

List of tables and figures

Chapter 1

1.1. Introduction

Brucellosis also known as **contagious abortion** or **Bang's disease** is a bacterial disease of cattle and other domestic animals, caused by *Brucella* species of bacteria. *Brucella* species are Gram-negative, coccobacillary, aerobic and facultative intracellular bacteria. The genus include, *B. abortus, B. melitensis, B. suis, B. canis, B. ovis, B. microti, B. neotomae* and *B. inopinata*. (Madhukar *et al.*,2014). Among these species, *B. abortus* (cattle), *B. melitensis* (goats and sheep), *B. suis* (pigs), *B. canis* (dogs), *B. ovis* (sheep) are the most important species that cause Brucellosis in animals.

Brucellosis is widely spread within African countries (Mangen, *et al* 2002). In sub-saharan Africa, brucellosis is also an important disease in both humans and livestock, in humans, brucellosis or undulant fever is caused by *B. abortus* which is chiefly an occupational disease, occurring most often in Veterinarians, stock inspectors, abattoir workers, laboratory personnel and farmers (Coetzer & Tustin, 2004). The pathogenic species are *B. melitensis,* which predominantly infects goats and sheep; *B. abortus,* which principally affects cattle; *B. suis* that infects swine and *B. canis* that infects dogs. Although any of 4 species of *Brucella* can cause systemic disease in humans, *B. melitensis* has the lowest infective dose, requiring as few as 10 organisms to cause infection. (Magwedere, 2011)

The disease is most commonly spread between herds by the movement of infected animals and between animals by contact of susceptible animals with infective discharges at the time of calving or abortion of infected animals, and for up to 1 month thereafter.

1

Bovine brucellosis cause characteristic mid-late term abortion and infertility in cows, orchitis inflammation of the accessory sex glands in bulls.

In South Africa for a number of years the first reliable information regarding the existence of Brucellosis was given by Gray in 1906 when he referred to a serious outbreak of abortion among cattle near Johannesburg, the source of infection was traced to an infected cow introduced into the herd (Henning, 1932).

Bovine brucellosis in South Africa is a controlled disease according to the Animal Diseases Act, 1984 (Act No. 35 of 1984). For control of the disease Strain 19 and RB51 vaccines are the only brucellosis vaccines currently allowed for use in R.S.A. Strain 19 vaccine induce post-vaccinal antibodies that are detected in serological tests (Coetzer & Tustin, 2004), this can interfere with interpretation of laboratory results. No accurate figures are available on the prevalence of brucellosis in cattle in Southern Africa, as most reports are based on non-representative laboratory results.

To understand the economic impact of brucellosis and public health hazard it poses, it is important to study the prevalence of the disease in the Mopani district where there is movement of cattle legally and illegally between farms. This study was aimed at estimating the prevalence of brucellosis within the Mopani district.

1.2. <u>Objectives</u>
- To determine the prevalence of Bovine brucellosis in the Mopani district.

- To review the current diagnostic techniques for detection of wild strain of *Brucella*.

Chapter 2

2.1. Literature review

Brucella species are small, Gram-negative coccobacilli that lack capsules, endospores or native plasmids. The cell wall consists of an outer layer of lipopolysaccharide (LPS) protein approximately 9 nm thick (Gillespie & Hawkey, 2006). *Brucella* species are non-motile facultative intracellular pathogens of the phagocytic, reticuloendothelial, and specialized epithelial cells of mammalian hosts (Banai & Corbel, 2010 and Olsen *et al* 2010).

2.1.1. Aetiological agents
Brucella species is composed of eight terrestrial species and at least two marine species. Terrestrial *Brucella* species include *B. abortus, B. melitensis, B. suis, B. canis, B. ovis, B. neotomae, B. microti, B. inopinata*. And *Brucella* isolated from marine mammals are, *B. ceti* and *B. pinnidialis* (Yu & Nielsen 2010). *B. canis* and *B. ovis* are designated as rough due to their lack of surface expression of the O polysaccharide, while *B. abortus, B. melitensis, B. neotomae, B. suis,* and marine isolates are designated as smooth strains because of their surface expression of the O polysaccharide on their lipopolysaccharide.

2.1.2. Transmission
The mechanism of transmission for *Brucella* can generally be described by dividing the genus into virulent (*B. abortus, B. melitensis)* and low pathogenic (*B. ovis, B. canis*) strains. For the virulent strains, transmission primarily occurs through fluids or tissues associated with the birth or abortion of infected foetuses or to offspring through milk. Venereal transmission is not considered to be important for transmission of *B. abortus* or *B. melitensis*. By contrast, venereal transmission is an important route of infection for *B. canis*, *B. ovis,* and *B. suis.* Animals infected with *B. canis* effectively shed *Brucella* for longer times from

mucosal surface and urine when compared to animals infected with *B. abortus* or *B. melitensis*. (Olsen *et al* 2010). Cattle usually become infected after ingesting contaminated feed or water or licking an infected placenta, calf or foetus, or the genitalia of an infected cow soon after it has aborted or calved. At this time very large numbers of *B. abortus* are present, particularly in the placenta lochia. Infected animals usually abort only once, subsequent calves are carried to full term although they may be infected. *Brucella abortus* infections in sheep and goats may occasionally cause them to abort, but the infection does not spread in these species. Several species of wildlife ó African (Cape) buffalo (*syncerus caffer*), hippopotamus (*Hippopotamus amphibious*), Zebra (*Equus burchelli*), eland (*Taurotragus oryx*) and impala (*Aepyceros melampus*) have tested serologically positive for brucellosis but these species are probably not of great importance in the epidemiology of bovine brucellosis in southern Africa.

The establishment of infection is influenced by the size of the infective dose, virulence of the bacteria, and the resistance, age, sex and reproductive status of the animal. *Brucella* readily penetrates mucous membranes, such as those of the pharynx and alimentary tract and survives and multiplies particularly in cells of the reticuloendothelial system. (Godfroid *et al* 2004)

2.1.3. Pathogenesis
Upon entry of *brucella* through the plasma membrane they modify the endosomal compartment of phagocytic cells to allow replication and long term survival, use various mechanisms to modify the host environment, resist oxidative killing, and modify their metabolism to survive in their preferred intracellular environment. The bacteria are then transported to regional lymph nodes. Localization in the reproductive or mammary glands is associated with the most severe pathology and capability to transmit infection. Erythritol is the preferred carbon source for *Brucella* strain, and it has been proposed that the presence of

4

this compound is an important factor driving the extensive intracellular replication exhibited by the *brucellae* in placental trophoblast during the latter trimester of pregnancy. Elevated levels of erythritol occur in the placenta and foetal fluids from about the fifth month of gestation. The abundance of erythritol in the pregnant uterus results in the massive multiplication of *Brucella* organisms in this organ. (Godfroid *et al* 2004). Erythritol stimulates the growth of some strains of *B. abortus.* Other strains, including *B. abotus* strain 19, are not stimulated by erythritol, yet these strains are capable of causing genital infection and abortions (Nielsen and Dunkan, 2006). The mechanisms of *Brucella* induced abortions are poorly understood (Nielsen and Dunkan, Godfroid *et al* 2004) *Brucella* may also reside in a dormant, non-reproductive state in phagocytic cells. Bulls can be infected but they do not readily spread the disease. The *brucella* organism localizes in the testicles of the bull and cause orchitis (Richey and Harrell, Olsen *et al* 2010)

2.1.4. Brucellosis worldwide
Brucellosis exists worldwide in domestic and wild animals, in many areas such as sub-Saharan Africa, brucellosis is known to exist, but the prevalence is unknown, owing to a lack of diagnostic and reporting mechanisms (Gillespie & Hawkey, 2006). *B. abortus* has been eradicated in Japan, Canada, Australia, New Zealand and several Northern and Central European countries (OIE, 2011)

Brucellosis is one of the world's major zoonoses that still is of veterinary, public health and economical concern in many parts of the world. Although brucellosis in livestock and transmission to humans has been decreasing following intervention by vaccination-based control and prevention programmes in parts of the world, it remains an uncontrolled problem in regions such as the Mediterranean, Middle East, Africa, Latin America and parts of Asia (Smits & Kadri, 2005)

5

In many high income countries, brucellosis has been successfully controlled or eliminated in livestock populations. In low-income countries of Sub-Saharan Africa and South Asia, brucellosis is endemic and neglected, with large disease and livelihood burdens in animals and humans and almost no effective control. (McDermott *et. al,* 2013, Yohannes *et al,* 2013).

2.1.5. Human Brucellosis

According to WHO Brucellosis is regarded as a ‑neglected zoonosisø in Sub-Saharan Africa that is closely associated with livestock production and widely endemic in rural Africa. Currently about half a million human brucellosis cases are annually reported worldwide but the estimated number of unreported cases due to the unspecific clinical symptoms of the disease is supposed to be 10 times higher. In endemic countries prevalence rates often exceed 10 cases per 100,000 population. (Godfroid *et al*.2013).

In humans, *Brucella* causes undulant fever, a disease characterized by intermittent fever, headaches, fatigues, joint and bone pain, psychotic disturbance, and other symptoms. *Brucella melitensis* is usually considered to be the *Brucella* species with the highest zoonotic potential, although *B. abortus* appear to be widely distributed among African catlle, *B. melitensis* seems to be relatively rare in sub-Saharan livestock (Marcotty *et al* 2009). The study done by Achebe *at al*. (2009) assessed the prevalence of major causative agents of acute febrile illness in 653 patients of four health centers in Northern Ethopia. Among these febrile patients*, B. abortus* was detected in 9.3% of the patients (Yohannes *at al* 2013).

Human Brucellosis was first suspected in South Africa in 1898 by Dr Simon Frezer, District Surgeon of Gordonia Hospital, who sent forward a public health report in which forty cases were recorded. When *Brucella* infection was demonstrated in cattle by Hall (1913) and Robinson(1918) it was believed that the Transvaal bushveld (now divided into Limpopo, Gauteng, Mpumalanga and North west provinces) was the mostly heavily infected area, but it

6

soon became apparent that this contagion had spread throughout the whole of Southern Africa(van Drimmelen 1961).

In January 2011 the National Institute for Communicable Diseases South Africa released an article about the farmer and one of the farm-workers who were diagnosed with Brucellosis in December 2010 in Gauteng Province South Africa. Abortions in the same farm have been reported in goats, and both the farmer and the farm worker contracted the disease from the goats (Brooke, 2012)

2.1.6. The prevalence in South Africa

In South Africa there is a legislative requirement that all heifers between 4 and 8 months old be vaccinated with the live, attenuated S19 vaccine which has been in force since 1968, or the RB51 vaccine for older cows. This has significantly reduced the national prevalence of Brucellosis, from 10.5% in 1976 to 6% 3 years later. By 1984/5 the figure was further reduced to 1.9% and by 1988/89 it was down to 1.4%. In recent study by Njiro *et al* (2011), carried out in Gauteng province 237 cattle were tested for Brucellosis by complement fixation test and only 9 tested positive. Currently the country is at 3.8% prevalence, higher that that recorded in 1988/89, that is why there is enforcement of the National eradication scheme against bovine Brucellosis.

2.2. Diagnosis of Brucellosis

The diagnosis of the disease can be done by history and clinical findings, identification of the bacteria and serological tests.

2.2.1. History and clinical findings

The clinical findings depend upon the immune status of the herd. In highly susceptible non-vaccinated pregnant cattle, abortion after 5 months of pregnancy is the typical feature of the disease in cattle. Second or even third abortions may occur in the same cow and retention of

7

the placenta and metritis are common sequel to abortion. In the bull, orchitis and epididymitis occur (Radostits, 2006)

The *" Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*ö published by the OIE lists diagnostic tests in two categories: õprescribedö and õalternativeö. Prescribed tests are required by the OIE Terrestrial Animal Health Code for the international movement of animals and animal products and are considered optimal for determining the health status of animals (Godfroid *et. al. 2013)*

2.2.2. Identification of the agent

2.2.2.1. Direct smear microscopic examination

A presumptive bacteriological diagnosis of *Brucella* can be made by means of the microscopic examination of smear from vaginal swabs, placentas or aborted foetuses, milk, and semen, stained with the Stamp modification of the Ziehl-Neelsen staining method (Kaltungo *et al* 2014, OIE 2008). *Brucella* species are not truly acid-fast, but they are resistance to decolorizing by weak acids, and stain red against a blue background. *Brucellae* are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. Morphological-related microorganisms such as *Chlamydophila abortus* and *Coxiella burnetti* can mislead the diagnosis because of their superficial similarities. (Kaltungo *et al* 2014, and Wobeser, 2009)

2.2.2.2. Culture of the organism

Brucella organism can be cultured from body tissues or secretions like blood, milk and vaginal discharge, the organism can also be cultured from cerebro-spinal fluid, joint and pleural fluids (Megid et. al., 2010). Several media can be used to grow *Brucella* organism. *Brucella* medium base, Tryptose-soy agar (TSA), blood agar base, serum-dextrose agar (SDA) or glycerol dextrose agar can be used. Appropriate antibiotics are added to suppress the growth of organisms other than *Brucella*. The most widely used selective medium is the Farrellǿs medium, which is prepared by adding 6 antibiotics to a basal medium. The following quantities are added to 1 litre of agar: polymyxin B sulphate (5 mg), bacitracin (25 mg), natamycin (50 mg), nalidixin acid (5 mg), nystatin (100 mg), vancomycin (20 mg).The enriched medium should be incubated at 37 degrees C in air supplemented with 5-10% CO_2 for 6 weeks, with weekly subculture on the solid medium.

2.2.2.3. Serological tests

Serological diagnosis of Brucellosis began more than 100 years ago with a simple agglutination test, it was realized that this type of test was susceptible to false positive reactions resulting from, for instance, exposure to cross reacting microorganisms (Nielsen and Yu, 2010).

The procedures are divided into 2 categories, the Conventional Tests and Primary Binding Assays. All conventional tests rely on the antibody performing a secondary function, for instance fixation of complement while in primary binding assays the only function of the antibody is attachment to its antigen (Nielsen and Yu, 2010).

2.2.2.4. Milk ring test

Milk Ring Test is basically a rapid agglutination test carried out on whole milk or cream. Haematoxylin stained *Brucella* cells are added to whole milk and incubated for reaction to

9

take place. Immunoglobulins present in the milk will, in part, be attached to fat globules via Fc portion of the fat molecule. The immunoglobulins detected by this test are IgM and IgA. The test may be applied to individual animal or to pooled milk samples using a larger volume of milk. The test is prone to false reaction caused by abnormal milk due to mastitis, presence of colostrums and milk from the late gestation (Kaltungo *et al.* and Nielsen and Yu, 2010)

2.2.2.5. Serum Agglutination test

Serum agglutination test is generally not used as a single test but rather in combination with other tests. The test is considered to lack specificity (Godfroid *et. al.*2009). In South Africa SAT is still very usefully employed as a supplementary test for indicating the level of serum IgM, the predominant Immunoglobulin after vaccination with strain 19 vaccine. This test is based on the reaction of antibodies against the smooth lipopolysaccharide of *Brucella*. The test is performed at a near neutral pH, which makes it more efficient in detecting IgM antibody. Hence it is best used to detect acute infections.(Godfroid *et. al.*2009, Kaltungo *et. al* 2014. and OIE, 2008)

2.2.2.6. Rose Bengal test

This test is used to screen serum samples. It does not differentiate between field and strain 19 vaccine strain reactions. Positive reaction with this test should be investigated using suitable confirmatory tests. The test uses a suspension of *B. abortus* smooth cells stained with Rose Bengal dy, buffered to pH 3.65. It is an internationally recommended test for the screening of brucellosis in small ruminants, but lacks standardisation of the antigen (Kaltungo *et. al* 2014)

2.2.2.7. Polymerase chain reaction

Polymerase Chain reaction (PCR) is a highly sensitive, very specific, rapid, and easily adapted to high volume demand diagnostic tool to detect slow growing bacteria including *Brucella*. This method can detect a few bacteria in a sample and are as sensitive as classical culture-based techniques. Furthermore, it is possible to detect dead bacteria reducing the necessity of careful sample conservation before analysis.

2.2.2.8. Complement fixation test

The CFT detects mainly the IgG1 isotype antibody as IgM isotype are partially destroyed during the inactivation process. In most cases, the CFT is used on RBT positive sera, but like the RBT, it is also affected to a large extend by the misuse of strain 19 vaccine. The basic test consists of *B. abortus* whole cell antigen incubated with dilutions of heat-incubated serum and a titrated source of complement, usually guinea pig serum. After a suitable time a pretitrated amount of sheep erythrocytes coated with rabbit antibodies is added. If a primary immune complex formed due to the presence of certain antibody isotypes mainly IgG, in the serum, complement is activated and therefore not available to react with the secondary immune complex of sheep erythrocytes and rabbit antibody, resulting in no or only slight lysis of the erythrocytes. Alternatively, if no primary immune complex was formed, complement would cause all the sensitized sheep erythrocytes to lyse. Thus the amount of haemoglobin in solution is a measure of anti-*Brucella* antibody-activity.

2.2.2.9. Indirect enzyme-linked immunosorbent assay

Indirect ELISAs are tests in which antigen is bound to a solid phase, usually a polystyrene micro-titre plate so that the antibody, if present in a sample, binds to the immobilised antigen and may be detected by an appropriate ant-globulin-enzyme conjugate which in combination with a chromogenic substrate gives a colour reaction indicative of the presence of antibody in the sample. The indirect ELISA is a highly sensitive test but it is sometimes not capable of

11

differentiating between antibody resulting from S19 vaccination or other false-positive reactions induced by pathogenic *Brucella* strains. There it should be considered as a screening test than a confirmatory test in the testing of vaccinated herds affected by false-positive results.

2.2.3 Control and prevention

Treatment

Cattle suffering from Bovine Brucellosis are generally not treated. The species may undergo L-transformation when exposed to certain antibiotics, such as penicillin and oxytetracycline.

Vaccination

Strain 19 vaccine. Vaccination with s19 increases resistance to *B. abortus* but does not induce absolute immunity, and vaccination with it is not curative. The main disadvantage of s19 vaccine is the induction of post-vaccinal antibodies that are detected in serological tests. Strain 19 and RB51 are the only brucellosis vaccines currently allowed for use in South Africa (Godfroid *et. al.* 2009) heifer calf are vaccinated subcutaneously at four to eight months of age.

RB51 vaccine

The vaccine was approved for use in South Africa in 2002. The vaccine does not usually induce the production of antibodies in cattle that can be detected in the classical Brucellosis serological tests regardless of their age, the dose they received and the frequency of injection.

Chapter 3

3.1. Materials and methods

3.1.1. Study area and population

The study was done in the Republic South Africa (RSA) in Limpopo province, Mopani District. RSA is located in the Southern part of Africa with its coastline stretching along the South Atlantic and Indian oceans. The neighbouring countries are Namibia to the west followed by Botswana, Zimbabwe Mozambique and Swaziland to the east, and within lies Lesotho (Wikipedia)

The human population is 51.8 million people, according to Statistics South Africa October 2011. Mopani district is one of the 5 districts of Limpopo province, the district contains 5 local municipalities namely Maruleng, Greater Tzaneen, Greater Giyani, Greater Letaba, Ba-Phalaborwa municipality. The area is 20,011 km2. There are many game reserves in the province with the largest game reserve, the Kruger National Park located in the Ba-Phalaborwa municipality.

The Limpopo province has a population of 1 061 997 (the Department of Agriculture census, 2013) cattle. The Mopani district has 187 040 cattle, Maruleng 29 983, Letaba 33 914, Greater Giyani 87 324, Greater Tzaneen 25 395, and Ba-Phalaborwa with 11 424 cattle. Cattle production in the Mopani district is mainly beef, both communal and commercial beef production. The small holder farmers in the communal area usually own few animals, less than or even more than 20 cattle per herd. With some having sheep and/or goats in their herds.

13

3.1.2. Data collection

A retrospective study was done by collecting the *Brucella abortus* serum laboratory test results from the state veterinarians of all the five municipalities in the Mopani district. The serum laboratory results compiled were from the year 2010 to the year 2013. The results that were available were recorded and tabled according to their respective municipality.

The laboratory results have the number of animals that were tested and also given the number positive and those negative. Rose Bengal Test is the first test that they do, sometimes Serum Agglutination Test is done, but no data of this test was available. The serum positive on Rose Bengal test was then tested further with Complement Fixation Test.

As a control and surveillance programme in the country, the state veterinarians and the animal health technicians test for Brucellosis by bleeding cattle in communal areas and also in the commercial herds. The serum is sent to the Government laboratories to undergo *Brucella* serological tests. The tests that are conducted at the laboratory include Rose Bengal Test (RBT), sometimes Serum Agglutination Test and Complement Fixation Test (CFT). The serum which reacts positive with RBT is then tested again for confirmation with CFT.

3.1.3. Rose Bengal test

This very sensitive test is used to screen serum samples. It does not differentiate between field and S19 vaccine strain reaction, but is quick, inexpensive and easy to perform. False negative reactions are rare but may sometimes be due to excessive heating in storage or transit. Positive reactions should be investigated using suitable confirmatory and/or

complementary strategies (including the performance of other tests and an epidemiological investigation).

The antigen stained with Rose Bengal stain, is buffered at a pH of 3.65. At this level of activity non-specific agglutinins are destroyed and IgG, the most abundant antibody in the serum of infected animals, agglutinates strongly. The test is prescribed by the OIE for international trade in cattle.

3.1.4. Complement fixation test (CFT)

The complement fixation test is very specific and sensitive and is regarded throughout the world as being the confirmatory test of choice for serological detection of infected animals. In most cases, the CFT I used on RBT positive sera, but like the RBT, it is also affected to a large extend by the misuse of s19 vaccine, particularly when recent or repetitive vaccinations have been used in sexually mature heifers and cows. It is almost impossible to prescribe strict cut-off readings that indicate infection particularly when S19 vaccine reactions play a role due to its misuse. Results are expressed in International Units (IU), and the following table bellow **(table 1),** indicate the conversions of CFT titres to IU. The units are then used to interpret the results:

TABLE 1: *CONVERSION OF CFT TITRE TO INTRERNATIONAL UNITS (IU): reaction at a 1/220 dilution equals 1 000 IU/ml*

Serum	¼				1/8				1/16				1/32				1/64				1/128			
End-point	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
IU/ml	15	18	21	24	30	36	43	49	60	72	86	96	120	145	172	196	246	290	344	392	480	591	688	784

16

In South Africa the recommended CFT positive levels have been set at **30** and **60 IU/ml** for correctly vaccinated and adult vaccinated animals respectively. I used the **30 IU/ml** because the lab results did not indicate the age of the animals which the samples are from, and also the vaccination status because the results are from a mixture of communal and commercial farmers. The interpretation of CFT results are used using this **table 2** below. *N.B* this table must not be applied before the infection status of the herd has been established (which can be difficult with communal farms).

Table 2: *INTERPRETATION OF CFT TITRES.*

Vaccination status	CFT IU/ml	Interpretation
Unvaccinated or calf-hood vaccination or unknown	15 18-24 30	Negative Suspicious Positive
Adult vaccinated	24 30-49 60	Negative Suspicious positive

Chapter 4

4.1. Results

In 2010(table 3) Giyani tested 651 cattle, and Tzaneen 94 cattle were tested. Tzaneen had 26 animals that tested positive with RBT despite the low number of cattle tested as compared to Giyani with 13 cattle testing positive with RBT. 23 cattle tested positive for Tzaneen and 8 tested positive for Giyani with CFT. In total 745 cattle were tested in 2010 and 31 of those cattle tested positive for Brucellosis.

In 2011(table 4) Tzaneen tested 1137 cattle and 45 tested positive with RBT and 22 tested positive for Brucellosis (CFT results). And Letaba tested 491 cattle 14 of those tested positive with RBT and 10 were positive for Brucellosis. In total, in 2011 32 cattle tested positive for Brucellosis higher than in 2010 where 31 cattle tested positive for Brucellosis (table 3).

In 2013 (table 5), Giyani which tested 27 cattle, and Maruleng 243, both the districts tested negative for RBT and CFT. Tzaneen tested 2728 cattle and 47 cattle tested positive with RBT and 24 positive for Brucellosis. Letaba tested 182 cattle and 8 cattle tested positive for Brucellosis. In total more cattle were tested in 2012 as compared to 2010 (table 3) and 2011 (table 4). And in 2012 32 cattle tested positive for Brucellosis making the results same as in 2011 (table 4) where 32 cattle tested positive for Brucellosis.

In 2013 (table 6), the number of cattle tested in all the districts is higher than in 2010, 2011 and 2012. 4615 cattle were tested and 39 tested positive for Brucellosis, the number is higher than the number recorded in previous years by 8 cattle in 2010 and 7 cattle higher in 2011 and 2012.

 The results compiled are tabulated in the tables below:

18

Table 3: _2010 results_

municipality	No tested	RBT positives	CFT		
			<18	18-24	24-30
Tzaneen	94	26	1	2	23
Giyani	651	13			8
Maruleng	-				
Letaba	-				
Total	745	39	1	2	31

Table 4: _2011 results_

Municipality	No tested	RBT positives	CFT		
			<18	18-24	24-30
Tzaneen	1137	45	2	1	22
Giyani	-				
Maruleng	-				
Letaba	491	14			10
total	1628	59	2	1	32

19

Table 5: *2012 results*

Municipality	No tested	RBT positives	CFT		
			<18	18-24	24-30
Tzaneen	2728	47	10	7	24
Giyani	27	-			
Maruleng	243	-			
Letaba	182	12		1	8
Total	3180	59	10	8	32

Table 6: *2013 results*

| Municipality	No tested	RBT positives	CFT		
			<18	18-24	24-30
Tzaneen	2 840	45	19	5	20
Giyani	-	-	-	-	-
Maruleng	739	11	-	-	6
Letaba	1 036	22	-	-	13
Total	4615	78	19	5	39

The table below represent the prevalence of Brucellosis in all the municipalities. The prevalence was calculated using the positive results on CFT (**figure 1**) of the total number of cattle each year. The total prevalence from 2010 to 2011 reduced drastically from 4.16% to 1.966% in 2011 **(table 7).** From 2011 to 2013 the prevalence reduced by less than 1% each year (**table 7**).

Table 7: _**The prevalence table**_

Year	2010	2011	2012	2013
Total number tested	745	1628	3180	4615
Number positive	31	32	32	39
prevalence	4.16%	1.966%	1.006%	0.845%

The results of the two tests were compared using the line graphs below, with figure 1 indicating the prevalence tested by CFT and figure 2 indicating the prevalence tested by RBT. The results are as follows:

Figure 1
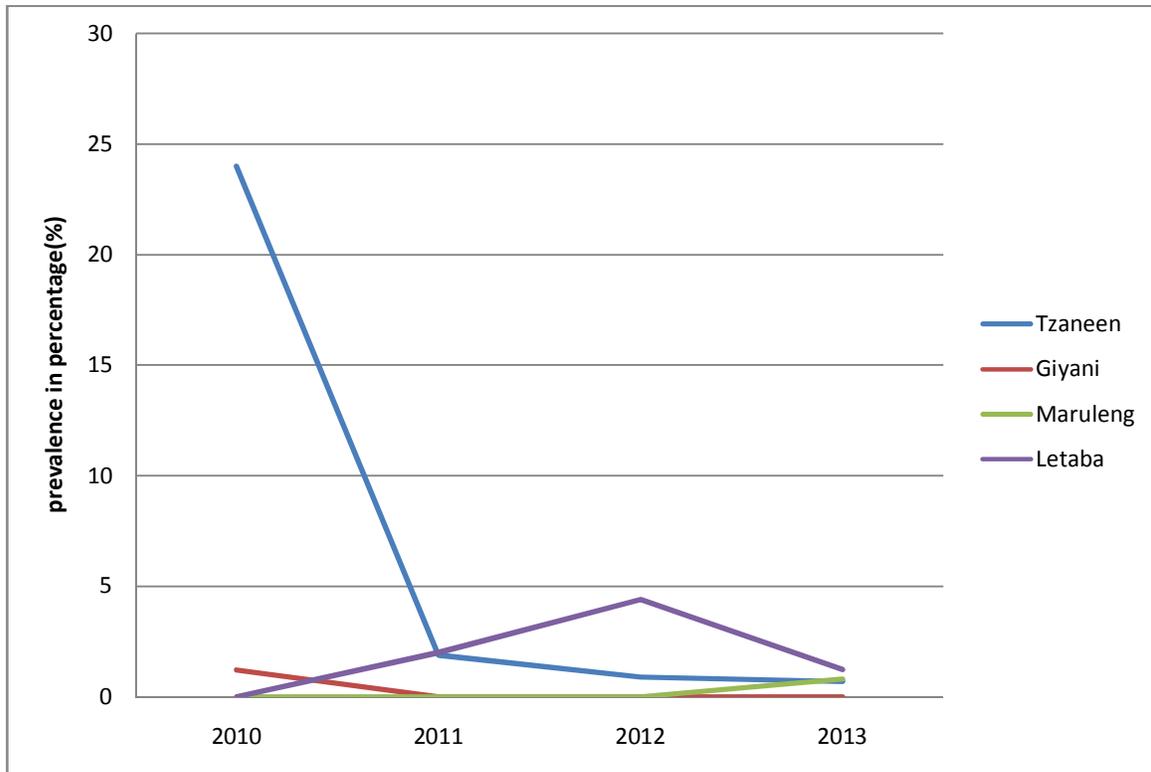
The prevalence of brucellosis in the tested cattle with CFT
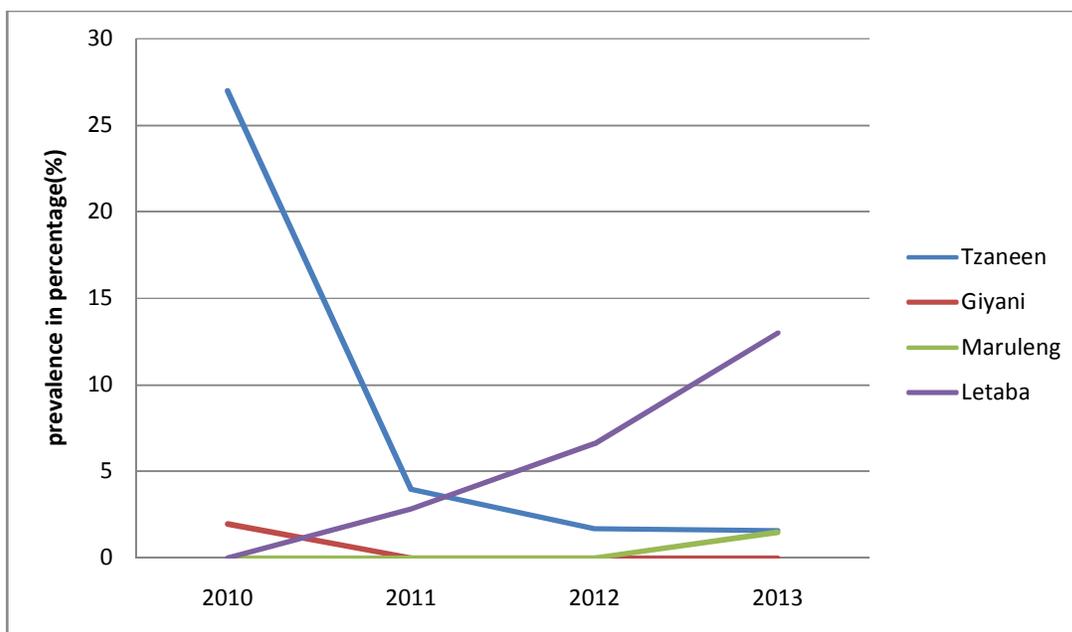
(2010-2013)

The prevalence of *Brucella,* tested by CFT (figure 1) in Tzaneen reduced to 1.9% in 2011 from 24% in 2010 and from then less than 1% reduction is seen in 2012 and 2013.The reason for the sharp drop is because Tzaneen did not have a State Veterinarian for several years before Dr K.V Letsoalo was posted in Tzaneen in 2010. Giyani had low prevalence in 2010 despite the large number of cattle tested (651 cattle, **table 3 above**) as compared to Tzaneen

23

with 94 cattle tested. No data was available for 2011 and 2013, in 2012 out of 27 cattle tested non tested positive to Brucellosis.

Maruleng showed positive results in 2013 (**figure 1**) where 0.8% prevalence of 739 cattle tested, and negative results in 2012 where 243 cattle were tested with CFT. This shows that new cases of *Brucella* outbreaks have been reported and may be increasing to date. In 2011 (**table 4**) Letaba had 2% prevalence which elevated to 4.4% in 2012 and reduced to 1.25% in 2013, making Letaba the municipality with the highest prevalence in 2013 followed by Maruleng(0.8%) and Tzaneen(0.7%)   (**figure1**)

The information for Maruleng and Giyani are difficult to conclude because the data available is only for a 2 years, therefore there is no enough data to compare with other years, both have negative tests with Giyani testing negative in 2012 and Maruleng also in 2012 (**figure 1**).

Figure 2

The prevalence of brucellosis of the tested cattle with RBT (2010-2013)

In 2010 Tzaneen recorded the highest prevalence of 27% as compared to other municipalities and in 2011 the prevalence reduced to 3.96% while that of Letaba was at 2.85%. The prevalence of Tzaneen reduced and Letaba increased in 2012 from 2.85% to 6.6%, and reduced to 2.12% in 2013. Tzaneen and Maruleng had only 0.09% difference in the prevalence in 2013 on RBT.

The trend of Letaba increases with years from 2010 to 2013 of RBT results, the prevalence is the opposite with Tzaneen which is showing reduction in the prevalence every year.

4.1.1. The kappa statistics

Is the proportion of potential agreement beyond chance exhibited by two or more tests. The value of Kappa ranges from -1.0 (perfect disagreement) through 0.0 (chance agreement only) to +1.0 (perfect agreement). By convention, Kappa values of 0.0-0.2= slight agreement, 0.2-0.4= fair agreement, 0.4-0.6= moderate agreement, 0.6-0.8= substantial agreement, and 0.8-1.0= almost perfect agreement between tests.

Calculations comparing the agreement between Rose Bengal Test and Complement fixation Test which are used in *Brucella* diagnosis was done using the formula K= <u>OA- EA</u> divided by 1-EA.

The Kappa was calculated to be equal to 0.0307 which falls in between the value of slight agreement between the tests. The two tests used RBT and CFT are both affected by the misuse of S19 vaccine but complement fixation tests is more specific than Rose Bengal Test.

Chapter 5

5.1. Discussion

Accessing information about the prevalence of Brucellosis in South Africa is a challenge

because most of the State Veterinarians do not file the laboratory results for better access.

One needs to visit their offices which might not be practical to arrange the *Brucella* data.

Brucella remains a contagious disease In South Africa and the rest of the African continent,

in both animals and man.

The study area has 5 municipalities which the data of Ba-Phalaborwa was not available and

the data of Greater Giyani municipality and Maruleng were not complete for analysis.

Serum Agglutination Test and RBT show false negatives in chronic infected animals

(Godfroid *et. al* 2004). This makes it difficult to rely more on the two tests, because those

animals with low *Brucella* titre in case of chronicity will continue shedding the bacteria and

testing negative. The use of CFT is very helpful because the test is not affected by the

chronicity of the disease and those animals that have chronic infection will test positive with

the test.

The RBT results in chart 2 shows all the municipalities with decreasing number of animals

showing positive results, but Letaba shows an increase throughout the years. This can be

affected by several factors like illegal movements of cattle within the municipality or from

other municipalities into Letaba, or maybe due to poor surveillance and monitoring of the

disease and misuse of the vaccine especially s19 vaccine within the municipality. The RBT is

not a specific test like CFT whereby as the results shows there is reduction in the prevalence

of the disease tested with CFT. All the municipalities are showed a prevalence of less than

2% with Letaba at 1.25% greater than other municipalities in 2013. The results shows how specific CFT is in testing for ÷ø true infection øø having in mind the use of s19 vaccine.

Lack of previous data on the disease makes it difficult to compare the trend of the disease in the past and the efficiency of the control programme.

Chapter 6

6.1. Conclusion
Record keeping is essential for future references and comparison. Controlling the spread of zoonotic diseases requires a good control measure and surveillance, and this can be achieved by monitoring the previous results to reduce the prevalence of the disease.

The use of a new improved serological tests that can be applied in the field and laboratory settings if put in place can be helpful in diagnosing Brucellosis early and the use of tests that are not affected by the misuse of s19 vaccine which is legal to use in the Republic of South Africa.

6.2. Recommendations
Data keeping is very important when dealing with a zoonotic disease that can impose danger to both animals and human beings. It was one of the challenges that I faced while collecting data from other municipalities. Computerization of results can make data availability easily shared between individuals.

The use of strain 19 vaccine must be stopped because it leads to unacceptable large numbers of false positives, because the vaccine induces serological titres (Godfroid *et. al*, 2004)

27

vaccine titre tend to decline faster than those due to infection with wild strain, therefore retesting is required in those herds that test positive on serological tests.

Haphazard vaccination of heifers and vaccination of adult animals may result in much confusion in interpretation of laboratory results therefore accurate records of vaccination and birth dates are required (Godfroid *et. al*, 2004), but this can be difficult in communal areas where farmers do not keep records of their animals, but use their knowledge.

With addition to the serological tests already available the following once will help in the rapid sensitive and specific diagnosis of Brucellosis:

- ELISA test is more sensitive for detecting antibodies to Brucella species than RBT, SAT, CFT. This test can be introduced to Government laboratories to better the diagnostics of Brucella organism.

- Fluorescene Polarization Assay is a simple and rapid technique for measuring antigen and antibody interaction and may be performed in a laboratory setting or in the field (Godfroid *et. al*, 2004). This will provide rapid diagnosis in the field more in the commercial farms because they generate income from the cattle.

-Other tests like demonstration of allergic reaction to *B. abortus* can be used by using the Brucelin injected into the skin and the reaction is observed, this method is very useful in the test for bovine Tuberculosis.

References

- Brooke B., Birkhead M., Cohen C., Msimang V.D., Eidex R., Koornhof H., Quan V., Schoub B., *The Communicable Disease Surveillance Bulletin, National Institute for Communicable Disease (NICD),* 2012,17 pages.

- Godfroid J, Bosman P.P, Herr S. and Bishop G.C (2004) **Bovine Brucellosis in Infectious Diseases of Livestock 2nd edition volume** 3, edited by Coetzer J.A.W and Tustin R.C, Oxford South Africa, page 1510- 1523.

- Godfroid J., Al Dahouk S., Pappas G., Roth F., Matope G., Muma J., Marcotty T., Pfeifferj D., Skjerve E., *A "One Health" surveillance and control of brucellosis in development countries: Moving away from improvisation*, 2013, 8 pages.

- Kaltungo B.Y., Saidu S.N.A, Sackey A.K.B. and Kazeem H.M. (2014) *A review on diagnostic techniques for Brucellosis*, African Journal of Biotechnology vol. 13(1) page 1-10 http://www.academicjournals.org/AJB

- Marcotty T., Matthys F., Godfroid J., Rigouts L., Ameni G., van Pittius N.G., Kazwala R., Muma J., van Helden P., Walravens K., deKlerk L.M., Geoghegan C, Mbotha D, Otte M., Amenu K., Samra N.A, Botha C., Ekron M., Jenkins A., Jori F., Kriek N., McCrindle C., Michel A., Morar D., Roger F., Thys E. and van den Bosshe P., (2009*) Zoonotic tuberculosis and brucellosis in Africa: neglected zoonoses or minor public-health issues? The outcome of a multi-disciplinary workshop*, Annals of Tropical medicine & parasitology, vol. 103, no. 5, 401-411

- Megrid J., Mathias L.A. and Robles C.A.*, Clinical Manifestations of Brucellosis in Domestic Animals and Humans*, The Open Veterinary Science Journal 2010, 4, 119-126, http://benthamopen.com/contents/pdf/TOVSJ/TOVSJ-4-119.pdf

29

- Nielsen K., Yu W. L., *Serological Diagnosis Of Brucellosis, Ottawa Laboratories*,
Canadian Food Inspection Agency, 25 pages, http://manu.edu.mk/prilozi/4kn.pdf

- Nizeyimana G., Mwiine F.N. and Ayebazibwe C, (2013) comparative Brucella abortus
antibody prevalence in cattle under contrasting husbandry practices in Uganda*, Journal of
the South African Veterinary Association* 84(1), Art. 943, 5 pages.
http://dx.doi.org/10.4102/jsava.v84i1.943

- Njiro S.M., Kidanemariam A.G., Tsotetsi A.M., Katsande T.C, Mnisi M., Lubisi B.A., Potts
A.D., Baloyi F., Moyo G., Mpofu J., Katale A. and Williams R, (2011), *A study of some
infectious causes of reproductive disorders in cattle owned by resource-poor farmers in
Gauteng Province*, South Africa. Journal of the South African Veterinary Association, 82(4),
213-218

- OIE Terrestrial Manual, Bovine Brucellosis, Chapter 2.4.3., 2009, 35 pages,
http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.04.03_BOVINE_BRUCELL.pdf

- Radostits O.M., Gay C.C., Hinchcliff K.W. and Constable P.D., *Brucella in Veterinary
Medicine a textbook of the diseases of cattle, sheep, goats, pigs and horses*, 10th edition,
edited by Rodenhuis J. and Demetriou-Swanwick R., Oxford illustrations, page 963-994

- Smits H.L. and Kadri S.M, (2005), *Brucellosis in India: a deceptive infectious disease*,
Indian J MED RES, 10 pages

- The Department of Agriculture Limpopo, Cattle Dipping facilities in Mopani district,
census, 2013.

- Wikipedia, Mopani District Municipality,
http://en.wikipedia.org/wiki/Mopani_District_Municipality

30

- Wobeser G. ***The Centre for Food Security & Public Health, Institute for International Cooperation in Animal Biologics***, Iowa State University, Brucellosis, 2009, 13 pages, http://www.cfsph.iastate.edu/Factsheets/pdfs/brucellosis.pdf

- Yohannes M, Degefu H., Tolosa T., Belihu K, Cutler K. and Cutler S, (2013) ***, Brucellosis in Ethiopia***, African Journal of Microbiology Research vol. 7(14), academic Jounals 8 pages. http://www.academicjournal.org/AJMR

31